Abstracts for the 18th Annual Meeting of the European Society for Dermatologic Research

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Monday, 20 June, 1988

8.00-10.30

Concurrent session – Cuvilliés

R. MARKS, Chairperson

Psoriasis (Abstracts 1-12)

1

BIOCHEMICAL CHARACTERIZATION OF CORNIFIED ENVELOPES FROM NORMAL AND PSORIATIC SKIN. Serge Michel, Valerie Legrain*, Rainer Schmidt, Braham Shroot, Jean-Paul Ortonne* and Uwe Reichert. Centre international de Recherches Dermatologiques (CIRD), Sophia-Antipolis, Valbonne, and Laboratoire de Recherches Dermatologiques, Université de Nice, Nice - France.

The cornified envelope is an insoluble protein structure characteristic of terminally differentiating keratinocytes. It is formed beneath the plasma membrane as the result of the cross-linking of precursor proteins by the calcium dependent activation of plasma membrane associated transglutaminase. Recent findings suggest that the biochemical composition of the cornified envelope is not absolutely constant and that envelopes exist in two major morphologically distinct forms: an irregularly shaped, fragile type and a polygonal rigid type. These two forms represent successive stages of envelope maturation.

Cornified envelopes from the stratum corneum of healthy individuals and from involved and uninvolved skin of psoriatic patients were electrophoretically purified and their biochemical composition analyzed by polyacrylamide gel electrophoresis after cyanogen bromide cleavage. The resulting envelope peptide patterns (EPPs) were compared.

In normal subjects, slight, mainly quantitative differences of the EPP were observed between different individuals. Intra-individually, the EPP allowed us to distinguish two main topographical groups: the palm and sole samples presented identical patterns which were different from the patterns found on all other body sites.

In psoriatic patients, EPPs from uninvolved skin resembled closely those of healthy epidermis but showed striking differences when compared with lesional skin. These differences occur mainly at the level of peptides with molecular weights between 5 and 11 kd. The EPP from lesional skin returned to normal in the course of PUVA therapy, indicating that the changes in the biochemical composition of the cornified envelope are correlated to the clinical status of the disease.

2

AN EPIDERMAL ELASTASE INHIBITOR INDUCED BY SPONTANEOUS OR EXPERIMENTAL INFLAMMATION. J. Schalkwijk, A.M. Lammers, A. Chang, P.C.M. van de Kerkhof and P.D. Mier, Department of Dermatology, University of Nijmegen, The Netherlands.

The enzyme elastase occurs in the granules of the polymorphonuclear leukocyte and has been used as a marker for this cell in cutaneous infiltrates (Lammers et al, Br.J.Dermatol, 115: 181, 1986). In the presence of the cationic detergent cetrimide which complexes endogenous glycosaminoglycans, extracts of normal human skin cause little or no inhibition of elastase. However, we have observed that epidermis from the lesions of psoriasis or other chronic inflammatory skin diseases contains high levels of an inhibitory substance. Further, we have shown that an acute insult such as sellotape stripping induces the transient appearance of this inhibitor which reaches maximum levels about 48h after injury.

The inhibitor is stable to heat (5 min, 100°C) and to extremes of pH (0.1N HCl or NaOH, 30 min, 20°C). The molecular weight, determined by gel permeation on Sephadex G50, is around 9 KD, and the isoelectric point, measured by focussing on an Ampholine PAGplate, is about 7.4. Related proteases such as porcine pancreatic elastase and leukocyte cathepsin G are unaffected by the inhibitor. The inhibition of human leukocyte elastase is stochiometric, and assuming equimolar kinetics the concentration of the inhibitor in psoriatic epidermis averages 25 µ/g.

These properties suggest that the inhibitor described here is distinct from any previously reported antiprotease. The observation that its induction occurs relatively late in the sequence of events following trauma suggests that it may function as an "OFF" switch in the inflammatory mechanism.

3

A SEQUENTIAL DOUBLE IMMUNOENZYMATIC STAINING PROCEDURE TO OBTAIN CELL KINETIC INFORMATION IN NORMAL AND HYPERPROLIFERATIVE EPIDERMIS. S. de Mare, P.E.J. van Erp, J.J. Rijzewijk, J.B.M. Boezeman, P.C.M. van de Kerkhof and F.W. Bauer(1), Department of Dermatology, University of Nijmegen/NL; (1) Hoffmann-La Roche, Basle, Switzerland

A sequential double immunoenzymatic staining procedure was developed using the monoclonal antibody anti-BrdU and Ki67 to visualize respectively DNA synthesizing cells (S-phase) and proliferating cells at one and the same frozen section. This technique is used to determine whether hyperproliferate skin disorders, such as psoriasis, are characterized by an increased growth fraction rather than a much faster cell cycle time of all germinative cells. Biopsies were taken from 15 psoriatic patients (all of lesional skin and in 4 also of uninvolved skin), 3 atopic dermatitis patients and 7 healthy volunteers. Although in hyperproliferative epidermis the absolute numbers of BrdU-positive cells (= Ns: normal skin = 8.64; atopic skin = 48.8; psoriatic skin = 69.1) as well as Ki67-positive cells (= Nc: normal skin = 24.7; atopic skin = 135.5; psoriatic skin = 194.1) were grossly increased, the ratio of these values was not changed compared to the ratio found in the epidermis of the clinically uninvolved skin of psoriatic patients and in normal epidermis (Ns/Nc ~ 0.35). The results obtained with this technique confirm the existence of a population of resting (Go) cells controlling the production of keratinocytes in normal as well as psoriatic skin. Assuming that the cycling cells are randomly distributed through the various phases of the cycle, Tc (duration of the cell cycle) can be calculated out of the equation: Ns/Nc=Ts/Tc in which Ts is the duration of the S-phase, which is relatively constant averaging about 10h. Tc was calculated to be ~ 28h for both normal and hyperproliferate epidermis which means that speeding up of the cell cycle time in psoriasis and other hyperproliferative skin disorder is not the major regulation process.

4

A MEMBRANE-BOUND INOSITOL-SPECIFIC PHOSPHOLIPASE C IN NORMAL AND PSORIATIC EPIDERMIS. P.D. Mier, Mieke Bergers and R. Happle. Department of Dermatology, University of Nijmegen, NL

The phosphoinositol cycle probably controls the proliferation of keratinocytes (via phosphorylation of the Na^+-H^+ antiport) and the release of inflammatory eicosanoids (via phosphorylation of lipocortin). The rate-limiting enzyme is a membrane-bound phospholipase C specific for phosphatidylinositol 4,5-bisphosphate ($PtdInsP_2$). Here we report the quantification of this enzyme in epidermis from healthy skin psoriatic lesion and psoriatic uninvolved skin.

Biopsies (1 cm^2 x 0.3 mm) were homogenized in tris buffer using a Potter-type grinder, and the homogenate centrifuged at 40,000 g. The pellet was washed and resuspended in buffer to yield a crude membrane preparation. Phospholipase C was assayed by measuring the water-soluble radioactivity released from cetrimide-stabilized micelles of Inositol-2-^{3}H(N)-$PtdInsP_2$. Results (means ± SD, n = 8) were:

	Normal	Ps uninv.	Ps lesion
pmol/min/mg wt	25 ± 18	31 ± 21	76 ± 25
pmol/min/ug protein	1.3± 0.9	1.7± 1.3	2.3± 0.9

Regardless of reference variable the level of phospholipase C in the lesion was increased with respect to healthy skin ($P < 0.01$, Wilcoxon), but the activity in psoriatic uninvolved skin was normal. These findings are in line with those recently reported for a soluble enzyme active against PtdIns (Bartel et al, J Invest Dermatol, 88:447, 1987), and support the concept that psoriasis may be associated with an imbalance in the phosphoinositol cycle.

5

15-HETE SPECIFICALLY INHIBITS THE LTB_4-INDUCED INFLAMMATORY SKIN RESPONSE. Thomas Ternowitz, Peter H. Andersen, Peter Bjerring and Knud Kragballe. Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark.

15-HETE, a 15-lipoxygenase product of arachidonic acid, inhibits leukocyte 5-lipoxygenase activity and leads, therefore, to an inhibition of leukotriene formation. Previously, we have shown, that psoriatic plaques injected intralesionally with 10^{-5}M 15-HETE have cleared completely during three weeks of treatment, and that 15-HETE inhibits LTB_4-induced chemotaxis of PMN. In this study we examined the effects of intradermal injections of LTB_4 and of LTB_4 + 15-HETE. Simultaneously, we performed intradermal injections of human complement split product C5a and of C5a + 15-HETE and C5a. LTB_4 was injected at the concentration of 200 ng/ml. At this concentration the skin response of LTB_4 and C5a was of the same order of magnitude. The final concentration of 15-HETE in the mixture with LTB_4 and C5a was 10^{-5}M. The injection volume was 100 µl, and the injections were performed on the volar forearm of healthy subjects. The skin response was evaluated by measuring the diameter of the wheal, the area of the flare and by erythema index. In all subjects the skin response was significantly inhibited when LTB_4 was injected together with 15-HETE. The decrease of wheal, flare and erythema index averaged 81.9%, 56.6% and 53.6%, respectively, when all parameters were obtained at maximal skin response. On the contrary, the C5a-induced skin response was not affected by addition of 15-HETE, even when the final concentration of 15-HETE was increased 10 times. The present results demontrate that 15-HETE is a specific inhibitor of inflammatory skin response by LTB_4. By inhibiting not only the formation, but also the effect of LTB_4, 15-HETE may have a profound effect on skin inflammation elicited by LTB_4.

0020-202X/88/$03.50

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PROPRANOLOL POSITIVELY AFFECTS PSORIATIC POLYMORPHONUCLEAR LEUCOCYTE MIGRATION. T.S. Sonnex and M.W. Greaves, Institute of Dermatology, St Thomas Hospital, London, England.

Many reports have now implicated various beta-adrenergic receptor blocking drugs in the precipitation and exacerbation of psoriasis. As polymorphonuclear leucocytes (PMN) are a well recognised feature of early psoriatic lesions we investigated the effect of propranolol on PMN mobility.

a) The chemotactic activity of propranolol was investigated in vitro (modified Boyden Chamber technique) using PMNs from 14 psoriatics and 14 age and sex matched controls and in vivo (skin window chamber technique) in 10 psoriatics and 10 age and sex matched controls;

b) The effect of propranolol on the ability of PMNs to respond to a standard chemotaxin (E. coli endotoxin activated serum) was investigated in vitro by incubating PMNs from 25 psoriatics and 25 age and sex matched controls in different concentrations of propranolol before the chemotaxis assay was performed. Propranolol in a concentration of 3.4×10^{-5}M ($p = < 0.05$) in vitro and at concentrations of 3.4×10^{-9} ($p = < 0.02$), 3.4×10^{-7} ($p = < 0.05$) and 3.4×10^{-5}M ($p = < 0.02$) in vivo was significantly more chemotactic for psoriatic PMNs as compared to control PMNs. Also in vitro, propranolol at concentrations of 3.4×10^{-6} ($p = < 0.05$) and 3.4×10^{-5} ($p = < 0.001$) significantly enhanced the ability of psoriatic PMNs (as compared to controls) to respond to the standard chemotaxin.

This positive effect of propranolol on psoriatic (but not control) PMN chemotaxis provides at least 1 mechanism by which beta blockers could influence psoriasis, particularly as the therapeutic level of propranolol is 3.4×10^{-7}M. It may be mediated by PMN beta-receptors and the cyclic nucleotide axis.

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DITHRANOL INHIBITS THE TRANSEPIDERMAL MIGRATION OF POLYMORPHONUCLEAR LEUKOCYTES. J.A.C. Alkemade, A. Chang, P.D. Mier, R. Happle and P.C.M. van de Kerkhof, Department of Dermatology, University of Nijmegen, The Netherlands.

The aim of the present investigation was to study the effect of dithranol with and without the addition of salicylic acid, on the migration of polymorphonuclear leukocytes (PMN) into the epidermis 'in vivo' after a standardized chemotactic signal i.e. (i) a standardized surface trauma by sellotape stripping; (ii) epicutaneous application of leukotriene B_4 (LTB_4).

Skin areas of 11 healthy volunteers were treated with dithranol creams, according to a standard schedule for short-contact therapy (20 min application) for 8 days. In each subject 3 areas (10 x 5 cm^2) were treated with: (i) dithranol (0.5%; 1.0%; 2% or 3%) each containing salicylic acid 2% in cream base (Psoralon^R MT, Hermal Kurt Herrmann, Reinbek, Germany); (iii) dithranol (0.5% - 3%) without salicylic acid in the same cream base; (ii) the cream base only. Approximately 12h after the last treatment sellotape stripping was carried out and LTB_4 (10 ng) was applied on each of the 3 test areas. Microbiopsies were taken, using a razorblade, 8 and 24h respectively after these challenges. After homogenization of the biopsies elastase was measured, using the fluorogenic substrate MeOSuc-Ala-Ala-Pro-Val-N-methylcoumarin, and the activity of this enzyme used to calculate the number of infiltrated PMN.[1]

In all subjects the concentrations of dithranol could be increased up to 3%. The additions of salicylic acid did not significantly modify burning, erythema or staining. The PMN accumulation following the application of LTB_4 was significantly decreased within the test areas pretreated with either dithranol and salicylic acid ($P < 0.005$, Wilcoxon ranking test for paired data) or dithranol only ($P < 0.025$). The addition of salicylic acid was accompanied in some subjects by a more substantial inhibition. In contrast, the trauma-induced PMN accumulation was not affected by pretreatment with dithranol.

In conclusion, dithranol treatment of normal skin prevents the intraepidermal accumulation of PMN following challenge with LTB_4.

1. Lammers, AM., van de Kerkhof PCM, Schalkwijk J, Mier PC, Br J Dermatol 115:181-186, 1986.

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ACIDIC LIPID LYMPHOCYTE CHEMOTACTIC ACIVITY IN LESIONAL PSORIATIC STRATUM CORNEUM. K. Bacon, R. Camp and F. Cunningham, Institute of Dermatology, London, England.

The lymphocyte infiltrates in psoriasis may be induced by locally generated chemoattractants including lipoxygenase products of arachidonic acid metabolism. Since the dose-related lymphocyte chemotactic activity found in aqueous extracts of lesional psoriatic stratum corneum (s.c.) and normal s.c. was similar, the lymphocyte chemotactic activity in acidic lipid extracts of normal and psoriatic s.c. has now been compared.

Samples of s.c. (15 mg each) were recovered by abrasion of psoriatic lesions ($n = 4$) and the heels of normal volunteers ($n = 5$), and lipid extracts obtained by homogenisation in an ethyl acetate / 0.1M sodium acetate (pH 3.5) mixture. Organic residues were then assayed in serial dilution by a lymphocyte microchemotaxis method. Lipid extracts of psoriatic s.c. (30 mg) were also purified by HPLC on a Nucleosil 50-5µm column eluted with hexane/propan-2-ol/methanol/-acetic acid (88:7:5:0.1 by volume) at 1 ml min^{-1}. Fractions (1 ml) were collected, evaporated and assayed for lymphocyte chemotactic activity.

Dose-related activity was obtained in response to the psoriatic s.c. acidic lipid extracts between 16 to 256 fold dilution, the maximal migration index being 1.8 ± 0.1 (mean ± s.e. mean, $n = 4$, random migration = 1). No dilution-related lymphocyte chemotactic activity was found in lipid extracts of normal s.c. Lipid extracts of ultrafiltered supernatants (< 10 kd) obtained by homogenising psoriatic s.c. (15 mg) in aqueous medium (0.8 ml), contained no dilution-related lymphocyte chemotactic activity ($n = 5$), indicating that the lipid activity is not recoverable from psoriatic s.c. by aqueous extraction. HLPC of psoriatic s.c. lipid extracts showed consistently increased lymphocyte chemotactic activity in fractions 4-5, co-eluting with a 12-HETE standard ($n = 4$). Two consistent peaks of more polar activity, neither of which co-eluted with standard leukotriene B_4 (retention time 9 - 9.4 min), were seen in fractions 7-8 and 14-15 ($n = 4$).

Thus lipid lymphocyte chemoattractants, including a 12-HETE-like compound and at least two other unidentified compounds, may be responsible for the increased lymphocytic emigration into lesional psoriatic epidermis.

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FUNCTIONAL AND PHENOTYPIC CHARACTERIZATION OF CLONALLY EXPANDED LYMPHOCYTES FROM PSORIATIC LESIONS. Rudolf E. Schopf, Wiltrud Meyer, Marianne Rohder, Konrad Bork, Martin Böckers, Bernd Morsches, Department of Dermatology, Johannes Gutenberg-University, D-6500 Mainz, W. Germany.

Epidermal lymphocytes are thought to regulate the activity of psoriasis. The functional properties of these cells have thus far not been examined in vitro. In order to obtain sufficient numbers for both phenotypic and functional in vitro examinations, we attempted to isolate lymphocytes for clonal expansion from psoriatic lesions. Pure epidermal cells were isolated by suction blister methods. Epidermal cell suspensions were cultured by limiting dilution techniques (1 lymphocyte/culture) using irradiated mononuclear blood cells plus EBV-transformed B cells (EBV-B) as feeder cells. Phytohemagglutinin was added at the beginning of the culture period. RPMI-1640, 5% AB serum culture medium supplemented with fresh interleukin-2 was changed every 3 days. After 2 to 3 x 10^6 lymphocytes/clone had grown, the phenotype was determined by FITC-labeled monoclonal antibodies. Functional tests performed included cell-mediated cytotoxicity and natural killer (NK) activity employing ^{51}Cr-labeled EBV-B and K562 target cells, resp. 94% of 48 expanded clones reacted with anti-CD4 and 6% with anti-CD8 monoclonal antibodies. 70% of 33 clones examined exerted cytotoxicity against EBV-B target cells. 48% of 48 clones tested exhibited NK activity. 100% of NK-active clones reacted cytotoxic against EBV-B target cells, while 50% of NK-inactive clones failed to exhibit cytotoxicity. 56% of cytotoxic clones were NK-active, the remainder inactive. Clones lacking cytotoxic activity all were NK-inactive. The cytotoxic and NK activities ot the clones correlated ($P < 0.001$). The high prevalence of CD4+ clones with cytotoxic activity is consistent with an activation of T cells by HLA-DR+ keratinocytes. The fact that NK cells also recognize cell proliferation-associated cell surface molecules supports the concept that CD4+ cells are closely linked to the development of psoriatic lesions.

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SIMULTANEOUS INTERMEDIATE FILAMENT/DNA ANALYSIS OF EPIDERMAL CELL SUSPENSIONS DERIVED FROM NORMAL AND PSORIATIC SKIN USING FLOW CYTOMETRY. Piet E.J. van Erp, Joris J. Rijzewijk, John Leenders, Frans C.S. Ramaekers(1), Peter C.M. van de Kerkhof, Frans W. Bauer(2). Department of Dermatology and Pathology (1), University of Nijmegen, NL; Hoffmann-La Roche, Basle, Switzerland (2).

Monoclonal antibodies against intermediate filaments are now widely used as cell-type markers in histopathology and for studies of cell differentiation. Proper and accurate identification of epidermal subpopulations in normal and pathological skin using these antibodies should lead to a better understanding of the complex mechanisms by which epidermal growth control is achieved. In the present study epidermal subpopulations were analyzed in normal skin (N), psoriatic lesional skin (IP) and distant uninvolved skin (UP) of psoriatic patients using four monoclonal antibodies against intermediate filaments and flow cytometric measurement of immunofluorescence and relative DNA content simultaneously. Cell supensions were prepared by trypsinization and fixed. The phenotypes of the cells were identified by an indirect immunofluorescence technique. Aliquots of the epidermal cell suspensions were incubated with RKSE60 (keratin 10, suprabasal keratinocytes), Pab601 (keratin 5/14, basal keratinocytes), K8.12 (keratin 13/16, hyperproliferation-associated expression in suprabasal keratinocytes) and MVI (vimentin, non-keratinocytes). Propidium iodide was used as a fluorescent DNA stain.

Relative pool sizes of RKSE60-positive cells were significantly decreased in IP and in UP as compared to N. Results obtained with Pab601 were complementary to RKSE60 bindings. In the case of vimentin-positive cells a significant decrease was observed only in IP. The difference between normal and psoriatic skin with respect to K8.12 bindings was striking. In N and UP from psoriatic patients K8.12-positive cells were found in very low amounts. In IP, however, the percentage K8.12-postive cells varied from 10-60%. Percentage cells in S-phase and G_2M-phase of the cell cycle in IP was significantly increased compared to N. The findings in this study confirm and reinforce previous observations of keratin abnormalities and increased proliferation in psoriatic skin. Multiparameter flow cytometry combining intermediate filament staining and measurement of relative DNA content allows rapid and accurate analysis of epidermal cell populations.

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PSORIATIC AND TRAUMA-INDUCED HYPERPROLIFERATION ASSESSED BY THE MONOCLONAL ANTIBODY K_s8.12 ON FROZEN SECTIONS. P.C.M. van de Kerkhof, S. de Mare and P.E.J. van Erp, Department of Dermatology, University of Nijmegen, The Netherlands.

Keratin 16 is of special interest in studies on epidermal proliferation in psoriasis. Firstly, this keratin is present exclusively in hyperproliferative skin and is not expressed in normal skin. Secondly, this keratin has been claimed to be the most reliable marker of clinical resolution of the lesion.[1] Recently a new monoclonal antibody has been reported, K_s8.12m which may be regarded as specific for keratin 16 in skin. Here we report on the distribution and degree of expression of this keratin, using K_s8.12, in lesional and clinically uninvolved skin of psoriatic patients and trauma-induced hyperproliferative epidermis of healthy volunteers.

Biopsies were taken from normal skin, 40h and 64h after sellotape stripping, and from psoriatic lesions. Following cryostat sectioning fixation was carried out in acetone/ether. Air-dried sections were then incubated in PBS containing Tween 80 and subsequently K_s8.12, diluted 1:40 in PBS was added. After washing in PBS a peroxidase-conjugated antimouse antibody was developed with the 3-amino-9-ethylcarbazole/H_2O_2 reaction. Sections were counterstained in glycerine gelatine.

In the psoriatic lesion keratin 16 was expressed in the whole suprabasal compartment except the stratum corneum. The clinically uninvolved skin of psoriatic patients and the skin of healthy volunteers showed no suprabasal staining and a slight basal staining. Following sellotape stripping of normal skin a pronounced suprabasal staining was seen. The wide range between normal and lesional skin of psoriasis in terms of K_s8.12 staining makes it a very interesting parameter for studies on this disease.

1. Holland DB, Wood EJ, Cunliffe WJ, West MR, Turner DM. J Invest Dermatol 1987; 89: 328.

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THE ROLE OF THE EPIDERMIS IN THE KOEBNER REACTION. A.V. Powles, B.S. Baker, J. McFadden, H. Valdimarsson and Lionel Fry. Department of Dermatology, St Mary's Hospital, London W2, U.K.

It is not known why some patients with psoriasis are Koebner positive and others Koebner negative. Since it has been reported that the Koebner phenomenon follows the 'all or none' rule it has been assumed that there is likely to be a central factor influencing the outcome. However, the nature of the injury may also be important in determining the result, and we have therefore investigated the Koebner reaction using three different types of injury. These were a) sellotape-stripping, b) suction cup blisters, leaving blisters intact, c) suction cup blisters with roof removed. Patients with psoriasis were randomly allocated to the three study groups. 12 had sellotape-stripping alone, 10 had multiple suction cup blisters, in which a proportion of the blisters were left intact but in others roofs removed, and 9 had sellotape-stripping and multiple suction cup blisters simultaneously; again some were left intact and others had their tops removed. Positive Koebner results were produced in 1 (8%) of 12 patients who had sellotape-stripping and in 6 (60%) of 10 patients who had suction cup blisters with roofs removed, but not in blisters left intact. In the 9 patients who had all three types of injury, 3 (33%) were positive to both sellotape-stripping and suction cup blisters with roofs removed but not to intact blisters, and 2 more were positive to de-roofed blisters but negative to sellotape stripping and intact blisters.

The positive Koebner response was punctate in the sellotape injury (probably coinciding with clefts produced in the epidermis) but confluent in the suction cup blisters. These results show that the type of injury is important in determining whether patients are Koebner positive and must involve disruption of the epidermal layer.

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EPITHELIAL DIFFERENTIATION OF HUMAN SKIN EQUIVALENTS AFTER GRAFTING ONTO NUDE MICE. E. Tinois[1], A. Ramirez Bosca[2], M. Faure[1], J. Kanatakis[1] and J. Thivolet[1]. 1. Inserm U209, Pav R, Hop E. Herriot, Lyon, France, 2. Servicio de Dermatol, Facultad Med., Valencia, España.

Human skin equivalent was developed in vitro with an unidimensionally retracted dermal equivalent made of human type I+III collagen and human MRC5 fibroblasts, and a multilayered epithelium developing from normal human keratinocytes plated on the surface of the dermal equivalent. Since the latter adhered to the bottom of the plate, the keratinocytes in suspension could be seeded directly and grown submerged in medium DMEM + HAM F12 with 10% FCS, adenine, hydrocortisone, insulin, T3, transferrin, cholera toxin and EGF. The epithelia that were obtained were composed of a basal cell layer and several suprabasal layers which exhibited an incomplete differentiation. We investigated the degree of epidermal differentiation that could be achieved after in vivo grafting onto nude mice by means of light and electronmicroscopy as well as by immunohistochemistry. Twenty-four nude mice were grafted with this skin equivalent. All transplanted grafts showed a primary take. The grafts formed an epidermis with a stratum corneum and from day 7 or 14 after transplantation a distribution of a 56.5 Kd keratin protein, involucrin, profilaggrin/filaggrin, and MHC-I antigens that was similar to what is noted in normal human epidermis. These data indicate that a full terminal differentiation was only achieved after in vivo transplantation of the cultured epithelium. Pigmentation was present, but no marker of Langerhans cells was seen at four weeks. Although there was no longer evidence of the dermal equivalent after two weeks, we noted a strong adherence of the graft to the wound bed, with the presence of type-IV collagen, laminin and bullous pemphigoid antigen at the dermo-epidermal junction (day 7) and hemidesmosomes, a lamina lucida and a lamina densa (day 30). No epithelial damage was noted in spite of an inflammatory infiltrate in the underlying tissue. This represents a preliminary step in the use of such a skin-equivalent in the treatment of human patients with wounds.

Monday, 20 June, 1988

8.00-10.30

Concurrent session – Effner

P. FRITSCH, L. DUBERTRET, Chairpersons
Skin Culture (Abstracts 13-24)

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A FIBRILLAR SPONGE OF BOVINE COLLAGEN AS READY-FOR-USE SUBSTRATUM FOR HUMAN EPIDERMAL CELLS. B. Bonnekoh, B. Thiele, A. Wevers and G. Mahrle, Department of Dermatology, University of Cologne, FR Germany.

From our clinical experience with wound coverage by skin epithelium produced in cell culture we were interested to look for underlying dermal equivalents as stable supports. Thus, we tested epidermal growth on Lyostypt (Braun, Melsungen, FRG), a compressed texture of pure, native bovine collagen fibrils, which is already licenced for the local control of surgical bleeding.

Normal human epidermal cells were cultured according to routine procedures with lethally irradiated 3T3 feeder cells in supplemented medium (choleragen, hydrocortisone). Reaching subconfluency in first subculture epidermal cells were seeded on Lyostypt (optimally 10^6 cells per 1 cm2). The development of epidermal sheets was monitored by light and electron microscopy.

At day 10 of culture on Lyostypt a thick epithelium with up to 7 layers of cells had formed. The epidermal cells invaded the loosened upper part of the collagen forming pegs which to some degree anchored the epidermis rete-like to the Lyostypt-substratum. Beyond day 14 progressive degradation of Lyostypt was observed obviously due to an epidermal collagenase activity. The digestion of the collagen matrix was more pronounced in an air-liquid-interface culture assay. Under all conditions of the assay SDS-PAGE showed keratins of 48, 50, 52.5, 56 and 58 kD indicative for proliferative growth conditions.

In comparison to the classical collagen gel the Lyostypt matrix allows to some extent the formation of an undulating epidermal-dermal interface caused by a heterogenous distribution of epidermal collagen digestive activity.

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DUAL ORIGIN OF THE VASCULAR BASEMENT MEMBRANE IN HUMAN SKIN RECONSTRUCTED IN VITRO AND TRANSPLANTED ONTO THE NUDE MOUSE. Michael Domarchez, Daniel Asselineau, Marcelle Regnier, Daniel Hartmann*. Cell Biology Department, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, F-06565 Valbonne, France, *Centre de Radio-analyse, Institut Pasteur, Lyon, France.

In a previous study, we demonstrated that, in human skin grafted onto the nude mouse, the blood vessels were composed of mouse endothelial cells which rest on a basement membrane containing human and mouse type IV collagen. Because of the disappearance of the human endothelial cells in the grafts, the maintenance of the human type IV collagen and its origin was questionable. The present study was designed to investigate whether the human type IV collagen would be the remaining type IV collagen of preexisting blood vessels or constantly produced by non-endothelial human cells. For this purpose, we have grafted, onto nude mice, different human skin equivalents reconstructed in vitro. They were composed of human keratinocytes deposited either on a dead human dermis, or on a lattice composed of GM10 fibroblasts embedded in type I collagen, or else on a dead lattice. At different stages after grafting, the transplants were harvested and processed for an immunohistological study. It was observed that, at one month after grafting, the three types of artificial dermis contained blood vessels whose vascular basement membranes were labeled with a mouse-specific anti-type IV collagen antibody. However, with a human-specific anti-type IV collagen antibody, labeling of the vascular basement membrane was only observed in the lattice containing GM10 fibroblasts while the dermal epidermal junction underneath the human epidermis was labeled in the three types of reconstructed skin. From this study, it is concluded that, in human skin grafted onto the nude mouse, human fibroblasts would interact with mouse endothelial cells to produce the basement membrane of the vessels. This observation is in favor of a dual origin of the blood vessel basement membrane.

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IN VITRO RECONSTRUCTED EPIDERMIS COMPARED WITH HUMAN SKIN USING FREEZE FRACTURE EM. Holman* B.P., Ponec** M., Boddé* H.E. and Spies*** F., *Center for Bio-Pharmaceutical Sciences, P.O. Box 9502, NL-2300 RA Leiden, The Netherlands, **Dept. of Dermatology, University Hospital, Leiden, The Netherlands, ***Dept. of Electron Microscopy, Psychiatric Hospital Endegeest, Oegstgeest, The Netherlands.

In vitro reconstructed skin may be of use both for scientific purposes (e.g. as a research model for metabolisms and transport studies) and in the clinical field (e.g. for burn treatment). For skin reconstruction it is vital that cells undergo complete differentiation. This and various other aspects were investigated in reconstructed epidermis using freeze fracture electron microscopy. Keratinocytes from juvenile foreskin were cultured using the Rheinwald-Green feeder layer method. Growth medium composition was varied as follows:
1. Dulbecco-Vogt's and Ham's Medium (3:1) enriched with 5% FCS and various growth factors (medium M). 2. Arachidonic acid added (medium A). 3. Linoleic acid added (medium L). For epidermal reconstruction keratinocytes were seeded on to de-epidermized human cadaver skin and cultured under air exposure. As soon as a confluent cornified apical layer had formed, samples were cut and prepared for freeze fracture EM. The reconstructed epidermis (case M) very well mimicked skin properties such as: the differentiation pattern; uninterrupted cornification rather than depostion of cornified 'patches'; disposition of lamellar intercellular (i.c.) lipids. However some differences with human skin were observed: irregular desmosomal particle arrangements, locally abnormal orientation of i.c. lamellae, and the sporadic presence of lamellar bodies inside corneocytes. Addition of Arachidonic acid (case A) or Linoleic acid (case L) both improved desmosomal structure.

We conclude that the reconstructed epidermis mimicks a number of skin features essential for the barrier function, and that its quality may even be improved by optimizing the lipid content of the growth medium and/or other growth conditions.

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SIMILAR EPIDERMAL KERATINOCYTE DIFFERENTIATION IN CULTURED HUMAN SKIN, PSORIATIC LESIONAL SKIN AND WOUND SKIN. M.R. West, S.J. Fogarty, J.M. Page, H.T. Rupniak, Department of Cell Biology, Glaxo Group Research, Greenford Road, Greenford, Middlesex U.K.

Explants of normal human skin cultured at an air/medium interface undergo rapid morphological and biochemical changes so that within 1-3 days the resultant epidermal phenotype is strikingly similar to that of psoriatic lesions by a number of criteria.

Morphologically, the epidermis develops a layer of parakeratosis underneath the stratum corneum as previously reported.[1] Immunocytochemical studies with a monoclonal antibody that recognises envelopes in cultured keratinocyte and psoriatic lesions, but not envelopes in normal or uninvolved interfollicular epidermis, have shown that envelopes in the parakeratotic area in the culture skin epidermis are of the cultured keratinocytes type.

Analysis of the keratin profile of cultured skin by one dimensional SDS PAGE has shown a rapid loss of the 67-69Kd keratin (keratin 1) and the appearance of the 48Kd keratin 'hyperproliferation marker' (keratin 16). Pulse labelling studies revealed that changes in the synthesis of keratins occurred within 14 hours.

The keratin profiles and envelope and involucrin antibody reactivity of wound skin have also been investigated. Preliminary studies indicate that the 48Kd keratin expression is induced, and that antibody reactivity of the skin at the wound edge is very similar to that seen in organ cultured skin and in psoriatic lesions.

In conclusion, by the above criteria, normal human skin in organ culture rapidly adopts the psoriatic lesional epidermal phenotype. As similar changes appear to occur in wound skin where normal homeostatic mechanisms are perturbed, a partial or total breakdown in homeostasis may account for the development of this altered phenotype in the psoriatic lesion.

1. Levine, M 1972: BR.J.Dermatol. 86, 481-490.

18

CULTURED ALLOGENIC KERATINOCYTES DO NOT SURVIVE TRANSPLANTATION TO A DEEP DERMAL BED. Brain A., Hancock K., Coats P., Navsaria H., Purkis P.E., Leigh I.M., Experimental Dermatology (ICRF), London Hospital, London E1 2BL.

Cultured Keratinocytes can be used to graft burns, surgical wounds and chronic leg ulcers. Both autologous and allogenic keratinocytes have been used clinically, and encouraging clinical results have suggested that there is clinical take of culture allografts. The loss of the Langerhans cells during keratinocyte culture, has been suggested as the mechanism for this postulated tolerance. However it is necessary to establish biological take by an unequivocal method. We have grafted a) deep surgical wounds following shave excision of tattoos and b) superficial wounds following excision of donor site, with sheets of sex-mismatched cultured allogenic keratinocytes. Post graft biopsies habe been taken at weekly intervals to healing and then studied by in situ hybridisation using a biotinylated probe to part of the y chromosome. There has been no evidence of long term survival of allogenic keratinocytes, and wounds were healed with cells of the recipient rather than that of the donor sex. Thus the beneficial effects of keratinocyte grafting are not due to biological take of keratinocytes.

19

ALLOGENEIC CULTURED EPIDERMIS FOR WOUND GRAFTING. B.-U. Specht[1], B. Strittmatter[1] and N. Böhm[2], 1 Department of Surgery, University of Freiburg, FRG, 2. Department of Pathology, University of Freiburg, FRG.

According to the method described by H. Green we grew epithelial sheets in tissue culture from single cell supension obtained after trypsinization of human foreskin specimen.

Allotransplantation of ten patients with burn lesions or with traumatic skin ulcers has been performed, with no rejection being observed up to one year. One graft has been lost due to infection. Immunohistochemical investigations showed that cultured epithelial cells express HLA class I antigens but not class II antigens. Class II antigen expression could be induced by addition of γ-Interferon to the culture medium for two days.

Histological examination of a biopsy taken three months after allotransplantation to a sex-mismatched recipient showed a straight basal membrane with almost no scar formation. Skin appendages as well as rete ridges were absent. Immunohistochemical staining showed the lack of melanocytes and Langerhans cells. Y chromosome analysis of the biopsy is under present investigation.

We conclude that allogeneic cultured epithelium can be used successfully for coverage of skin lesions.

20

RETINOIDS MODULATE THE DERMO-EPIDERMAL INTERACTIONS ON THE SKIN EQUIVALENT CULTURE MODEL. S. Sanguer, B. Coulomb, C. Lebreton, L. Dubertret, INSERM U 312, Dept of Dermatology, Hôpital Henri Mondor, F-94010 Créteil.

The pharmacological effects of retinoic acid (RA), isotretinoin (ISO) and etretin (ET) have been studied using a recently described tridimensional cell culture system: the human skin equivalent model.

First, dermal equivalents (DE) made up with normal dermal fibroblasts seeded into a collagen matrix were treated on days 1 and 4 after casting. After 1 week, either RA, ISO and ET at concentrations 10^{-7}M, 10^{-6}M, 5.10^{-6}M induce a dose-dependant fibroblast hyperproliferation and a dose-dependant decrease of the DE contraction. As DE contraction increases with the number of fibroblasts, we may conclude that retinoids modify fibroblast-matrix interactions.

Secondly, epidermalization is initialized by sticking 1 mm diameter normal human skin punch biopsies on contracted DE. Epidermal cell proliferation is quantified by 4 parameters: epidermal surface, tritiated thymidine incorporation (Thy*), DNA content and the ratio of Thy* by DNA. The treatment started at the first day of epidermalization and repeated every 4 days. At the end of experiments (13th day) we observed that at 10^{-7}M the three retinoids decrease the epidermal growth, at 10^{-6}M and 5.10^{-6}M RA and ISO maintain the inhibition of the cell proliferation but with ET at the same concentration the inhibition disappears. From these differences on cell growth, we may conclude that the activity of RA, ISO and ET is related to their chemical structure with a parallel effect of the two isomers (RA-ISO) and one different for the aromatic compound (ET).

Third, the dermo-epidermal interactions have been visualized by the comparison of epidermalization on living DE (LDE) and killed DE (KDE). After retinoid treatment (10^{-7}M, 10^{-6}M and 5.10^{-6}M), a better epidermalization is obtained on KDE that on LDE. Furthermore, in absence of living fibroblasts (KDE) we do not observe significant difference between RA, ISO and ET on epidermal cell proliferation in contrast with epidermalization on LDE.

Our results demonstrate that retinoid effects on epidermal cell proliferation are mediated by dermal fibroblasts.

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A COMPARATIVE STUDY OF ANTISEPTIC TOXICITY ON BASAL KERATINOCYTES, TRANSFORMED HUMAN KERATINOCYTES AND FIBROBLASTS. F.M. Tatnall, I.M. Leigh and J.R. Gibson. The Skin Department, The London Hospital, London, England.

We have previously shown that virally transformed keratinocytes (SVK14 cells) may be of value in providing a ranking order of antiseptic cytotoxicity. In this study, we compare the cytotoxic effects of antiseptics on SVK14 cells with those on human fibroblasts and basal keratinocytes, cells which are of fundamental importance in wound healing.

Passaged human keratinocytes cultured in low calcium medium to induce destratification, fibroblasts and SVK14 cells were grown in subconfluence in multiwell plates. The cells were exposed for 15 minutes to serial dilutions of the therapeutic concentration of chlorhexidine, hydrogen peroxide, and sodium hypochlorite. The antiseptic agents were washed off and the cells were reincubated in culture medium for 24 hours. Cytotoxicity was then evaluated using the colorimetric MTT assay in which a yellow tetrazolium salt is cleaved to a blue formazan product in living cells. The optical densities were read using a spectrophotometer and compared to untreated controls.

At therapeutic concentrations, all of the antiseptic agents produced 100% killing of all cell types. An appraisal of respective LD50 values and therapeutic concentrations showed the relative toxicities on all cell types to be sodium hypochlorite>hydrogen peroxide>chlorhexidine.

These results are in agreement with in vivo studies of impairment of wound healing by antiseptic agents. LD50 values suggest that basal keratinocytes and SVK14 cells have similar susceptibilities to antiseptics and thus SVK14 cells, which have the advantage of unlimited growth potential, may appropriately be substituted for normal keratinocytes when studying antiseptic toxicity in vitro. Finally, antiseptic cytotoxicity to basal keratinocytes and fibroblasts indicates that caution should be exercised when using such agents in wound management.

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QUANTIFICATION OF ANGIOGENESIS IN HEALING WOUNDS OF HUMAN SKIN. R. Marks, J. Baverstock, *J. Giddings, S. Barton, Department of Medicine and *Haematology, University of Wales College of Medicine, Cardiff, Wales, U.K.

Revascularisation of wounden areas is thought to be a major rate-determining factor in the healing process. Measurement of the degree on new vessel formation would be helpful in evaluating the effects of environmental and physiological changes or the effects of proposed treatment on wound healing. This paper describes a morphometric method and an immunoradioactive assay (IRMA) to assess neoangiogenesis and their inter-relationship in normal and wounded skin. 3 mm punch biopsy wounds were produced on either leg in 6 normal human volunteers. Half was processed for histology and the remainder homogenised prior to IRMA. After 4 days of healing, a 6 mm punch biopsy of the wound was removed and likewise halved and processed for two assays. Histological sections were assessed stereologically using 100 point sampling lattice (3000 point total) to derive the relative volume of vascular endothelium per unit volume dermis (^{V}vend). The homogenate was assessed using a rabbit antibody to Von Willebrand Factor Antigen (VWFAg) as the basis of the IRMA. Results from this assay were expressed as counts per minute/mg weight. The amount of endothelial tissue per unit volume dermis (^{V}vend) had a mode of 0.1027 and was increased after wounding (mode 0.2076). VWFAg had a greater range in normal samples (mode 71.9 cpm/mg) and decreased after wounding (mode 33.1 cpm/mg). Correlation coefficient between the two methods was 0.42 in normal samples and 0.36 in wounds. It is not surprising that the values of VWFAg are decreased in the wounds since the whole of the biopsy is homogenised; in contrast the stereological method analysed only the surviving dermal component. This also explains a differing relationship between the methods in normal and wounded skin. These data on small numbers encourages us to pursue this relationship further.

23

LYMPHOCYTE CHEMOTACTIC ACTIVITY IN NORMAL HUMAN STRATUM CORNEUM. K. Bacon, R. Camp, F. Cunningham, *A. Gearing and *C. Bird, Institute of Dermatology, London England, and *National Institute for Biological Standards and Control, South Mimms, England.

Prior to a study of the role of lymphocyte chemoattractants in the pathogenesis of inflammatory skin disease, we have analysed samples from normal skin for the presence of lymphocyte chemotactic material, including interleukin 1 (IL 1).

Supernatants obtained from aqueous homogenates of normal heel stratum corneum samples (15 mg each) were successively ultrafiltered in microconcentrators to yield >30kD, 10-30kD and >10kD fractions. Lymphocyte chemotactic and IL 1 activities were measured by use of purified peripheral blood lymphocytes in a microchemotaxis assay, and in a specific IL 1 bioassay incorporating EL-4 NOB-1 and CTLL cells, respectively.

Dilution related chemotactic activity was found in unfiltered supernatants and in the >30kD and >10kD fractions, maximal migration indices (m.m.i.) being 2.3 ± 0.2, 2.5 ± 0.2 and 2.1 ± 0.2 respectively, at 16-fold dilution in each case (means ± s.e.m., n = 6, random migration = 1). In contrast, little chemotactic activity was detected either in the 10-30kD fractions (m.m.i. = 1.3 ± 0.1, n = 6) or in acidic lipid extracts of the >10kD fractions (n = 5). Variable but readily detectable IL 1-like biological activity was seen in the >30kD and 10-30kD fractions (representing 182 ± 63 and 272 ± 199 ng ml^{-1} recombinant [r] IL1 equivalents respectively; mean ± s.e.m., n = 4). Assay of rIL 1 α and β showed dose related lymphocyte chemotactic activity from 0.25 - 10 ng ml^{-1} (n = 4 of each, m.m.i. being 2.6 ± 0.4 and 2.3 ± 0.6 at 10 ng ml^{-1} rIL 1 α and β respectively).

Thus the lack of lymphocyte chemotactic activity in the 10-30kD stratum corneum fractions suggests the presence of an inhibitor, or IL 1-like material which is different from the recombinant forms tested. The >30kD lymphocyte chemoattractant activity may at least in part be due to an IL 1 precursor peptide. Lymphocyte chemoattractants which are not acidic lipid in nature may therefore be recovered from the superficial layers of normal skin. These substances have yet to be characterised, but may represent material which is produced in deeper layers and which may play a physiological role in eliciting lymphocyte traffic through normal skin (Bos et al, J Invest Dermatol 88: 569-573, 1987).

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TUMOR CELL SURFACE ANTIGENS ASSOCIATED WITH ADHESION: THEIR ULTRASTRUCTURAL LOCALISATION. Roland Kaufmann, C. Eberhard Klein, Christel Westphal* and Lutz Weber, Department of Dermatology and Electronmicroscopy, University of Ulm, FRG.

An adhesion related role has been postulated for the VLA3 cell surface glycoprotein - a member of the integrin gene family (Rettig et al, J Exp Med 164: 1581, 1986; Takada et al, PNAS 84: 3239, 1987), the melanoma proteoglycan (Garrigues et al, J Cell Biol 103: 1699, 1986) and a transformation associated 90 kD glycoprotein (gp 90) (Klein et al, submitted). However, the precise function of these molecules remains to be elucidated. All three antigens are highly expressed in many human melanoma and other tumor cell lines suggesting a function in tumor cell adhesion in general. We have studied the ultrastructural distribution of the antigens on the surface of tumor cells by transmission and scanning EM using specific monoclonal antibodies and immunogold labeling. Antigen expression was analyzed on smooth membranes, peripheral cell processes, cell-cell- and cell-substrate contact sites. Cells were cultured on a new melamine-resin foil and in situ labeled by mouse monoclonal antibodies (J143 directed to VLA3; Ao122 and B5.2 directed to the melanoma proteoglycan and F8 directed to gp 90). After coating with rabbit anti-mouse IgG coupled to 15 and 40 nm gold, samples were attached to marked grids and studied by whole-mount TEM. For subsequent evaluation by scanning EM, cells were further processed without critical point drying leading to excellent preservation of peripherical structures. This protocol allowed the analysis of identical cell surface microdomains by both techniques and testing, whether the binding sites were located on the upper or lower surface of structures. We demonstrate differential localisation of the three antigens on the surface of tumor cells. Furthermore, we show preferential expression of the VLA3 glycoprotein at cell-cell but not at cell-substrate contact sites suggesting a role in cell-cell interaction but not in adhesion in general.

Monday, 20 June, 1988

8.00-10.30

Concurrent session – Holl

R. MACKIE, C. SCHMOECKEL, Chairpersons

Melanocytes (Abstracts 25-36)

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MODULATION OF NORMAL HUMAN MELANOCYTE GROWTH IN VITRO, M. Benathan and E. Frenk, University Dept of Dermatology, Lausanne, Switzerland.

Under usual culture conditions, with TPA and cholera toxin, normal human melanocytes (NHM) can only be grown to subconfluent densities. The factors responsible for this growth-limiting phenomenon are not yet known. We investigated possible control mechanisms.

Culture of NHM were established from neonatal foreskin according to Eisinger and Marko (Proc Natl Acad Sci 79, 2018, 1982). NHM were subcultured during 5 months before performing the experiments. By that time, the NHM population was predominantly composed of bipolar cells and grew with a mean doubling time of 4 days up to a subconfluent density of 1.1×10^4 cells per cm^2. - When the NHM were grown in medium harvested from a stationary culture, they displayed a doubling time increased to 9.7 days and an increase in pigmentation. - When the volume of fresh medium was adapted to the increasing NHM density, an extension of the growth phase from two to more than three weeks could be observed; at three weeks the NHM density was 3 to 4 fold that observed in cultures with a constant medium supply. - The NHM density reached in subculture was found to decrease as a function of the age of the cell line: $1.0 \pm 0.2 \times 10$ cells $/cm^2$ between 200 and 400 days, 0.61×10^4 at 467 days, 0.38×10^4 at 536 days, 0.18×10^4 at 693 days. Senescent cultures did not show the bipolar phenotype and consisted predominantly of stellate cells.

Our results indicate that the growth behaviour of NHM in vitro is controlled at least in 2 ways: 1) by exchange of growth inhibiting and promoting factors with the medium 2) by progressive decrease of their replicative potential.

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THE FLOW CYTOMETRY OF MELANOCYTIC SKIN LESIONS. Julia A. Newton*, R.S. Camplejohn** and D.H. McGibbon*, *Dowling Skin Unit, UMDS, **Dimbleby Cancer Research, UMDS.

DNA flow cytometry (FCM) permits the detection of DNA aneuploidy which is held to be implicit of neoplasia. We have performed FCM on formalin fixed, paraffin embedded melanocytic naevi to determine if the presence of DNA aneuploidy has prognostic or diagnostic significants.

60 µm thick sections of tissue were processed as described previously. The DNA was stained with DAPI and the results were only deemed aneuploid if a distinct peak of aneuploid cells was seen. Results with a coefficient of variation (CV) of the diploid peak greater than 7% were rejected.

Three types of naevus were studied, see table. Results for Bowen's disease are included for comparison.

TYPE OF NAEVUS	TOTAL NUMBER	MEAN CV	% REJECTED	% ANEUPLOID
Benign	58	4.4	24	5
Dysplastic	28	5.8	25	25
Giant congenital	8	5.3	18	63
Small congenital	9	5.3	18	11
Bowen's disease	18	4.8	6	90

DNA aneuploidy was detected in all types of naevus but was significantly more likely in those naevi accepted as precursors of malignancy, that is, dysplastic and giant congenital pigmented naevi. The presence of DNA aneuploidy may therefore have prognostic significance in melanocytic naevi.

The quality of results for melanocytic lesions was in general, poorer than for other skin lesions processed by us (see Bowen's results above). Melanin autofluorescences when formalin fixed and therefore experiments were carried out to determine if this phenomenon was contributory. Unstained cell suspensions were passed through the cytometer with a voltage across the photomultiplier tube. Those samples with the highest level of autofluorescence had the poorer quality results, providing some evidence that melanin autofluorescence was at least contributing to the problem. Caution should therefore be exercised in the interpretation of flow cytometry results from formalin fixed melanocytic lesions.

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CORRELATION BETWEEN HLA-DR AND Ki67 ANTIGENS IN MELANOCYTIC LESIONS. S. Moretti, L. Brogelli, M. Novelli*, R. Massobrio*, Clinica Dermatologica II, University of Florence, and *Clinica Dermatologica I, University of Turin, Italy.

An association between HLA-DR antigen expression and advanced steps of tumor progression has been described in melanoma. Advanced phases of tumor progression are known to be characterized by an increase of the proportion of neoplastic cells that continue to proliferate actively. Our aim was to evaluate the proliferation fraction in melanoma and to correlate its entity with the expression of HLA-DR antigens. We used the Ki67 monoclonal antibody that reacts with nuclei of cyclic cells and an anti HLA-DR monoclonal antibody by both an indirect immunoperoxidase and immunofluorescence technique amplified by avidin-biotin system. We examined 71 melanocytic lesions: 15 melanocytic nevi, 35 primary melanomas, divided into three groups on the basis of the histologic type (19 superficial spreading melanoma -SSM-, 21 superficial spreading melanoma with a nodular area -SS+NM-, 5 nodular melanoma-NM-) and 11 metastases. HLA-DR antigens were more frequently expressed by SS+NM, NM and metastases. Ki67 antigen expression paralleled this result. The degree of concordance in the reactivity pattern of individual lesions stained for both HLA-DR and KI67 antigens was 75% (53/71 lesions). Such a concordance was observed in 93% of nevi, 74% of SSM, 76% of SS+NM, 60% of NM and 55% of metastases. Both antigens were expressed by 7% of nevi, 21% of SSM, 52% of SS+NM and 37% of metastases. Neither HLA-DR nor Ki67 antigens were expressed by 86% of nevi, 53% of SSM, 24% ofSS+NM, no NM, and 18% of metastases. When the reactivity pattern for the two antigens was not concordant Ki67 positive lesions outnumbered HLA-DR positive lesions. This study shows that Ki67 antigen expression in melanoma correlates with the tumor progression phases; in spite of the different biological values of HLA-DR and KI67 antigens, one may suggest the joint evaluation of both antigens as a useful marker of aggressive behaviour in melanoma.

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REGULATION OF TYROSINASE EXPRESSION DURING EUMELANIN AND PHAEOMELANIN SYNTHESIS IN MOUSE HAIR FOLLICULAR MELANOCYTES. Burchill SA, *R. Virden and A.J. Thody, Department of Dermatology and *Biochemistry, University of Newcastle upon Thyne, U.K.

Tyrosinase activity in hair follicular melanocytes is higher during the synthesis of eumelanin than that of phaeomelanin. In this study we have examined whether this difference in tyrosinase activity involves changes in the synthesis or some post-translational change in the enzyme.

Hair growth was initiated in C_3H-$He^{a^{vy}}$ mice by plucking and hair follicular tissue taken during anagen. Tyrosinase synthesis was measured by isolating tyrosinase as an immunocomplex with polyclonal antityrosinase after labelling with [^{35}S]-methionine. Glycosylation of tyrosinase was measured in the same way using [^{3}H]-glucosamine. Tyrosinase activity was measured by the method of Pomerantz (1966).

Tyrosinase activity increased during eumelanogenesis and this was paralleled by an increase in tyrosinase synthesis. Subcellular localization studies showed that approximately 50% of this activity was in the melanosomal fraction. However during phaeomelanogenesis the increase in tyrosinase activity was smaller despite there being no change in tyrosinase synthesis. Virtually all of this activity was confined to the soluble fraction indicating a reduction in binding of tyrosinase to the melanosome membrane. Glycosylation, which is necessary for tyrosinase transport and binding, was also considerably reduced and only 10% of that seen during eumelanin production. Cyclic AMP increases the glycosylation of tyrosinase in eumelanin and phaeomelanin producing follicles but in each case there was no increase in the uptake of glycosylated tyrosinase by the melanosome membrane.

These results suggest that the reduced expression of tyrosinase activity during phaeomelanogenesis is not due to changes in synthesis of tyrosinase but may be due to changes in activation of the enzyme. This change in activation could be due to a decrease in the glycosylation of the enzyme and its control by cyclic AMP. There would also appear to be a reduction in the uptake of tyrosinase by the melanosome membrane. How this is controlled is not yet clear.

Reference Pomerantz SH (1966) The tyrosine hydroxylase activity of mammalian tyrosinase. J Biol Chem 241: 161-168.

29

ULTRAVIOLET-MEDIATED MELANOGENESIS IN CULTURED HUMAN MELANOCYTES IS NOT MODULATED BY PROSTAGLANDINS E1, E2 OR TPA, F. Wren, K. Thompson and P.S. Friedmann, Dermatology Department, University of Newcastle upon Tyne, U.K.

We have previously shown that cultured human melanocytes (HuMC) and S91 melanoma cells respond to UVR with increased melanin synthesis and reduced growth[1]. To examine the physiological regulation of this response we studied 2 known modulators of cellular activation - prostaglandins (PG's) and protein kinase C (activated by TPA). HuMC were cultured as described[1] and irradiated with a bank of Helarium fluorescent sunlamps (spectral output 78% UVA, 22% UVB) daily in the presence and absence of PG's or TPA. UVR alone caused a dose-related increase in melanin synthesis up to three fold. PGE1 (10^{-10}-10^{-6}M) alone, stimulated both formation of dendritic processes and proliferation (up to 2 fold) but had no effect on melanogenesis. PGE2 had no effect on growth or melanin synthesis and neither PG altered the response to UVR. TPA (1-100ng/ml) alone stimulated dendrite formation, increased growth by up to 50% but had no effect on melanin synthesis and in addition, TPA did not alter the response to UVR (see table). By contrast, melanin synthesis by S91 melanoma cells was stimulated both by PGE1 (260%) and TPA (50%) although PGE2 had no effect. UVR-induced melanogenesis by S91 cells was enhanced by up to 100% by the addition of TPA but PGE1 and PGE2 had no effect.

Effects of TPA on UV-mediated melanogenesis

UV-Dose (UVA+B)	0		550mJ/cm2		820mJ/cm2	
TPA	-	+	-	+	-	+
Hu. Melanocyte	100%	117±37*	151±43	152±48	180±42	170±46
S91 melanoma	100%	135±39	179±80	214±82	191±3	439±95

* % of control ± SE

Our results indicate that PGE1 and PGE2 are not involved in the UV-mediated response of HuMC and as TPA did not modify this response either it is unlikely that protein kinase C plays a role. By contrast, in S91 melanoma cells the response to UVR appears to be subject to regulation by protein kinase C.

1. Friedmann P.S. and Gilchrest B.A. (1987) J Cell Physiol 133: 88-94.

30

A PIGMENTED HUMAN SKIN EQUIVALENT, *B. Bertaux, *P. Morlière, **G. Moreno, *A. Courtalon, *L. Dubertret, *U 312 Hôpital Henri Mondor, F-94010 Créteil, France, **U 201, MNHN, Paris, France.

The human skin equivalent, a model recently developed in our lab, is obtained by inserting a punch biopsy as a source of epidermis into a dermal equivalent. Although the punch biopsy contained melanocytes, only keratinocytes are present in the newly formed epidermis.

Using 2 mm-diameter foreskin punch biopsies (from donors of 8 and 15 months, 4, 5 and 6 years of age) implanted into a dermal equivalent and stimulating the melanocytes with 8-MOP + UVA or UVB we described the experimental conditions which induced the melanocyte outgrowth from the implanted biopsy. The cultures were performed in EMEM with 10% FCS, EGF, choleratoxin, hydrocortisone. At day 5, the dermal equivalents were cultured at the contact of air. The reconstructed skins (RS) were treated with 8-MOP + UVA or UVB as follows: some RS were incubated at 37° C for 60 min in a freshly repared 8-MOP solution (5×10^{-7}M). The latter solution was removed from the culture and the RS were irradiated at 365 nm with UVA dose = 500mJ/cm2. The other RS were irradiated (without culture medium) at 313 nm with UVB dose = 200mJ/cm2. Controls RS were incubated either in: Hank's medium without irradiation (C), 8-MOP without irradiation (8-MOP) or Hank's medium with UVA irradiation (UVA). UVA or UVB irradiation was performed at day 7 and thereafter every 2 days (5 irradiations in total). The experiment was stopped on day 17. Dopa reaction was performed on epidermal sheets of RS. The brownish black dopa-stained melanocytes were quantitatively evaluated in radial zones extending from the central punch. Results were expressed as the mean number ± SE of dopa-positive melanocytes per radial zone.

Dopa positive melanocytes were scattered both in treated and control epidermal sheets obtained from donors less than 2 years old. Melanocytes remained separated from each other similarly to in vivo but were in lower density. They were numerous around the punch biopsy and then decreased in number progressively toward the periphery of the newly formed epidermis. Dopa-positive melanocyte density was higher in RS treated with 8-MOP + UVA (15.50 ± 1.20) or UVB (26.50 ± 5.60) than in controls : C (2.25 ± 0.40) 8-MOP (5.15 ± 0.70) UVA (2.85 ± 0.65). Semi-thin and thin sections of 8-MOP + UVA or UVB-treated RS confirmed the presence of melanocytes in the basal layer of the differential multilayered epidermis.

In conclusion, the pigmented reconstructed human skin is a useful model for investigating the biology and the photobiology of human skin pigmentation.

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ARE DYSPLASTIC NEVI AN ENTITY? AN APPROACH USING COMPUTERIZED HIGH-RESOLUTION IMAGE ANALYSIS. Wilhelm Stolz, *Wolfgang Abmayr, Christian Schmoeckel, Fritjof Eckert, Ulrich Hohenleutner and Otto Braun-Falco, Department of Dermatology, University of Munich and *Fachhochschule Munich, Department of Informatics, FRG.

The term 'dysplastic nevus' (DN) is currently controversially discussed. Some authors still reject the term 'DN' outside the setting on the DN syndrome. In contrast, most authors nowadays use the term also with regard to solitary lesions and support the concept of DN as a possible stage in the development of malignant melanoma (MM).

The aim of this study using computerized IA was to classify DN in light microscopy according to these features, which have been proven to be significant ($p < 0.0001$) for the discrimination between MM and compound nevocytic nevi (NN) in the stepwise linear discriminant analysis programm BMPD7M.

653 intraepidermal nuclei (in each case at least 50) in semithin sections of 12 dysplastic nevi were digitized using a TV camera and the IA system IPS KONTRON for data acquisition. The stored images were interactively segmentated, stored on 12 inch discs, and transferred to a VAX computer 11/750 for statistical analysis. For each nucleus 22 parameters of hetero- and euchromatin as well as for karyometry (area, circumference, shape factors) were calculated.

The mean value of condensed chromatin (FHP 136) and the standard deviation of dispersed chromatin (SHP 118) for each individual case were the most important features in the linear discriminant analysis for the discrimination between MM and NN. Applying these identical criteria to the DN group, six cases were classified as benign, whereas the other six cases were diagnosed as malignant.

We conclude therefore, that the group of DN is heterogeneous and might consist of very initial malignant melanoma, which can not be diagnosed with the currently applied histological criteria, or of common benign NN with some unusual histological signs. Computerized IA can distinguish between benign and malignant melanocytic lesions and might be therefore a useful objective and reliable technique for the evaluation of concepts with different steps of tumour development in histopathology.

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DIMINISHED VIABILITY OF HUMAN MELANOMA CELLS AFTER GLUTATHIONE DEPLETION WITH BUTHIONINE SULFOXIMINE. Heidi Jackson, M. Benathan and E. Frenk, University Department of Dermatology, Lausanne, Switzerland.

The chemotherapeutic efficacy of several antitumor agents has been shown to be influenced by the glutathione (GSH) status of the target cells. In this work, the viability of a human melanoma cell line (Me8) was assessed after selective inhibition of GSH synthesis by buthionine sulfoxamine (BSO) and after GSH recovery.

Me8 cells were grown as adherent cultures in EMEM plus 5% FCS, 1% non-essential aminoacids. They were passaged once per week at 0.2 x 10^4 cells/cm2. GSH was depleted by adding 0.1 x, 0.5 or 2.5 mM BSO. GSH recovery was induced by omitting BSO. Morphology, colony formation and cell growth were determined in each group. GSH was assessed according to Tietze (Anal Biochem 27, 502, 1969).

While 0.1 mM BSO was well tolerated by Me8 cells, 0.5 and 2.5 mM BSO induced an increase of the mean cell size and a sharp decrease of the cell yield when applied for two consecutive passages. Only the 0.1- and the 0.5-BSO groups could be maintained in long-term cultures. After treatment for 6 weeks, GSH values per 10^6 Me8 cells were strongly decreased : control 4.1; 0.1-BSO 0.35; 0.5-BSO < 0.1 nmol GSH. The viability of the cells depleted in GSH was substantially reduced, with a cloning fraction of 0.69 in the 0.1 and 0.28 in the 0.5-BSO series. Despite these results mean doubling times remained unchanged. Two weeks after ceasing BSO exposure, suprabasal levels of GSH were observed in the formerly depleted cells : control 3.3; 0.1-BSO 6.2; 0.5-BSO 16.9 nmol GSH. The viability of Me8 cells was improved, the cloning efficiency being now 0.94 in the 0.1- and 0.78 in the 0.5-BSO group.

These results show that melanoma cell viability can be modulated by acting selectively on the GSH content. As observed in other tumor cell lines, GSH depletion could enhance drug cytotoxicity upon melanoma cells.

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INFLUENCE OF CULTURE FACTORS ON GROWTH PATTERNS OF MALIGNANT MELANOMA CELL LINES. ESTABLISHMENT OF A MODEL FOR TUMOR STUDIES. Christos Zouboulis, Claus Garbe, Constantin E. Orfanos, Department of Dermatology, University Medical Center Steglitz, Free University of Berlin, Berlin (West), F.R. Germany.

We evaluated the influence of several factors on the growth of malignant melanoma-derived cell lines (StML-11, StML-12a, StML-12c, StML-12d, SKMel-28), cultivated in a monolayer culture system.

The following factors were tested: 1) cell number, cell density (cells/mm2) and cell concentration (cells/ml); 2) intervals of medium change; 3) growth media (RPMI-1640, DMEM, MEM, Ham's F-10, McCoy's 5A, M-199); 4) concentration of fetal bovine serum (FBS); 5) conditioned medium. Two main parameters were studied: a) cell proliferation evaluated by cell counting and liquid scintigraphy of ^{3}H-thymidine incorporation into DNA; b) cell attachment assessed by determination of the plating efficiency. Statistical evaluation was performed by the t-Student test.

Each of the cell lines presented a particular behaviour in culture, but common reactive patterns were found. Elevated plated cell numbers and cell concentrations led to shortening of the population doubling time (PDT), of to faster entrance in the logarithmic growth-phase (LOG-Phase) ($p < 0.001$), while changes of cell densities had no influence on the cell proliferation. Frequent medium renewing delayed the cell proliferation ($p < 0.01$). Higher proliferation rates were observed while using RPMI-1640 and McCoy's 5A media ($p < 0.001$), however DMEM extended the duration of cell proliferation from 10 to 15 days. Low concentration of FBS decreased the cell proliferation. The use of conditioned medium increased the plating efficiency (53 ± 4% to 86 ± 9%). The scintillation counting of ^{3}-H-thymidine incorporation was shown to be a reliable method for assessment of cell proliferation only during the LOG-Phase.

In conclusion we demonstrated that the in vitro-growth of melanoma cell-derived lines is influenced from the several factors tested, but under strictly controlled conditions, a coherent and reliable standardised model was achieved. This model can be used for drug testing and studies on the pathogenesis of malignant melanoma.

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CORRELATION OF MITOTIC RATE AND KI-67 POSITIVE CELLS IN PRIMARY MALIGNANT MELANOMAS. H. Ostmeier, L. Suter, Fachklinik Hornheide, D-4400 Münster/FRG.

The validity of the mitotic rate as a prognosticator in malignant melanomas has been confirmed in a number of studies. The monoclonal antibody (Mab) Ki-67 reacts with a nuclear antigen present in all proliferative cells (G1, S, G2 and M phase of the cell cycle). We wanted to find out, if the mitotic rate and the number of Ki-67 positive cells are exchangeable as parameters of proliferative activity.

139 primary malignant melanomas were labelled in frozen sections with the MAb Ki-67 and stained by the alkaline-phosphatase-anti-alkaline-phosphatase (APAAP)-method.

A poor correlation was found between both parameters (correlation coefficient: $r = 0.55$). Proliferative activity was not always homogeneously distributed in the studied tumors. The mitotic rate and Ki-67 positive cells correlated much better in thin tumors (< 1.5 mm) with homogeneous (correlation coefficient: $r = 0.76$) compared to inhomogeneous (correlation coefficient: $r = 0.17$) proliferative activity. On the contrary in intermediate (1.5 - 3 mm) and thick (> 3 mm) melanomas the correlation coefficients were not substantially different between homogeneous and inhomogeneous proliferative tumors. In thick tumors the mean value of Ki-67 positive cells was 199/mm^2 compared to 110/mm^2 in intermediate and 28/mm^2 in thin melanomas.

We conclude that both parameters - mitotic rate and Ki-67 positive cells - are not exchangeable. Obviously inhomogeneous proliferative activity alone does not explain the poor correlation. Tumor cells in prolonged arrested states in all phases of the cell cycle have been found by other investigators. Probably these arrested cells are Ki-67 positive, thereby contributing to the poor correlation between both parameters. The dependance of the number of Ki-67 positive cells on tumor thickness indicates its prognostic value. So far our follow-up periods are too short to assess, if the number of Ki-67 positive cells gives additional prognostic information that cannot be obtained by the other known prognosticators.

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INTERLESIONAL PHENOTYPIC HETEROGENEITY AND ANTIGEN EXPRESSION OF METASTATIC MELANOMA IN RELATION TO THE CLINICAL STATUS. L. Suter, H. Ostmeier, E.B. Bröcker, Fachklinik Hornheide and Univ.-Hautklinik, D-4400 Münster/FRG.

Results of animal experiments suggest that wide-spread dissemination of metastatic tumors at least in part depends on the ability of tumor cells to modulate, thereby gaining the capacity to grow in different organs. We asked, if similar conditions for wide-spread metastatic dissemination exist in human malignant melanoma.

We therefore studied interlesional phenotypic heterogeneity in metastatic human melanoma as an indicator for the capacity to modulate by use of the monoclonal antibodies anti M-2-2-4, anti K-1-2, anti H-2-5-47, anti H-2-4-7-33, anti A-1-43 and anti HLA-DR. Interlesional phenotypic heterogeneity was assumed, if a substantial difference of at least one of the corresponding antigens could be detected between two different skin or lymph node metastases from the same patient.

Phenotypic heterogeneity was found in 22/23 patients (67%) with disseminated metastatic melanoma including internal organs, but only in 4/26 patients (15%) whose metastases were restricted to skin and peripheral lymph nodes ($P < 0.01$, α2-test). No significant increase of the frequency of interlesional heterogeneity was observed in patients who had received antineoplastic chemotherapy compared to those without this treatment. The expression of 3 of 10 tested antigens (A-1-43, K-1-2, H-2-8-10) was related to the clinical situation of the patients.

The results indicate that biological peculiarities of tumor cells - especially their capacity to modulate - influence the clinical course of metastatic human melanoma. We had no evidence that antineoplastic chemotherapy enhances phenotypic hetereogeneity by killing subpopulations of tumor cells.

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A NEW AND LIFE-LIKE IN VITRO MODEL OF MALIGNANT MELANOMA. A. Majid, P.W.M. Copeman, K. Henry, Skin and Therapeutics Research Laboratories and Histopathology Department, Westminster Hospital, London, U.K.

Human malignant melanoma (MM) cells were cultured on the surface of a dermal equivalent (DEq), seeded as a cell suspension or as an explant. MM cells proliferate in both vertical and radial patterns of growth on the DEq.

DEq, composed of fibroblasts in a three-dimensional collagen lattice, lends itself for physiological, pathological and pharmacological study (Saiag et al, Science 230, 669, 1985; Rowland Payne et al, J Invest Dermatol 983, 314, 1987).

DEq was prepared as described (Coulomb et al, Br J Dermatol 114, 91, 1986). The MM component was provided by either 4 x 10^{-5} cells suspended in 0.5 ml of complete medium plated onto DEq in droplets delivered from 100 µl pipetman, or by a 3 mm punch biopsies of MM implanted into the DEq. At day 4, and then twice weekly, the medium was changed. The medium contained epidermal growth factor, cholera toxin, hydrocortisone, insulin and phorbol acetate. At day 18, the culture was arrested. Preliminary results have shown that there was new outgrowth of MM. This MM outgrowth was assessed by planimetry, light microscopy (haematoxylin and eosin and S100 protein) and also by electron microscopy.

Animal models of MM are not satisfactory because MM, in many animals, is a slow growing neoplasm which seldom metastasises. Monolayer cell culture of MM do not function under the same conditions that prevail in vivo.

MM cells grow on DEq to provide a lifelike in vitro tool for investigating the biology of MM, and in particular for studying the interactions between tumour cells and the dermis.

Monday, 20 June, 1988

10.45-11.45

Plenary session – Cuvilliés

W.J. CUNLIFFE, Chairperson (Abstracts 37-40)

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CHARACTERIZATION OF LIPOCORTIN GENE EXPRESSION IN HUMAN SKIN AND CULTURED KERATINOCYTES. James T. Elder, Barbara P. Wallner, *R. Blake Pepinski and John J. Voorhees, Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan and *Biogen Research Corporation, Cambridge, Massachusetts.

Lipocortins I and II are 35kd proteins which display phospholipase inhibitory activity in vitro. In addition, lipocortin I is a major substrate of the epidermal growth factor (EGF) receptor tyrosine kinase, while lipocortin II is a major substrate for the $pp60^{v-src}$ tyrosine kinase. Since corticosteroids have been reported to induce the secretion of phospholipase A_2 inhibitory activity in several systems, and since topical steroids have been reported to reduce phospholipase A_2 activity in human skin and cultured human keratinocytes, we have analyzed the expression of lipocortin I and II RNA and protein in human skin and cultured human keratinocytes using Northern, Western, and dot blotting techniques in the presence and absence of clinically-effective corticosteroids. In addition, we have characterized the expression of lipocortin in situ in human skin by immunofluorescence.

Our results indicate that lipocortins I and II are highly expressed in human skin and in primary *keratinocytes cultures, and that keratinocyte expression of lipocortin I is enhanced in the basal and suprabasal levels of human epidermis. However, the steady-state expression of lipocortin I and II mRNA and protein is not detectably affected by corticosteroid treatment in vivo or in vitro. The implications of these findings for the mode of action of corticosteroids in inflammatory skin disease will be discussed.

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MODULATION OF OKT3-INDUCED ACTIVATION OF HUMAN T LYMPHOCYTES BY INTERLEUKIN 6 AND EPIDERMAL CELL DERIVED-CONTRA-INTERLEUKIN 1. *J. Krutmann, **T. Schwarz, *R. Kirnbauer, *A. Urbanski, *T.A. Luger, *Dept. Derm. II and LBI for Dermven. Lab. for Cellbiol., Univ. of Vienna, **Dept. Derm., Hospital Lainz-Vienna, Austria.

The monoclonal antibody OKT3 activates human T-lymphocytes via the CD3-T cell receptor complex thereby mimicking antigen-induced T cell blastogenesis. In this system, accessory cells (AC) augment T cell activation by the release of Interleukin 1 (IL 1). The present study was designed to examine whether AC derived cytokines distinct from IL 1 were able to modulate OKT3-induced T cell proliferation. Two recently recognized factors, IL 6, produced by both monocytes and epidermal cells (EC) and EC-contra-IL 1, released by EC following exposure to UV light, were tested. Human peripheral blood mononuclear cells (PBMC) were stimulated with OKT3 and ^{3}H-thymidine incorporation measured as an index for T cell activation. When PBMC were cultured with OKT3 in the presence of an antiserum to human IL 6 (αIL 6), a decrease in blastogenesis was observed. The suppressive effect of αIL 6 appeared to be specific to T cell receptor mediated T cell activation, since no inhibition could be observed, when OKT3 was replaced by Con A. When αIL 6 was added to OKT3-stimulated PBMC in combination with a monoclonal antibody against human IL 1, synergistic rather than additive inhibition of T cell proliferation occured. Finally, various concentrations of EC-contra-IL1 suppressed OKT3-induced T cell activation in a dose-dependent manner. These studies indicate that AC augment OKT3-induced activation of human T cells by secreting IL1 and IL 6 and that both cytokines may be functionally linked. Furthermore, the ability of EC-contra-IL 1 to inhibit blastogenesis suggests that AC derived cytokines may also down-regulate activation of human T-lymphocytes.

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GAMMA/DELTA-RECEPTOR BEARING LYMPHOCYTES IN NORMAL HUMAN EPIDERMIS: ANALOGUES OF MURINE THY-1+ DENDRITIC CELLS ? J.D. Bos', M.B.M. Teunissen'', J. van der Kraan', S.R. Krieg'', M.L. Kapsenberg', P.J. Peters* and J. Borst**, Department of 'Dermatology, ''Histology and Cell Biology and ''Pathology, University of Amsterdam, Academisch Medisch Centrum, Amsterdam, *Department of Cell Biology, Faculty of Medicine, University of Utrecht, Utrecht, **Department of Immunology, The Netherlands Cancer Institute, Amsterdam.

The human integument harbours a wide variety of immuno-response associated cells (including T lymphocytes) forming a complexity denominated the skin immune system (SIS). Human T cells may be subdivided in common alpha/beta heterodimer receptor and less common gamma/delta receptor expressing subpopulations. The development of a monoclonal antibody (anti-TCR-gamma/delta-1: 11F2) reactive with TCR gamma/delta in native form enabled us to identify a human TCR gamma/-delta expressing and previously unrecognized lymphocyte subpopulation in normal human epidermis.

We applied anti-TCR-gamma/delta-1 ascites in a three step immuno-peroxidase assay with neonatal thymus and lymph node tissue as positive controls. Staining of normal human skin sections with anti-TCR-gamma/delta-1 ascites 1:100 revealed the occasional presence of cytoplasma membrane stained cells within epidermis and adnexal epithelium. Immunolabelling of normal human skin sections, human epidermal sheet preparations and human epidermal cell suspensions demonstrated that TCR gamma/delta expressing lymphocytes are regularly present in normal human epidermis as well as in adnexal epithelia of the skin. They constituate 0.80 ± 0.24 ‰ of epidermal cells but are not easily detectable in vertical skin biopsy sections.

It seems reasonable to assume that these T cells are the human analogue of Thy-1+ 'dendritic' epidermal cells observed in mice, which recently demonstrated to express the gamma/delta complex type of T cell receptor. From a functional point of view, present evidence is in favour of a cytokine-dependent non-MHC restricted cytotoxic capacity. Functional studies using human epidermis-derived gamma/delta TCR bearing T cell lines are in progress. It might be possible that these cells have rearranged into gamma/delta receptor expressing cells from immature bone-marrow derived precursors within the epidermal micro-environment.

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PARTIAL CHARACTERIZATION OF A 34KD PROTEIN INVOLVED IN CELL-COLLAGEN INTERACTIONS OF HUMAN SKIN FIBROBLASTS. *M. Borchert, **C. Mauch, *M. Pfäffle, *K. von der Mark and **Th. Krieg, *MPI für Biochemie; Martinsried, **Dermatologische Klinik der LMU München.

Fibroblast interactions with the extracellular matrix are important in regulating fibroblast metabolism during connective tissue remodelling and wound healing. These are mediated by membrane-associated proteins binding to collagens, fibronectin and laminin. Previous work has shown that a 3kD collagen-binding protein from sheep fibroblasts related to anchorin CII is associated with severe healing defects in dermatosparaxis. In trying to establish a similar model for human wound healing two proteins with MW = 36kD and 34kD have been isolated from human skin fibroblasts. The 34kD protein has been identified as calpactin I, an intracellular membrane-associated protein in the same supergene familiy as anchorin CII. The identity of the 34kD protein is as yet unknown. However, similarly to the 34kD sheep fibroblast protein, it is immunologically related to anchorin CII. Immunofluorescence of human fibroblasts shows that the protein is indeed synthesized by human fibroblasts. Furthermore, it can be specifically immuno-precipitated from surface-iodinated human fibroblast cell extracts. Collagen binding could be demonstrated for both the 34kD and 36kD proteins in an overlay assay with iodinated type I collagen. The 34kD fibroblast protein may also be involved in the regulation of cell morphology. Evidence for this stems from the fact that dermatosparactic fibroblasts, which lack the 34kD sheep protein, have a more polygonal shape than normal fibroblasts in cell culture. Partial amino acid sequencing of the 34kD fibroblast protein indicates that it is identical with calpactin II, protein II or anchorin CII, members of the same supergene family. It is thus a candidate as a new member of the protein family which may play an important role in mediating the effects of the extracellular matrix on fibroblast cell shape and biosynthetic functions during tissue repair.

Monday, 20 June, 1988

15.00-17.00

Concurrent session – Cuvilliés

H. SCHAEFFER, M. PONEC, Chairpersons **Keratinocytes** (Abstracts 41-50)

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ANDROGEN RESPONSES IN CULTURED HUMAN HAIR PAPILLA CELLS. A.G. Messenger, *M. Julie Thornton, Kathy Elliott, *Valerie A. Randall, Department of Dermatology, Royal Hallamshire Hospital, Sheffield and *School of Biomedical Sciences, University of Bradford, Bradford, U.K.

We have previously found that cells cultured from the dermal papillae (DP) of human beard follicles proliferate more rapidly in vitro than DP cells from scalp follicles suggesting that the action of androgens on hair growth is mediated via dermal papilla. To investigate this further we have studied: 1) the effect of testosterone on DNA synthesis and: 2) the uptake of testosterone by human DP cells and dermal fibroblasts from skin sites with differing sensitivities to androgens.

Cells (2 x 10^4) were grown in 24-well plates for 24 hours and then exposed to testosterone (10^{-10}M-10^{-4}M) plus H^3-thymidine for a further 24 hours. Physiological concentration of testosterone failed to alter the incorporation of H^3-thymidine into DNA in any of the cell lines studied although high concentrations (10^{-4}) suppressed DNA synthesis by DP cells and dermal fibroblasts. In a second series of experiments confluent cultures were incubated with H^3-testosterone after 36 hours in serum-free medium. Radioactive uptake was measured at intervals up to 24 hours after extensive washing and either triton digestion or chloroform: methanol (2:1) extraction. DP cells of beard and scalp derivation showed increasing uptake of labelled testosterone up to c.2h. However, the uptake was 5-10 x greater in beard cells than in scalp cells and this could be inhibited by unlabelled testosterone, 5a-dihydrotestosterone and cyproterone acetate. The uptake of radiolabelled testosterone by beard and scalp dermal fibroblasts was similar to that of scalp DP cells.

The failure to demonstrate a mitogenic effect of testosterone on cultured DP cells may indicate that a maximal response has already occurred in vivo. Suppression of DNA synthesis by high testosterone concentration may be a toxic effect. However, the results of testosterone uptake studies show that cultured DP cells contain saturable androgen receptors. The greater uptake of testosterone by beard DP cells provides further evidence of the importance of the DP in mediating androgen responses.

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IN VITRO CELL TO CELL COMMUNICATION OF HUMAN KERATINOCYTES, DIFFERENT TECHNICAL APPROACHS. Denis Salomon, **David Spray, *Marc Chanson, Jean-Hilaire Saurat, *Paolo Meda, Clinic of Dermatology, Hôpital Cantonal Universitaire, *Institute of Histology, University of Geneva, CH-1211 Geneva 4, Switzerland, **Department of Neuroscience Albert Einstein College of Medicine, Bronx, NY 1046, USA.

Cell-to-cell communication between keratinocytes was previously demonstrated by microinjecting the fluorescent tracer, Lucifer Yellow (LY), in intact human skin. To study the modulation of keratinocyte gap junction permeability, an in vitro approach was developed. Cultures of multilayered primary human keratinocytes were prepared according to the Green technique. At confluence, the cells were either microinjected with LY as in intact skin, or scrape-loaded in presence of LY. The pattern of communication observed in vitro using the microinjection technique was similar to that in vivo, i.e. coupling was present between the different keratinocyte layers, and was more extensive between the suprabasal than between the basal cells. Keratinocyte coupling was blocked by a few minutes exposure to 3.5 mM heptanol and 10^{-4}M all trans retinoic acid (RA) but not by a 5 to 60 min treatment with 10^{-6}M to 10^{-8}M RA. Identically results were obtained using the scrape loading method. In addition this technique allowed for the screening of cell coupling on a very large number of keratinocytes and further quantification by microspectrofluorimetry. In parallel dual voltage clamp whole-cell recording was used as a third approach to characterize electrical coupling in pairs of human keratinocytes. Using this method, the conductance of keratinocyte gap junctions was assessed directly and kinetically. Measurements indicated again the presence of permeable gap junctions, the lack to effect of 10^{-6}M - 10^{-8}M RA, and the rapid uncoupling caused by 10^{-4}M RA.

In conclusion, we have used three different techniques to characterize the in vitro pattern of human keratinocyte communication, and its modulation by pharmalogical and therapeutics agents.

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MC 903, A NON-CALCIOTROPIC VITAMIN D_3 ANALOGUE STIMULATES DIFFERENTIATION AND INHIBITS PROLIFERATION OF CULTURED HUMAN KERATINOCYTES. Knud Kragballe, Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark.

1α,25-dihydroxy-vitamin D_3 (1α,25-$(OH)_2$-D_3), the naturally occuring active form of vitamin D_3, causes an increase in differentiation and a decrease in proliferation of cultured human keratinocytes. Unfortunately, the use of 1α,25-$(OH)_2$-D_3 to control keratinocyte growth is limited by its potent effect on calcium metabolism. MC 903 is a novel vitamin D_3 analogue which is at least 100 times less active than 1α,25-$(OH)_2$-D_3 in its effects on calcium homeostasis (Biochem Pharm, in press). The purpose of the present study was to determine the effects of MC 903 on DNA synthesis and morphologic differentiation of cultured human keratinocytes. Trypsinized epidermal cells were suspended in McCoy's 5A medium supplement with 10% human type AB serum and plated on culture dishes precoated with a collagen type I gel. After 1 week in culture fresh medium containing vehicle alone (0.1% ethanol), 1α,25-$(OH)_2D_3$, 1α,OH-D_3 or MC 903 were added at concentrations from 10^{-12}M to 10^{-9}M to each dish at each change of medium. After incubation for 2 weeks terminal differentiation was examined morphologically by formation of a cornified envelope. The rate of DNA synthesis was quantified by the uptake of ^{3}H-thymidine (60Ci/mmol) into culture exposed for 6 to 1 μCi/ml. The number of cornified envelopes was stimulated dose-dependently by both 1α,25-$(OH)_2$-D_3 and MC 903. At a concentration of 10^{-9}M the stimulation was 345% and 278% respectively compared to control. DNA synthesis was also inhibited dose-dependently by 1α,25-$(OH)_2$-D_3 (51% at 10^{-9}M) and MC 903 (40% at 10^{-9}M). 1α,OH-D_3 caused a modest increase of cornified envelope formation (140%) at the highest concentration used (10^{-9}M), while DNA synthesis was unchanged. It appears that MC 903 is a potent regulator of differentiation and proliferation of human keratinocytes. Therefore, MC 903 may be of value in treating differentiation and hyperproliferation disorders of epidermis.

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THE EFFECT OF TUMOR NECROSIS FACTOR (TNF) ON ISOLATED EPIDERMAL CELLS. Hans-Joachim Schulze, *Michael Schaadt, *Volker Schenk, Gustav Mahrle, Department of Dermatology and *Medicine, University of Köln, FRG.

The monokine TNF is synthetized by activated mononuclear phagocytes. It has a direct and indirect cytostatic or cytotoxic effect on non-epidermal cells (Beutler B, Cerami A: N Engl J Med 316: 379-85, 1987) and has been reported to mediate cell death in skin lesions of acute graft versus host disease (Piguet PF et al: J Exp Med 166: 1280-9, 1987). The present study was designed to investigate the influence of recombinant TNF-alpha (Asahl, Japan) on human keratinocytes in vitro.

Keratinocytes from the germinative cell pool of normal human epidermis were isolated from punch biopsies (n = 6 volunteers) by the digestion with dispase (1.5 U/ml, 4°C, 6 h) followed by the incubation in trypsin (0.3%, 37°C, 1 min). The cells were seeded on collagen coated microdishes (o 0.6 cm) at a density of 2 x 10^5 cells per dish and grown in McCoy 5A Medium containing antibiotics and 10% FCS. TNF was added to the cell cultures at different concentrations (0.1, 0.5, 1.0, 2.0, 5.0 U/ml) one day after seeding. After another 3 days of continuous TNF exposition the cell number and the rate of ^{3}H-thymidine incorporation (1 h-pulse labelling) were determined. The toxicity of TNF was monitored by trypan blue staining and the morphological alterations were studied in an electron microscope.

Under low TNF conditions (0.1 - 1.0 U/ml) cell cultures did not differ significantly from untreated controls. Medium concentration of TNF (2 U/ml) reduced DNA synthesis (CPM/1000 cells: minus 36%) and the cell number (minus 45%). Inhibition of cell spreading and vacuolization of the cells became evident first at high concentrations of TNF (5 U/ml).

These results indicate that TNF exerted a direct effect on keratinocytes in vitro. Low doses of TNF (≤ 2.0 U/ml) were cytostatic and higher doses (≥ 5 U/ml) were cytotoxic.

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DIFFERENTIATION OF KERATINOCYTES AND THE EXPRESSION OF RECEPTORS FOR EPIDERMAL GROWTH FACTOR (EGF) AND LOW DENSITY LIPOPROTEIN (LDL). Marinus F.W.te Pas and Maria Ponec, Department of Dermatology, University Hospital Leiden, The Netherlands.

Our earlier studies revealed that the binding activity of the receptors for epidermal growth factor (EGF) and low density lipoprotein (LDL) is inversely related to the differentiation capacity of keratinocytes and a number of squamous carcinoma cells (SCC). In our investigation the cells were used with receptor binding capacity decreasing in the order: A431 > SCC-4 > SCC-15> SCC-12F2 = SVK14 = normal keratinocytes. The same rank order has also been observed for amplification of both the EGF- and the LDL-receptor genes.

In the present investigation we focused our attention on possible differentiation-related modulations of the EGF- and LDL-receptor expression on the level of the mRNA expression. Furthermore, the possible regulatory role of EGF on the mRNA expression on its own receptor has been investigated, too. For this purpose both the total cytoplasmic and the poly (A^+) selected RNAs were isolated and subjected to the S_1-nuclease analysis, in which either 150 ug of total cytoplasmic RNA or 1 ug of poly (A^+) selected RNA was used. In these experiments probes were used that consisted partly of EGF- or LDL-receptor cDNA and partly of plasmid DNA. The hybridization temperature was 51°C for the EGF- and 57°C for the LDL-receptor probes.

The mRNA expression for both the EGF- and LDL-receptors was found to decrease in the following order: A431 > SCC-4 > SCC-15 > SCC-12F2 = SVK14 > normal keratinocytes. An elevated level of the EGF-receptor mRNA expression was observed in cells cultured for 48 hours in the presence of 10 ng EGF/ml medium.

From the data presented here it can be concluded that i) both the EGF- and LDL-receptor gene amplification is reflected in the expression at the mRNA level and ii) the expression of EGF-receptor on the mRNA level is regulated by EGF.

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TOPICAL APPLICATION OF 1α,25-DIHYDROXYCHOLECALCIFEROL FAILS TO MODULATE EPIDERMAL GROWTH IN VIVO. M.G.J. van Bokhoven, *B.M. Czarnetzki, P.E.J. van Erp, S. de Mare, R. Happle and P.C.M. van de Kerkhof, Department of Dermatology, University of Nijmegen, The Netherlands, *Department of Clinical Research Dermatology, Hoffmann-La Roche, Basle, Switzerland.

Topical application of 1α,25-dihydroxycholecalciferol (1α, 25-DCC) has been reported to be effective in the treatment of psoriasis, the effective dosage range being 0.1 - 0.5 μg/g petrolatum. Here we studied the effect of 1α,25-DCC on epidermal growth control of human skin in vivo.

1α,25-DCC in medium chain triglyceride (2 μg/ml) and medium chain triglyceride only, were applied twice daily for one week on six sites of the back of 8 healthy volunteers. On day 9, after a 24h rest period, these sites were stripped by repeated applications of sellotape until the skin appeared glistening (35 - 45 applications). Treatment with 1α,25-DCC was resumed immediately thereafter, at 12h intervals up to the moment that a stripped site was biopsied. The time points for biopsies were at 26, 40, 44, 48, 52 and 56h after stripping. Biopsies were processed for cell cycle kinetic studies according to standard methods[1]. In brief, epidermal cell suspensions were prepared by trypsinization and cell suspensions were stained with propidium iodide. After filtration cellular DNA was determined with the flowcytometer 50H (Ortho instruments). A window was set in mid S phase relative to the peak of cells in G_0 and G_1 phase.

Analysis of percentage cells in S phase, at different intervals following sellotape stripping, revealed no significant difference between the areas pretreated with 1α,25-DCC and the areas pretreated with the vehicle only.

No interference of 1α,25-DCC with epidermal cell cycle kinetics could be shown in this in vivo model in human skin. This observation is contrasting with the successful treatment of psoriasis with 1α,25-DCC, reported by several investigators. Other antipsoriatic targets for the reported effectivity of 1α,25-DCC in psoriasis should be considered, including the immune system.

1. Boezeman J, et al, Cell Tiss Kin 20: 99-107, 1987.

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DEFECTIVE CALCIUM UPTAKE IN KERATINOCYTE CELL CULTURES FROM VITILIGINOUS SKIN. Karin U. Schallreuter, Department of Dermatology, University of Hamburg, Hamburg, FRG and Mark R. Pittelkow, Department of Dermatology, Mayo Clinic, Rochester, Minnesota, USA.

Cell cultures of human keratinocytes contain membrane associated thioredoxin reductase. This enzyme is extremely active in reducing free radicals. It has been shown that enzyme activity is under allosteric control by calcium. Because Ca^{++} is known to control significantly epithelial proliferation and differention, the effect of medium Ca^{++}- concentration on enzyme activity has been examined at low (0.1 mM) and high (2.0 mM) Ca^{++} on keratinocytes established from healthy control skin as well as from vitiliginous and normal skin of a patient with extensive vitiligo. At low Ca^{++} concentration double of activity has been found in control cells compared to activity in high Ca^{++} concentration. In cell cultures from vitiliginous and normal skin of a patient with vitiligo no differences in enzyme activity have been observed in low Ca^{++} concentration, meanwhile only a tenth of activity has been found in high Ca^{++} concentration. In order to examine the fate of Ca^{++}, the isotope 45 Ca has been used to follow the kinetics for the uptake, efflux and steady state in normal control keratinocytes and in keratinocytes established from involved and uninvolved skin of one donor with vitiligo. Cells grown from uninvolved skin yielded a very rapid uptake and efflux of 45 Ca before reaching steady state. A similar profile has been found for keratinocytes from normal healthy control skin. However cells established from vitiliginous skin showed a slow uptake of 45 Ca before reaching the same steady state as the controls. 45 Ca efflux has not been observed in vitiliginous keratinocytes. Furthermore vitiliginous keratinocytes yielded a higher concentration of extracellular bound 45 Ca compared to keratinocytes from uninvolved skin. Since Ca^{++} has been found to be an allosteric inhibitor of membrane associated thioredoxin reductase, this defect in Ca^{++}-transport may explain the proposed breakdown in free radical defense in vitiligo.

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CULTURED HUMAN KERATINOCYTES: EFFECT OF CULTURE CONDITIONS ON LIPID COMPOSITION. *J.Brod, **M. Regnier, ***M. Ponec, *M. Prunieras, *Dept. of Biology, L'Oreal Research Laboratories, Aulnay/Bois, France, **Cell Biology Dept., CIRD, Valbonne, France, ***Dept. of Dermatology, Universital Hospital Leiden, The Netherlands.

Culture conditions have been shown to modulate the differentiation of epidermal keratinocytes in terms of protein synthesis (keratins, envelopes); but informations on modulation of lipids are scarce. Therefore, in the present work, the influence of culture conditions on the lipid composition of cultured human keratinocytes has been studied:

Two culture systems were used: 1) high density cultures of keratinocytes grown on plastic and 2) reconstructed skin on de-epidermized dermis (DED). Lipid analysis were performed on either primary cultures or second passage cultures. DED-cultures were either exposed (EXP) to the air or immersed (IM) in the culture medium. The culture medium was supplemented with either normal serum (NS) or delipidized serum (DS). Lipids were analyzed by TLC using both one-and two-dimensional systems or by the Iatroscan method.

In primary cultures, sterol esters, triglycerides, free fatty acids, cholesterol (CH), lanosterol (LAN), cholesterol sulfate, ceramides including acylceramides (AC), glucosylceramides (GC) including acylglucosylceramides as well as phospholipids were found to be present on both systems and in similar amounts; however, CH was twice higher in culture grown on plastic than on DED. No difference was observed between IM and EXP cultures. Under all culture conditions, delipidation of serum resulted in an increase of triglycerides.

In secondary cultures grown on DED, the amounts of LAN, AC and GC were higher in EXP than in IM cultures. Furthermore, in keratinocytes grown on plastic in NS, the neutral lipids were low; LAN, AC and GC were not detectable. When DS was used, the lipid composition was similar to that seen in EXP-DED cultures. Moreover, retinoic acid added at 1 µM in DS affected the lipid composition of secondary cultures grown on plastic (i.e.: LAN, AC and GC no longer detectable).

Results suggest that serum lipids or lipid soluble substance such as vitamin A may control the synthesis of lipids which are involved in the terminal differentiation of epidermis.

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LIPID COMPOSITION OF CULTURED NORMAL AND MALIGNANT KERATINOCYTES: EFFECT OF CULTURE CONDITIONS. M. Ponec, A. Weerheim, J. Kempenaar, *P. Elias, and *M. Williams, Dept. of Dermatology, University Hospital Leiden, The Netherlands and *Dept. of Dermatology, Veterans Administration Medical Center, San Francisco, USA.

The effect of culture conditions on lipid composition has been investigated in normal keratinocytes and three squamous carcinoma cell (SCC) lines which showed a decreasing capacity to differentiate in the order normal keratinocytes > SCC-12F2 > SCC-15 > SCC-4. The extent of differentiation of these cells can be experimentally modulated by changing the culture conditions. When cultured under the conventional, submerged conditions the extent of cellular differentiation is low when the culture medium contains low calcium concentration and is increased when cells are cultured in medium containing calcium concentration present in standard culture media. At both calcium levels no significant differences were observed in total lipid composition among various types of cells. Furthermore, the induction of differentiation by culturing the cells at normal calcium concentration was not accompanied by significant changes in lipid composition. This suggests that differences in the ability of cells to differentiate are not reflected by differences in lipid composition.

Marked differences in cellular lipid composition were, however, observed when the cells were grown under air-exposed culture conditions using de-epidermized dermis as the substrate. Under these conditions the extent of cellular stratification was increased in all types of cell used; the effect being most profound in normal keratinocytes. The analysis of the lipid composition revealed marked differences between individual SCC lines and the normal keratinocytes and showed that the phospholipid content remained high in SCC-4 and SCC-15 cells and decreased in SCC-12F2 cells and even to a higher extent of stratification of normal keratinocytes. In all cell under study the triglyceride content was higher and cholesterol content was lower as compared with submerged cultures, this effect being most profound in SCC-12F2 cells. Compared with SCC cells, the higher extent of stratification of normal keratinocytes in air-exposed cultures was paralleled by results of lipid analysis. Namely, in SCC cells no detectable amounts were found of acylceramides (AC) and of acylglucosylceramides (AGC), as well as of lanosterol and lathosterol.

It can be concluded that air-exposed cultures approach the in vivo situation to a higher extent that the submerged ones do, and offer hereby an attractive possibility to study in great details the mechanisms controlling epidermal differentiation.

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EPIDERMAL GROWTH FACTOR AND TRANSFERRIN RECEPTORS IN HUMAN EMBRYONIC AND FETAL EPIDERMIS. G. Zambruno, G. Girolomoni, L. Benassi, V. Manca and A. Giannetti. Department of Dermatology, Unversity of Modena, Italy.

EGF and transferrin receptors (EGFR, TFR) are known to be associated with proliferating cells and to be expressed in normal human epidermis. As far as we know no data are available on embryonic and fetal epidermis. The aim of the present study was to investigate the distribution of EGFR an TFR in human developing epidermis. Embryonic and fetal skin specimens were obtained from 30 aborted fetuses of 7 to 31 weeks estimated gestational age. Anti-EGFR and anti-TFR monoclonal antibodies (MAb) were used in a three step immunfluorescence technique (biotin-streptavidin system) on frozen sections. In selected cases immunoelectron microscopy (IEM) using an indirect immunogold technique on dermo-epidermal sheets was also employed in order to visualize EGFR.

EGFR MAb uniformly labelled all cell layers in embryonic and fetal epidermis up to week 12. Primary epidermal ridges were regularly stained. By week 16 the labelling was mainly present on basal cells with a greatly reduced staining of the suprabasal layers. During the 3rd trimester the stratum corneum was negative. The germ and hair peg cells were positive with a reduced labelling at the level of the hair cone; hair canals were completely negative. Both the periphery of the sebaceous glands and the dermal ducts of eccrine sweat glands were stained. Periderm cell membranes were positive, as confirmed by IEM labelling on a week 10 fetus. TFR was expressed on basal cells throughout embryonic and fetal development. In palmo-plantar epidermis the staining intensity was higher in the primary epidermal ridges. In the bulbous hair peg the outer root sheet was faintly positive. Our data show a good correlation between EGFR distribution and areas of potential epithelial cell proliferation during fetal development. Peridermal cells, which are known to be mitotically active, are positive too. The preferential expression of TFR in primary epidermal ridges could be correlated to a higher proliferative activity in these areas.

Monday, 20 June, 1988

15.00-17.00

Concurrent session – Effner

H. HONIGSMANN, G. PLEWIG, Chairpersons
Photobiology (Abstracts 51-60)

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THE EVALUATION OF SUN PROTECTION FACTOR (SPF) USING A PORTABLE ERYTHEMA METER. A.D. Pearse, C. Edwards and R. Marks, Department of Medicine (Dermatology), University of Wales College of Medicine, Cardiff.

A portable erythema meter for measuring skin colour using reflectance spectrophotometry was described by Diffey et al (1984)*. Our group has constructed an improved and modified, compact hand-held solid state meter using light emitting diodes. Ultraviolet (UV) erythema is traditionally recorded using a subjective, visual scale of assessment. The purpose of this study is to compare our erythema meter with visual assessment of UV erythema in human skin.

Eight subjects were irradiated on one side of the back with 10 x 1,5 cm^2 of UVR from a bank of four Westinghouse FS20 fluorescent sunlamps, with each square having a 25% increase in irradiation time. The sites were subjectively assessed 24 hrs post irradiation using a 0 - 4 scale, 0 being no erythema, and 4 being strong erythema + oedema. At the same time the sites were measured using our erythema meter (Table). A 12 cm^2 area on the other side of the back of each subject was evenly covered with 2 µl/cm^2 of an SPF4 sunscreen, and 6 sites were irradiated with 2, 3, 4, 5, 6 and 7 times the minimal erythema dose (MED). Twenty-four hours later the SPF was visually assessed and found to be 4.75 - (rounded down: SPF4). The mean erythema meter reading for the eight subjects was 58 (SD 13). This compares well with the meter value for 1 MED of 60 erythema units (Table). It is proposed that our erythema meter is a suitable improvement for objectively determining the SPF of a sunscreen and that under the conditions of this experiment, 1 x MED = 60 erythema units above the reading obtained from adjacent normal skin.

Table: Comparison of subjective assessment of MED with an erythema meter.

Visual assessment	n	Meter reading (±SD)	
- 1 MED	8	25 ± 10	Spearmans's rank cor-
1 MED	8	60 ± 22	relation (all data
2 MED	7	121 ± 17	points:) Spearman's
3 MED	6	171 ± 46	rho = 0.93

* Diffey BL, Oliver RJ, Farr PM (1984) British Journal of Dermatology 111: 663.

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A COMPARISON BETWEEN ULTRA-VIOLET B INDUCED DAMAGE IN CULTIVATED HUMAN KERATINOCYTES AND MELANOCYTES. A.A. Schothorst, F. Bruynzeel and L. Evers. Department of Dermatology, University Hospital, Leiden, The Netherlands.

Until now experimental studies concerning ultra-violet irradiation in relation to the induction of melanoma in human skin are scarce. In this study we investigated the cell killing and dimer induction in cultivated human keratinocytes and melanocytes after UVB irradiation. Human diploid skin keratinocytes as well as melanocytes were derived from foreskin of normal caucasian boys. Keratinocytes were cultured in Dulbecco's Modified Eagle Medium supplemented with 15% foetal calf

serum. Melanocytes were cultured in Ham's F10 medium supplemented with 5% foetal calf serum, 12-0-tetradecanoyl phorbol-13-acetate (TPA), cholera toxin and 3-isobutyl-1-methyl-xanthine (IBMX). Exponentially growing cells were rinsed with phosphate buffered saline and irradiated with monochromatic UVB, 302 nm. UVB irradiation was provided by a 1000 W Mercury-Xenon arc lamp source, equipped with a narrow band-interference filter.

Dose response relationships were established for a) cell survival in terms of ability for clone formation. Immediately after irradiation cells were trypsinized and seeded at a density estimated to give 100-180 clones per 9 cm Petri dish, cocultured with 200.000 lethally irradiated mouse 3T3 cells. b) induction of pyrimidine dimers. The dimers were quantified by determining the number of DNA sites sensitive to T4 endonuclease V.

Keratinocytes irradiated with 280, 510 and 700 J/m2 left a surviving fraction of respectively 10, 1 and 0,1%. These surviving fractions chosen as a measure of lethal action, were obtained with melanocytes after an irradiation with 2-3 times higher doses of UV. Dimer induction in irradiated melanocytes was also at the same level as in keratinocytes after applying 2-3 times higher doses of UVB.

We can conclude that 1) cell killing as well as dimer induction, induced by UVB irradiation, can be estimated with the same methods in both cell types, 2) compared with keratinocytes melanocytes are very resistant for UVB irradiation.

The latter may play a role in the complicated relation between the induction of melanoma in human skin and exposure to UV irradiation.

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QUANTITATIVE SKIN REFLECTANCE CHROMAMETER MEASUREMENTS OF ERYTHEMA AND PIGMENTATION RESPONSE IN HUMAN VOLUNTEERS OF DIFFERENT SKIN TYPES IRRADIATED WITH SOLAR SIMULATED UV. Wiete Westerhof, Oscar Estevez Uscanga, Arthur Kammeyer, Joes Mears, Muriel Durocq and Irina Cairo. Departments of Dermatology and Medical Physics, Academic Medical Center, University of Amsterdam, The Netherlands.

The assessment of sensitivity of the human skin to sun rays is important in connection with photo-aging, photo-carcinogenesis and photo-protection. Fitzpatrick introduced a Working Classification of Sun-reactive Skin Types, which is based on a person's tendency to sunburn and capacity to tan, along with some racial parameters (1) which is not realistic and not practical for various reasons.

We determined the minimal erythema dose (MED) after solar simulated irradiation in 54 healthy volunteers belonging to the six skin types.

Whithin one skin type we found a wide range of MED's. Moreover there was an overlap in the ranges of MED's between the different skin types sometimes up to 100%. We therefore think that the MED, which is only 1 point on the dose-response curve, is not an accurate parameter to assess the sensitivity of the human skin to UV-irradiation. Rather than relying on 1 point in the dose-response relationship in one individual, we constructed a dose-response curve based on at least 4 points. The UV energy was each step increased by a constant factor $\sqrt{2}$, so that of the 5 spots on the forearm irradiated, at least 4 showed an increasing redness (without edema or blistering). For the quantitative measurement of erythema it was necessary to use a chromameter instead of the human eye (2). The chromameter readings were analyzed and graphically processed with a computer program Symphony.

The steepness of a dose response curve (< α) appeared to be distinctive per skin type (according to Fitzpatrick) although each group had some degree of heterogeneity. There was a better correlation between the constitutional skin type and the steepness of the dose-response curves. We also determined the minimal melanogenic dose (MMD) (1). The correlation between UV-radiation dose and pigmentation response was less clear than between UV-radiation dose and erythema response.

We propose as a parameter of the photosensitivity of the human skin the dose-response angle (< α) in combination with the MED for the use in clinical practice. 1. Pathak MA, et al. In: Dermatology in General Medicine. 3ed ed. 1987; 1507-1516. 2. Westerhof W, et al. In: Photodermatology. 1986; 3: 310-314.

54

PHOTOPROTECTIVE EFFECT OF AN E. COLI-FILTRATE (COLIBIOGEN^{R}) DEMONSTRATED BY PHOTOPROVOCATION THRESHOLD TESTING IN POLYMORPHOUS LIGHT ERUPTION AND BY INHIBITION OF 8-METHOXYPSORALEN PHOTOTOXICITY IN VITRO. B. Przybilla, M. Heppeler, R. Iglhaut, U. Vielwerth, H.C. Korting, T. Ruzicka, Dermatologische Klinik der Ludwig-Maximilians-Universität, Munich, F.R.G.

The therapeutic use of so-called biologic response modifiers of natural origin has gained increasing interest. We investigated the photoprotective effect of Colibiogen^{R} (Cb), a commercially available E. coli-filtrate.

In a total of 13 patients with polymorphous light eruption (PLE) the reaction threshold for the elicitation of skin lesions was determined by applying 10, 20, 40, or 80 J/cm2 UV-A to test areas on the extensor side of the upper arm on 3 consecutive days. Then 9 patients received Cb, 4 individuals were left untreated. Photoprovocation threshold testing was repeated three weeks after the initial procedure. For in vitro phototoxicity testing, broth cultures of Candida albicans incubated with 10^{-3}M 8-MOP were irradiated with various UV-A doses in the presence or absence of Cb.

A beneficial effect of therapy was found in 8 patients. The median of the cumulative UV-A threshold doses increased from 60 J/cm2 to 120 J/cm2 for any kind of reaction ($P < 0.01$) and from 80 J/cm2 to 240 J/cm2 for the appearance of papules ($P < 0.01$). Furthermore, the median of the time lag between the first irradiation and the development of papules increased from 2 to 3 days ($P < 0.05$). In the controls no improvement with regard to the reaction time lag nor the threshold doses for the development of papules was observed. - 8-MOP phototoxicity to Candida growth was inhibited by Cb up to more than 50 %.

Thus, Cb exhibits photoprotective properties. A controlled clinical trial of the drug in patients with PLE is currently in progress. Photoprovocation threshold testing is recommended as an objective method for the evaluation of therapeutic regimens in PLE.

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CIPROFLOXACIN-INDUCED PHOTOSENSITIVITY: IN VITRO AND IN VIVO STUDIES. J. Ferguson[1], J. McIntosh[2], E.M. Walker[1], and B.E. Johnson[1]. Departments of Dermatology[1] and Medicine[2], University of Dundee, Ninewells Hospital, Dundee, Scotland.

Ciprofloxacin (1-cyclopropyl-6-fluor-1, 4-dihydro-4-oxo-7-(piperazinyl)-3-quinoline carboxylic acid) is one of the new series of broad spectrum antibiotic quinolones, chemically related to nalidixic acid and therefore potentially photosensitizing for human skin. We have investigated the phototoxic potential of this drug using the standardized model systems of photosensitized destruction of histidine, killing of Candida albicans and mouse peritoneal macrophages, inhibition of PHA stimulated DNA synthesis in human lymphocytes, and photohaemolysis. Broad band UVA (315-400 nm) was obtained with an array of Sylvania (PUVA) fluorescent tubes. For action spectrum studies, an irradiation monochromator (½ max bandwidth 7.8 nm) was used. Routine phototesting procedures with an irradiation monochromator as source were used to determine the sensitivity of subjects taking ciprofloxacin as drug therapy (250-750 mg twice daily for a minimum of 10 days) to 305 ± 5 nm in the UVB and to 335 ± 30 nm and 365 ± 30 nm in the UVA.

Results obtained with Candida (solid drug or a 10% solution in DMSO on filter paper discs) and with photohaemolysis (up to 0.4 mg/ml; 1.2 mM and 14 J/cm2 UVA) were negative. With 14 J/cm2 UVA, there was a drug concentration dependent destruction of histidine, a 50% level being obtained with around 50 µg/ml (0.15 mM). There was a drug concentration (< 500 ng/ml:1.5 µM) and UVA dose (> 1.75 J/cm2) dependent inhibition of DNA synthesis in PHA stimulated lymphocytes. Mouse macrophages were damaged with 25 µg/ml (75 µM) drug and 14 J/cm2 UVA while with 50 µg/ml (150 µM) drug, they were damaged with 3.5 J/cm2 and killed with 7 J/cm2. An action spectrum for this killing effect showed a peak around 320-330 nm.

Six subjects taking ciprofloxacin showed normal MED values for 305 ± 5 nm. Two were abnormally sensitive at 335 ± 30 nm (MED values of 1.5 and 1.0 J/cm2 where low normal value is 1.8 J/cm2). Two others were more sensitive to 335 ± 30 nm while taking ciprofloxacin although still within the low normal range of MED. One subject showed abnormal photosensitivity at 365 ± 30 nm.

We have shown that ciprofloxacin has a phototoxic potential in vitro and that the most effective wavelength range is around 325 nm in the UVA. Phototesting showed that this phototoxicity might be manifest in human skin by a lowered MED to UVA wavelengths particularly around 335 nm.

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INTERACTION OF UVA AND UVB IN PHOTOCARCINOGENESIS. H. Slaper and J.C. van der Leun, Institute of Dermatology, University of Utrecht, The Netherlands.

Both UVA and UVB have been found to induce skin carcinomas in experimental animals. The comparative effectiveness of the two wavelength ranges is approximately known. Uncertainty remains about possible interactions of exposures to UVA and UVB: is there photoaugmentation, photoprotection, photorecovery? Such interactions may be informative about mechanisms involved in photocarcinogenesis, and also play a role in assessing the risks of cosmetic tanning procedures.

We performed an experiment on hairless albino mice Skh hr1. We arranged UVA and UVB regimens of approximately equal tumourigenic effectiveness, measured in terms of the median tumour development times. The UVA used was from fluorescent tubes Philips TL09, filtered through a 10 mm thick glass plate, selected to filter out the UVB rigorously; the remaining spectrum was composed essentially of wavelengths longer than 330 nm. The UVB was from unfiltered fluorescent sunlamps Westinghouse FS40. In both regimens, the mice were irradiated daily. The daily doses in the UVA regimen were 19 J/cm2, the daily doses of UVB (< 320 nm) in the UVB regimen 9.5 mJ/cm2. Besides groups of mice (A and B; n = 24 each) receiving the UVA and UVB regimens, respectively, one group (AB; n = 48) received the UVA and UVB regimens alternatingly, changing to the other regimen every week.

All surviving animals developed tumours, in all three groups predominantly squamous cell carcinomas. The median tumour development time in the AB-group was 313 ± 15 days, against 273 ± 14 days in the A-group and 281 ± 10 days in the B-group. This result implies that the alternating regimen was slightly less carcinogenic than the UVA and, UVB regimens separately. The carcinogenic effect of the alternating regimen was rather stronger, however, than what could be ascribed to only the UVB component in the combination.

In this particular experimental combination of UVA and UVB, both wavelength ranges contributed to the carcinogenic effect. The effectiveness of the combination was slightly smaller than what could be expected on the basis of photoaddition; photoaugmentation did not occur. Compared with the other groups, the mice under the UVA regimen showed more wrinkling and sagging of the skin, more scratching marks and a higher mortality ($P < 0.05$).

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THE CUTANEOUS BLOOD FLOW SLOPE AS A NEW MARKER OF SKIN SENSITIVITY TO UV-B. EVALUATION IN PATIENTS WITH ATOPIC ECZEMA AND IN CONTROLS. Rainer Gollhausen, Konrad Göttsberger, Hanno Winter, Bernhard Przybilla and Johannes Ring, Department of Dermatology, University of Munich, FRG.

We evaluated skin sensitivity to UV-B in patients with atopic eczema before and after UV-A phototherapy and in non-atopic controls using the determination of the MED and a new method, the cutaneous blood flow slope (CBFS).

The individual MED to polychromatic UV-B was determined on the lower back in 8 patients with atopic eczema (6 male, 2 female, 19 - 45 years) before and after UV-A phototherapy and in 10 non-atopic, healthy individuals (8 male, 2 female, 21 - 59 years). - Then 6 various doses of 0.5 till 3 MEDs rising in steps of 0.5 MED were applied on the lower back of each individual. The next day the cutaneous blood flow was assessed in the 6 irradiated spots using a laser doppler flowmeter. By simple regression analysis a dose response line was calculated: y=ax+b, the independant variable x being the MED, the dependant variable y being the cutaneous blood flow and the slope a being the CBFS.

The MED was in the same range in atopics before UV-A treatment and in controls, but it was significantly raised in atopics after phototherapy ($p < 0.05$). - The CBFS was significantly higher in atopics before treatment than in controls ($p < 0.05$), but was not significantly lowered by UV-A phototherapy.

Although unspecific skin sensitivity is supposed to be higher in atopics than in normals, the MED is in the same range in both groups. UV-A phototherapy can significantly raise the MED probably by inducing a tan, but skin sensitivity to UV-B is yet higher in tanned atopics than in untanned controls as indicated by the CBFS. So MED and CBFS are different markers of skin sensitivity to UV-B. MED is a marker of skin protection greatly dependant upon tan, whereas CBFS is a marker of skin sensitivity relatively independant of tan.

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THE EFFECTS OF UV-B AND UV-C IRRADIATION ON THE EXPRESSION OF NON-HISTONE NUCLEAR ANTIGENS ON KERATINOCYTES IN THE PATHOGENESIS OF LUPUS ERYTHEMATOSUS. E.G. Wildschut, P.J. Velthuis, H. Baart de la Faille, H. van Weelden, W.A. van Vloten, Department of Dermatology, University Hospital, Utrecht, The Netherlands.

The role of the nuclear antigens SSA, SSB, Sm and nRNP in the pathogenesis of LE is still unclear. Le Feber et al. (J Clin Invest 74:1545-1551) introduced the hypothesis that due to UV-irradiation these antigens migrate from the nucleus to the cell membrane. In contact with patient-serum an antibody-dependent-cellular-cytotoxicity reaction starts, which causes cell damage. We studied the effects of UV-B and UV-C on the distribution of nuclear antigens on keratinocytes in suspension, in order to find new evidence for this hypothesis. Cell suspensions were obtained by treating shave biopsies from the backs of healthy volunteers, with successively 0,76% EDTA (1-2 h), 0,3% trypsin (30 min) and 0,025% DNAse (3 min). The cells were maintained in RPMI-medium containing 10% FCS and 10% penicillin-streptomycin. Cell viability, assessed by trypan blue exclusion, varied between 70-85%. The keratinocytes, resuspended in PBS, were irradiated with doses ranging from 0-160 mJ/cm2 of UV-B from a Philips HP 3114 sunlamp or UV-C from 4 germicidal lamps (TUV). After 16 hours nuclear antigens were visualised by incubating the cells with patient-sera containing antibodies to non-histone or ds-DNA nuclear antigens, determined by immunoblotting or standard immunofluorescence techniques. Subsequently the cells were fixed with acetone on cytocentrifuge preparations, and incubated with FITC-labeled-anti-IgG-conjugate. Without irradiation the percentage of cells with granular staining of the cell membrane, determined by visual counting of 200-400 cells in each specimen by use of a Zeiss immunofluorescence microscope, varied between 5-30% (n = 41). There was no significant change following UV-B(n=26) or UV-C (n = 23) irradiation (Student-t-test). The membrane-antigen-expression was selective for the non-histone nuclear antigens; granular membrane staining did not show up after incubation with anti-dsDNA antisera or control sera. We could not find full evidence for the hypothesis of Le Feber et al. Contrary to their results, in our experiments not-irradiated keratinocytes showed nuclear antigen expression on cell surfaces and an influence of UV was not found. This discrepancy may be explained by the difference between normal epidermis cells used in our experiments and cultured neonatal foreskin keratinocytes of Le Feber's. The fact remains that LE-lesions may develop in the absence of antibodies to non-histone nuclear antigens and that these antibodies can circulate without giving skin problems.

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TOWARDS AN IMPROVEMENT OF PUVA DOSIMETRY, PSORIASIS TREATMENT WITH SUBERYTHEMOGENIC UVA. Adrian Tanew and Herbert Hönigsmann, Division of Photobiology, Department of Dermatology I., Univ. of Vienna, Austria.

According to be the standard European protocol for photochemotherapy (PUVA), UVA dosimetry relies on the patient's individual minimal phototoxicity dose (MPD), which is used as the initial treatment dose. This procedure reflects the common, but unconfirmed opinion that a faint phototoxic erythema reaction may serve as a suitable guideline for optimal UVA dosimetry. However, phototoxicity and antipsoriatic activity of PUVA treatment most probably are mediated via different photobiochemical mechanisms. Therefore it seems conceivable that effective photochemotherapy may not necessarily depend on erythemogenic UVA doses.

To test this assumption a paired comparison study was performed in ten patients with either chronic plaque type (n = 7) or acute guttate type (n = 3) of psoriasis. Each patient was treated with the usual regimen employing the MPD as the initial dose and subsequently slightly erythemogenic UVA doses on one side of the body and only two thirds of that irradiation dose on the other side of the body. Treatment was performed with a Waldmann PUVA 4000 lay-down unit.

After an equal number of exposures (mean: 9.8) both regimens resulted in complete clearing of psoriasis in all patients. The mean total UVA dose at the time of clearing was 59.6 and 39.3 J/cm2, respectively. During treatment a mild erythema reaction was clearly discernible on the side treated with routine PUVA, whereas the other side showed no erythema. Interestingly, however, irrespective of the patients' skin type no clear difference could be observed with regard to the intensity of PUVA-induced pigmentation.

In agreement with Hawk (Europ. Soc. Photobiol., Padua, 1987) our findings indicate that phototoxic erythema may not be the appropriate indicator for optimal dosimetry in photochemotherapy of psoriasis, and many patients may be, in fact, overtreated.

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PUVA AND UVB MODULATE THE INTRAEPIDERMAL ACCUMULATION OF POLYMORPHONUCLEAR LEUKOCYTES. A. Chang, J.A.C. Alkemade, P.D. Mier, R. Happle and P.C.M. van de Kerkhof, Department of Dermatology, Unversity of Nijmegen, The Netherlands.

The effect of PUVA and UVB therapy on the intraepidermal accumulation of polymorphonuclear leukocytes (PMN) in normal skin was assessed following a standardized chemotactic signal. Topical PUVA therapy, treatment with UVA only, application of 8-MOP only or UVB therapy was given on days 1, 2, 4 and 5 on test areas in 12 healthy volunteers. The dosage schedule for PUVA and UVB was according to a standard protocol for the treatment of psoriasis. On day 8 two chemotactic challenges were given to the treated areas: (i) standardized surface trauma by sellotape stripping; (ii) the epicutaneous application of 10 ng of leukotriene B_4 (LTB_4). Razorblade biopsies were taken 8 and 24 h respectively after the two challenges and the accumulation of PMN was quantified using the marker enzyme elastase.[1] Elastase activity was measured in the supernatant fractions using the fluorogenic substrate MeOSuc-Ala-Ala-Pro-Val-N-methylcoumarin, and was corrected for endogenous inhibition by the inclusion of an internal standard of elastase.

PUVA treatment caused a profound inhibition of the trauma- and LTB_4-induced accumulation of PMN ($P < 0.025$ and $P < 0,05$ respectively). Following UVB treatment these PMN accumulations were again significantly inhibited ($P < 0.025$). Treatment with UVA only or 8-MOP only had no effect. Inhibition of the internal standard was in all cases below 15%, and did not differ significantly from normal.

In conclusion, transepidermal migration of PMN could well be an antipsoriatic target of the light therapies. It is speculated that an interaction at the level of the capillaries, or the induction of inflammatory eicosanoids which counteract LTB_4, might play a role in this respect.

1. Lammers AM, van de Kerkhof PCM, Schalkwijk J, Mier, PD. Br J Dermatol 115: 181-186, 1986.

Monday, 20 June, 1988

15.00-17.00

Concurrent session – Holl

D.M. MACDONALD, M. BERNENGO, Chairpersons

Lymphoma (Abstracts 61-70)

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ACTIVATION OF T CELLS FROM SEZARY PATIENTS CAN OCCUR THROUGH BOTH ANTIGEN DEPENDENT AND INDEPENDENT PATHWAYS. O Baadsgaard, ER Hansen, V Ho, S Lisby, K Thomsen, JT Elder, D Fox, KD Cooper, G Lange Wantzin. Dept. of Derm., Univ. of MI, Ann Arbor, U.S.A., Lab of Immunol., Dept of Derm., Bispebjerg Hospital, Copenhagen, Denmark.

Sezary leukemia is characterized by proliferation of activated T-cells. The mechanism through which these T-cells achieve and maintain their activated state, is unknown. UM4D4 is a newly recognized T-cell surface molecule expressed on 25% of normal blood T-cells. The monoclonal antibody, anti-UM4D4 is mitogenic for most T-cell clones that express the molecule even in the absence of antigen presenting cells. Antigen dependent and independent activation pathways for blood T-cells and T-cells derived from the skin of a patient with Sezary leukemia were examined. The blood T-cells were composed of 97% $CD4^+2H4^-$ (helper cells) and 3% $CD8^+CD4^-$ (suppressor/cytotoxic cells). The same distribution was found in lesional skin. A T-cell line was derived from lesional skin and cloned yielding 80% $CD4^+2H4^-$ and 20% $CD8^+CD4^-$ clones. Southern blot analysis using a cDNA probe specific for the constant region of the T-cell receptor beta chain gene, demonstrated the same rearrangement in more than 85% of the blood T-cells indicating one dominant T-cell clone. None of the clones from the skin demonstrated the same rearrangement, suggesting that non-malignant T-cells grew preferentially in culture. More than 95% of the blood T-cells expressed UM4D4 and were induced to proliferate by anti-UM4D4 (stimulation index (SI) +/- SEM; 11 +/- 1). Furthermore 11 out of 12 oligoclones from the skin expressed UM4D4 and were induced to proliferate by anti-UM4D4 (SI +/- SEM; 32 +/- 2). Lesional epidermal cells stimulated the patients' own blood T-cells (SI +/- SEM; 116 +/- 13). However, in contrast to blood T-cells, lesional T-cells were not activated by autologous epidermal cells. In conclusion, 1) $UM4D4^+$ T-cells are enriched in the blood of Sezary leukemia and induced to proliferate by anti-UM4D4, 2) lesional epidermal cells activate autologous Sezary leukemia blood T-cells, 3) thus both antigen dependent and independent pathways may contribute to the activation and proliferation of T-cells in Sezary leukemia.

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EPIDERMAL CELLS FROM MYCOSIS FUNGOIDES DEMONSTRATE MHC-CLASS II DEPENDENT ACTIVATION OF THE $CD4^+CD8^-$ LYMPHOCYTE SUBSET. ER Hansen, O Baadsgaard, S Lisby, KD Cooper, K Thomsen, G Lange Wantzin. Laboratory of Immunology, Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark; Department of Dermatology, University of Michigan, Ann Arbor, U.S.A.; Department of Dermatology, Rigshospitalet, Copenhagen, Denmark.

Mycosis fungoides (MF) is characterized by skin infiltrations comprising activated $CD4^+$ T-cells. The mechanism by which the T-cells achieve and maintain their activated state is unknown. Antigen specific activation of T-cells are dependent on antigen presenting cells (APC) which express HLA-DR (DR) class II MHC molecules. The epidermal Langerhans cell is the only DR^+ cell population present in normal skin. However, a heterogeneous population of DR^+ epidermal cells (EC) is present in involved skin in MF plaque stage. We therefore determined the capacity of DR^+ EC present in involved and uninvolved skin to activate different autologous T-cell subsets. Involved skin contained in contrast to uninvolved skin a population of $OKM5^+DR^+$ EC (1.9%). The number of $T6^+DR^+$ epidermal Langerhans cells in involved skin (4.8%) was also increased compared to uninvolved skin (3.4%). To determine whether the increased number of DR^+ EC present in involved skin was associated with an enhanced capacity to activate autoreactive T-cell subsets, various numbers of involved and uninvolved EC were used to stimulate autologous $CD4^+$ and $CD8^+$ lymphocytes in the absence of added antigen. Involved EC potently induced proliferation of $CD4^+$ cells (stimulation index +/- SEM: 560 +/- 49). In contrast uninvolved EC only induced background level of proliferation (stimulation index +/- SEM: 7.7 +/- 3.3). Neither involved nor uninvolved EC were able directly to activate $CD8^+$ lymphocytes. The capability of involved EC to activate $CD4^+$ cells was dependent on expression of the DR molecules since addition of anti-DR monoclonal antibodies totally abrogated the reaction. In conclusion: Epidermal cells from involved skin of patients with MF potently induce class II restricted proliferation of $CD4^+$ lymphocytes but not $CD8^+$ lymphocytes. This may have implications for the preferential presence of $CD4^+$ cells in the skin of patients with mycosis fungoides.

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SOLUBLE IL-2 RECEPTOR SERUM LEVELS IN CUTANEOUS T CELL LYMPHOMA-CORRELATION WITH CLINICAL STAGE AND IL-2 RECEPTOR STATUS IN CUTANOUS INFILTRATES. Peter Kaudewitz, Olivera Josimovic-Alasevic, Tibor Diamantstein, Fritjof Eckert, Kristina Klepzig, Günter Jurg. Dept. of Dermatology, Munich, Inst. of Immunology, Berlin, West Germany.

There is evidence that some malignant cells in cutaneous T cell lymphoma (CTCL) express the interleukin-2 receptor (IL-2-R) and that these cells tend to be increased in advanced stages. Assessment of IL-2-R expression may therefore supply information on the stage and activity of a given T cell lymphoma. In situ immunohistochemical detection of IL-2-R positive cells is confined to the local situation at the site of biopsy. As a 55 kD-subunit of the IL-2-R is shed into the circulation, serum levels of soluble IL-2-R more accurately may reflect the IL-2-R status of both cutaneous and extracutaneous malignant T cell proliferation. It was the purpose of the present study to correlate serum IL-2-R levels, cutaneous in situ IL-2-R status and clinical stages of CTCL. Soluble human IL-2-R was measured by ELISA using AHT-107 as coating MAb and biotinylated AHT-54 as detector MAb. In situ expression of IL-2-R was detected by MAb ACT-1 using the APAAP technique. Sera and skin biopsies from 34 patients with mycosis fungoides (MF), subgrouped into eczematous(e), plaque (p) and tumor (t) stage were investigated. In the sera the following IL-2-R levels were found (mean values ± SD): e = 58.3 ± 27.9 U/ml (n =

11); p = 153.9 ± 74.7 U/ml (n = 14); t = 809.8 ± 346.5 U/ml (n = 9). Differences between all groups were statistically significant (p<0.0005). In skin infiltrates percentages of IL-2-R positive T cells (membrane staining) was 5 - 40% in e- and p- and 10 - 50% in t-stage. Differences were not significant. No clearcut correlation with serum IL-2-R levels was found. It is concluded from our results that soluble IL-2-R levels closely correlate to the clinical stage of MF in contrast to the percentages of IL-2-R positive T cells in cutaneous infiltrates. As advanced stages of MF are accompanied by a depression of the immune system., it is unlikely that elevated IL-2-R serum levels document an intensified cellular anti-tumor immune response. Alternatively they seem to be a direct correlate to an increased number of extracutaneous, activated malignant T cells and thereby represent an important clinical parameter for the stage of a given CTCL.

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MORPHOMETRICAL DIAGNOSIS OF MYCOSIS FUNGOIDES ON PARAFFIN SLIDES. Edgar Rieger, Josef Smolle, Stefan Hoedle, Freyja-Maria Juettner, Helmut Kerl, Department of Dermatology, University of Graz, Austria.

Quantitative histological techniques for the diagnosis of cutaneous T-cell lymphomas have repeatedly been applied on semithin and ultrathin sections. The aim of our study was to investigate, whether quantitative histology can provide objective criteria on routine paraffin material.

We have investigated 35 cases of mycosis fungoides (patch stage: 12; plaque stage: 11; tumor stage: 12) and 29 cases of contact dermatitis. The diagnosis of mycosis fungoides was based on histological, immunohistological and clinical follow-up data. From each case, H&E-stained paraffin sections were evaluated using an interactive image analysis system. (IBAS 1, Zeiss, FRG) connected to a Zeiss Axiomat bright field microscope at a final magnification of 4560 x. A total of 14 morphometric parameters was assessed. The results obtained were evaluated by the median test and the k-nearest-neighbour analysis.

The most significant differences between contact dermatitis and mycosis fungoides were found in the mean thickness of the infiltrate (TI) (0.076 mm ± 0.009 SEM compared with 0,722 ± 0.161; median test: p = < 0.0001) and in the nuclear volume fraction (VV) (13% ± 0.3 compared with 17% ± 0,9; p = < 0.01). The numerical density of the cells (ND), mean nuclear area (A) and nuclear contour index did not reveal significant differences. Non-parametric discriminant analysis (k-nearest-neighbour method) based on TI, VV, ND and A revealed a specificity in the diagnosis of MF of 93%, the sensitivity, however, was only 48 %. The calculation of an "infiltration index" given as TI * VV * ND * A provided a discrimination at a specificity of 90% and a sensitivity of 74% at a threshold value of 0.6.

Our study shows, that morphometrical data obtained on paraffin sections can provide helpful objective criteria for the diagnosis of mycosis fungoides. An unequivocal discrimination, however, cannot be achieved in each individual case. The nuclear contour index, which is of great importance in electron microscopy, does not contribute to the diagnosis of mycosis fungoides in routine histological material. The "infiltration index" as proposed in this study seems at present to be the most useful morphometrical parameter in H&E tissue sections.

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GENOTYPIC ANALYSIS IN LYMPHOMATOID PAPULOSIS - EVIDENCE FOR A CLONAL T-CELL ORIGIN. S.J. Whittaker, L. Foroni, N. Smith, L. Luzzatto, R. Russell Jones. Dept. of Dermatology & Molecular Genetics, Hammersmith Hospital and St. John's Hospital for Diseases of the Skin, London.

The exact histogenesis of lymphomatoid papulosis remains controversial. Immunohistochemical studies have indicated that the cellular infiltrate is heterogenous but these techniques cannot determine the presence of T cell clonality.

We have analysed the configuration of DNA for the Beta and Gamma T cell receptor genes and immunoglobulin heavy and light chain genes in 10 patients with lymphomatoid papulosis. Four patients had associated poikiloderma, one patient had a hypereosinophilic syndrome but none had evidence of classical mycosis fungoides.

High molecular weight DNA was prepared by homogenisation of cutaneous biopsies and from whole peripheral blood. DNA was also prepared from biopsies of nickel positive patch tests and lichen planus. DNA extracted from the granulocytes of normal donors was used as a control. Purified DNA was digested with bacterial restriction enzymes ECO R1, HIND 111, BAM H1 and BGI 11. Restriction fragments were separated by gel electrophoresis, transferred to nylon membranes and hybridised under high stringency conditions with P^{32} oligolabelled DNA probes. These consisted of T-cell receptor C β and J τ probes and immunoglobulin heavy (JH) and light chain (CK) probes.

Rearrangements of both the TCR C β and J τ chain genes were found in 6 patients in ECO R1 or HIND 111 digests. Rearranged bands were single and of differing sizes in all patients. In the remaining four cases TCR genes were present in a germ line configuration. One patient with lymphomatoid papulosis and poikiloderma also showed a TCR gene rearrangement in peripheral blood mononuclear cells. No patient had a rearrangement of immunoglobulin heavy or light chain genes.

These genotypic studies show that in the majority of cases of lymphomatoid papulosis a monoclonal T cell population is present in skin and also rarely in peripheral blood. No evidence of TCR or Ig gene rearrangement has been detected in the other inflammatory disorders studied.

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FOLLICULAR LYMPHOID HYPERPLASIA OF THE SKIN WITH MONOTYPIC PLASMA CELLS - A PRECURSOR LESION OF LYMPHOMA? F. Eckert, P. Kaudewitz, W. Stolz, and G. Burg, Department of Dermatology, University of Munich, W. Germany.

It is generally recognized that monoclonality of immunoglobulins (Ig) is not detectable in typical follicular pseudolymphoma of the skin. In contrast, surface and/or cytoplasmic Ig monoclonality has been equated with B-cell lymphoma. Our purpose is to describe a lymphoproliferative disorder showing features of both pseudolymphoma and lymphoma leading to considerable difficulties in assessing its biological behavior.

Seven patients were included in the present study. Clinically, the patients showed solitary tumors or single nodules. At the time of diagnosis no systemic involvement was detectable. Histologically, the lesions revealed a polymorphous lymphohistiocytic infiltrate with germinal center formation surrounded by distinct mantle zones. In addition, numerous plasma cells were intermingled in a sheet-like growth. Immunohistologically, infiltrates revealed a typical B- and T-cell compartmentalization. Follicular B-cells expressed weakly polytypic Ig while plasma cells showed restriction to IgG and one light chain (either kappa or lambda). Clinical follow-up studies of these patients have ranged from 6 months to 7 years. Serum or urine monoclonal gammopathy was not confirmed in any patient. After excision of the lesion 4 of our patients are still free of disease (up to 4 years), 1 patient showed recurrent lymphoid lesions without monoclonal B-cells and 2 patients subsequently developed malignant lymphoma of B- and T-cell type, respectively.

Our findings indicate that detectable monoclonality of plasma cells in follicular lymphoid hyperplasia of the skin does not represent overt lymphoid neoplasia but may arise from a clonally limited response to an antigenic stimulus. Subsequent development of malignant lymphoma may be due to an additional defect in the immune response.

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ASSESSMENT OF DIAGNOSTIC VALUE OF ACTIVATION- AND PROLIFERATION-ASSOCIATED ANTIGENS IN CUTANEOUS T-CELL LYMPHOMAS. Kurt Meissner, K. Michaelis, C. Mathis-Schütz, W. Rehpenning, Th. Löning, Dpts. of Dermatology, Pathology, and Mathematics in Medicine, University of Hamburg, Hamburg, FRG.

In order to evaluate their diagnostic value recently developed monoclonal antibodies (MCA) directed against activation- and proliferation-associated antigens (ClonabIL2R/CD25; OKT9; Ki1/CD30; Ki67) were applied to cryostat sections (3-step immunoperoxydase technique) of 35 patients with cutaneous T-cell lymphomas (CTCL) of different stages and of 19 patients with chronic dermatoses (benign chronic dermatoses, e.g. atopic dermatitis, psoriasis vulgaris:n=10; large plaque parapsoriasis:n=9). Reactivity was assessed quantitatively in epidermis (with the exception of MCA Ki67), and dermis separately and calculated as positive cells per mm2 of tissue section. Statistical analysis was performed with Mann-Whitney's U-test, stepwise discrimination analysis, and Simes' test. - No significant difference in the reactivity to the four MCA could be assessed between the groups of benign chronic dermatoses and large plaque parapsoriasis; reactivity to MCA Ki1 was absent in 17/19 cases. - Cells stained with MCA Ki1 were present in low numbers in the dermis of 19/35 cases, (x=62 cells/mm2) and in the epidermis of 10/35 cases (x=48 cells/mm2) of CTC. On an average, a more intense reactivity to the other three MCA was always observed in both dermis and epidermis of all cases with CTCL in comparison with the control groups. Significant differences between early stage CTCL and the groups of both benign chronic dermatoses and large plaque parapsoriasis were observed in the reactivity to MCA Ki1 in the dermis ($p<0,005$) and to ClonabIL2R in the epidermis ($p<0,05$). Grouping all CTCL stages together stepwise discrimination analysis revealed MCA Ki67 to discriminate best between CTCL and the control groups. Simes' test confirmed the results obtained. - It is concluded that the combined assessment of reactivity to the MCA Ki1 and Ki67 in the dermis and ClonabIL2R in the epidermis may be of diagnostic value in CTCL.

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ACCESSORY DENTRITIC CELLS IN SKIN LESIONS OF MYCOSIS FUNGOIDES: AN IMMUNOHISTOLOGICAL, ULTRASTRUCTURAL AND IMMUNOELECTRON MICROSCOPICAL CHARACTERIZATION. G. Kolde, St. Hanneken, and J. Knop, Dept. of Dermatology, University of Münster, Münster, F.R.G.

Several immunohistological studies have shown that accessory dendritic cells which express the CD1 antigen form a substantial component of the cutaneous infiltrate in mycosis fungoides (MF). However, it is still controversial whether this increase of dendritic cells is due to the CD1 reactive Langerhans cells (LCs), indeterminate dendritic cells (IDCs), or interdigitating reticulum cells (IRCs). We here report immunohistological, ultrastructural and immunoelectron microscopical findings showing a characteristic distribution and behaviour of the accessory dendritic cells in skin lesions of MF.

The investigation was performed on skin biopsies from 6 patients with untreated MF (4 plaque stage, 2 tumor stage). On immunohistology, all biopsies exhibited a slight increase of the CD1 positive dendritic cells in the epidermis and a pronounced increase in the dermal infiltrates. On electron microscopy, the epidermis contained few LCs, but numerous IDCs. Most dermal dendritic cells showed ultrastructural features of activated IRCs. There were often clusters of IRCs in the plaque stage of MF. LCs and IDCs, by contrast, were only rarely found in the dermal infiltrates. Immunoelectron microscopical analysis proved the CD1 antigen on the cell membranes of IRCs, LCs, and IDCs. The atypical lymphoid cells did not express this antigen. Dermal IRCs were often in close apposition to CD4 reactive lymphoid cells.

The present observations demonstrate that the CD1 positive IRCs are a characteristic component of the dermal infiltrate of MF. In the lymph node, IRCs play a significant role in the mitotic activity and blastic transformation of T cells. It is therefore reasonable to assume that the dermal accumulation of IRCs in MF participates in the pathogenesis of this probably reactive cutaneous T cell neoplasia.

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CUTANEOUS TUMOURS OF TRUE HISTIOCYTIC ORIGIN. Gunhild Wantzin, Nigel O'Connor, Elisabeth Ralfkiaer, Department of Dermatology, Bispebjerg Hospital, Denmark; Nuffield Department of Haematology, John Radcliffe Hospital, University of Oxford, England; and Department of Pathology, Rigshospitalet, University of Copenhagen, Denmark.

During the last 20 years it has been increasingly recognized that most lymphomas classified as "histiocytic" are in reality derived from transformed lymphoid cells rather than macrophages. In consequence there has been much debate as to the existence and incidence of true histiocytic lymphomas.

In this study, evidence is presented that rare cases of cutaneous lymphoma may originate from macrophages. Among more than 200 lymphomas, three cases showed immunophenotypic and genotypic properties consistent with their derivation from macrophages. These tumours presented as skin lesions, showed non-epidermotropic infiltrates of pleomorphic lymphoid cells that were negative for myeloid cell associated antigens, lacked pan-T-cell and pan-B-cell markers, showed germline configuration of both T-cell receptor and immunoglobulin genes, and expressed macrophage associated antigens, e.g. CD11b, CD11c, KB61, EBM11, FMC32, lysozyme, alpha 1-antitrypsin and/or the L1 antigen. One of the cases was positive for CD1 and may have originated from Langerhans cells/interdigitating cells. The remaining two tumours were CD1-negative and may be related to mononuclear phagocytic cells.

In all cases, the clinical course has been aggressive with rapid and wide-spread dissemination to visceral organs, poor response to chemotherapy and survival times ranging from 0.5 to 4 months. This suggests that although true histiocytic lymphomas are overall very rare, their recognition may be important for clinical and prognostic reasons.

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SEZARY CELLS BEHAVE LIKE PRIMARY IMMUNODEFICIENCY CELLS IN RESPECT TO THEIR FUNCTIONAL PROPERTIES. M.G. Bernengo, M. Meregalli, C. Jemma, G. Forni, and G.C. Doveil, Dermatologic Clinic and Microbiology Institute, University of Turin, Turin, Italy.

In this study we correlate the proliferative activity to IL-2 and IFN-τ production, activation of antigen expression and in vitro response to exogenous human recombinant IL-2 in PBMC from 10 untreated Sézary syndrome (SS) patients. A defective or absent proliferative response to PHA, ConA, PWM was observed in 9/10 patients. To determine whether the PHA unresponsiveness was related to a failure of the atypical cells to express IL-2 receptors, we evaluated the capacity of Sézary cells (SC) to display IL-2 receptors upon stimulation. CD25 antigens were virtually absent on fresh resting SC and PHA stimulation evoked the appearance of IL-2 receptors on 44.7 ± 24.7% Sézary cells. IL-2 receptors on SC were functional since the addition of purified IL-2 to the culture medium containing PHA led to an increase in the proliferative response in all but one patients. In addition IL-2 induced proliferation was partially inhibited by anti-CD25 antibody. DNA synthesis was not increased in the patient with normal proliferative activity, however SC from 9/10 cases did not respond directly to IL-2 suggesting that, like normal T-lymphocytes, SC had an absolute requirement for prior lectin or antigen activation. PBMC from all patients did not produce IL-2 spontaneously. Stimulation with PHA increased IL-2 production only in the patient who displayed normal proliferative activity. Although we observed an increase in IL-2 production with the addition of PHA + TPA in 5/10 cases, the increase was markedly lower than in normal controls. The addition of TPA, that could replace IL-1 in the activation process, did not increase the proliferative response to PHA. IFN-τ production by PBMC from SS patients stimulated with PHA + TPA was markedly reduced in 7/10 cases compared to normal controls. After 7-day cultures in exogenous recombinant IL-2, IFN-τ increased reaching the values produced by the normal controls in 4 cases. In the two cases where SC phenotype was CD7-, FACS sorted CD7-cells did not respond to PHA, however the addition of IL-2 to PHA led to an increase in the proliferative activity. FACS sorted CD7+ cells had a normal proliferative response without enhancement, after incubation with IL-2. No permanent lines could be obtained from any patient, the cells invariably dying after 20 - 60 days. Seven out of 7 patients were HTLV-I negative. In conclusion SC show a selective defect in IL-2 production, while they can respond to it. They behave like PBMC from immunodeficient patients, differing from T-CLL neoplastic cells.

Monday, 20 June, 1988

17.00-18.00

Plenary session – Cuvilliés

K. WOLFF, Chairperson
(Abstracts 71-74)

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Ia$^+$ MURINE EPIDERMAL LANGERHANS CELLS ARE NOT DEFICIENT IN SURFACE EXPRESSION OF THE CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX. Angelika Lenz, Hans-Georg Rammensee*, Christine Heufler, Herta Glassl**, Nikolaus Romani and Gerold Schuler, Departments of Dermatology and Internal Medicine**, University of Innsbruck, Innsbruck, Austria, and Max-Planck Institute for Biology*, Tübingen, FRG.

Class I major histocompatibility complex (MHC) antigens are expressed on virtually all nucleated cells and serve as recognition structures and restriction elements for CD8$^+$ T cells at the induction and the effector stage. Questioning previous findings (J.Exp.Med. 161: 1368-1393, 1985) it has recently been claimed that epidermal Langerhans cells (LC) when studied either in situ or in freshly prepared epidermal cell suspensions are deficient in surface expression of class I antigens (Proc.Natl.Acad.Sci.USA 83: 7438-7442, 1986). To clarify this obviously important issue we have studied epidermal sheets prepared from (B6x Balb/c) F1 -> B6 (i.e. H-2^{b,d} -> H-2^b) bone marrow chimeras 4,5, and 6 months after irradiation and bone marrow reconstitution. When sheets were stained for donor class I antigens [staining sequence: 1.) monoclonal antibody to H-2K^{d}D^d (clone 34-1-2S; mouse IgG2aK) or H-2D^d (clone 34-40-20S; mouse IgG2aK); 2.) biotinylylated F(ab')$_2$ sheep anti mouse Ig; 3.) streptavadin-FITC], donor LC [identified by double labelling with TRITC conjugated anti I-E^{k,d} (clone 14-4-S)], but not LC of recipient origin were found to express donor class I antigens. Appropriate controls for antibody isotype and MHC haplotype, e.g. anti H-2K^{k}D^k (clone 15-3-1S; mouse IgG2aK), were negative. We also used FACS analysis to quantitate surface class I antigen expression. Freshly prepared Balb/c (H-2^d) epidermal cell suspensions were stained as above except that streptavidin-phycoerythrin was used, and FITC anti I-E^{k,d} (clone 14-4-S) or I-A^{b,d} (clone B21-2) to identify LC. The staining of LC paralleled the brighter keratinocytes in our suspensions. Controls were negative.

Our data clearly show that LC do synthesize and express class I molecules. Our findings are in sharp contrast to those reported by S.W. Caughman et al. (Proc.Natl.Acad.Sci.USA 83: 7438-7442, 1986). As we have used appropriate controls including positive ones we are confident that our results are correct and that epidermal LC do indeed express class I molecules.

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CD1a MONOCLONAL ANTIBODIES (EXCEPT OKT6) DEFINE A NEW MOLECULE ON EPIDERMAL LANGERHANS CELL MEMBRANES. Colette Dezutter-Dambuyant, Daniel Schmitt, Marie-Jeanne Staquet, and Jean Thivolet, INSERM U209, Laboratoire de Recherche Dermatologique et Immunologie, Hôp. Ed. Herriot, Lyon, France.

The first cluster of differentiation (CD1) defines three distinct human thymic cell-surface antigens non-covalently attached to β_2-microglobulin, CD1a (M_r 49,000), CD1b (M_r 45,000), and CD1c (M_r 43,000) molecules. Immunocytochemical studies have shown that human epidermal Langerhans cells expressed CD1a and CD1c molecules on their membrane.

This study has been performed in order to demonstrate whether CD1a and CD1c monoclonal antibodies recognize similar molecules on epidermal Langerhans cells and cortical thymocytes. Langerhans cell-enriched epidermal cells were surface labeled with ^{125}I and then lysed in 0.5% Nonidet P-40 Tris-EDTA buffer. Immunoprecipitates were carried out with six CD1a (BL6, 10D12.2, L404, L544, Na1/34, and OKT6) and two CD1c (L161 and 7C6/162/3B10) monoclonal antibodies and were analyzed in reduced and non-reduced conditions. Biochemical analysis confirms the existence of the CD1a (M_r 49,000) chain and CD1c (M_r 43,000) associated with a M_r 12,000 chain (β_2-microglobulin). CD1a monoclonal antibodies (except OKT6) co-immunoprecipitated an additional band of M_r 27,000. Under reducing conditions CD1a antigen was born by the same chain of M_r 49,000 on cortical thymocytes and Langerhans cells whereas M_r 27,000 molecule was never found on thymic cells. Under non-reducing conditions the M_r 27,000 molecule had a faster electrophoretic mobility. After pre-clearing lysates with OKT6, we could still precipitate the protein band of M_r 27,000 with L544. On two-dimensional gel analyses, M_r 27,000 molecule showed a pattern including three major spots with a pI of 5.6, 5.9, and 6.2. By endoglycosidase F treatment, the M_r 27,000 molecule was found to contain one N-linked oligosaccharide residue and has a core size of M_r 25,000. Apparently the M_r 27,000 molecule seemed to differ from the MHC class II β chains (DR, DQ, and DP).

Langerhans cells express a membrane antigen otherwise characteristic of cortical thymocytes. The identity of this M_r 27,000 molecule is unknown and its presence suggests a potential degree of epitopic heterogeneity of some CD1a monoclonal antibodies.

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BIRBECK GRANULES OF UNSTIMULATED HUMAN LANGERHANS CELLS DO NOT EXPRESS HLA CLASS II, T6 OR CLATHRIN MOLECULES. A.M. Mommaas-Kienhuis, M.C. Wijsman, J.J. Out-Luiting and B.J. Vermeer, Department of Dermatology, University Hospital, Leiden, The Netherlands.

Langerhans cells, which represent a minor cell population of the human epidermis, play an important role in antigen presentation and immunoregulation in the skin. This is supported by the finding that they express several immunological markers on their plasma membrane such as HLA class II antigens, and receptors for Fe-IgG and C3. The Langerhans cells are characterized by a unique, ultrastructural cytoplasmic organelle, the Birbeck granule. The origin, fate and function of this Birbeck granule is still unknown. Various authors claim that they represent endocytotic vesicles. Others suggest that they play a role in the internalization of HLA-DR and T6 antigens (during antigen presentation). However, their experiments were performed with antibodies against HLA-DR and T6 on unfixed cells, thus allowing an induction of mobilization (c.q. internalization) of the antigens. We employed a post-fixation immunoelectromicroscopical technique to study the distribution of HLA class II T6 and clathrin molecules on Langerhans cells. Freshly isolated human epidermal cell suspensions were fixed and after cryoprotection ultracryosections were prepared. The sections were incubated with monoclonal antibodies against HLA class II, T6 or clathrin. For each of the described antibodies, a specific labeling was found, either on the plasma membrane, on intracellular vesicular structures, or within the cytoplasm. However the Birbeck granules were always devoid of label. Our data suggest that in unstimulated Langerhans cells:

1) the Birbeck granules do not take part in the internalization and recycling of HLA class II and T6 antigens.

2) Birbeck granules are not actively involved in the first steps of endocytosis via the coated pit-vesicle system.

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IN VITRO HUMAN EPIDERMAL INDETERMINATE CELLS (CD1a$^+$, 40 kD FcτR$^-$) ARE POTENT IMMUNOSTIMULATORY CELLS FOR ALLOGENIC LYMPHOCYTES. Diedier A. Schmitt, Daniel Hanau, Thomas Bieber, Gilles Pauly and Jean-Pierre Cazenave, INSERM U.311, Centre Régional de Transfusion Sanguine, Strasbourg, France; Dermatologische Klinik und Poliklinik der Ludwig-Maximilians Universität, Munich, F.R.G.; IRBD, Pulnoy-Nancy, France.

In human epidermis only Langerhans (LC) and indeterminate cells (IC) express the plasma membrane CD1a antigens. We have previously demonstrated, using the mouse monoclonal antibody (mMAb) anti-40kD Fc τ receptor (FcτR) CIKM5, that LC are FcτR$^+$ and that IC are FcτR$^-$. In this study, using these surface markers and immunomagnetic indirect depletion procedures, we have investigated the stimulatory capacity for T lymphocyte proliferative responses of IC and LC. Suspensions of epidermal cells (EC) were incubated in microtiter plates with allogeneic lymphocytes using 3 conditions: i) total EC; ii) EC depleted of LC (40 kD FcτR$^+$ cells); iii) EC depleted of LC and IC (CD1a$^+$ cells). EC suspensions were depleted of LC and/or IC using a 3 step method: i) pretreatment with CIKM5 (IgG1 subclass) or with the mMAb anti-CD1a, BL6 (IgG1 subclass); ii) addition of magnetic beads coated with sheep anti-mouse IgG1 (Dynal-SHAM); iii) isolation of the respective target cells rosetted with Dynal-SHAM by applying a magnet. The intensity of the skin cell-lymphocyte reaction (SLR) was determined by measuring the uptake of ^{3}H-thymidine for 24 h after 96 h of coculture. EC depleted of CD1a$^+$ cells (i.e. of LC and IC) did not stimulate allogeneic lymphocytes above background levels. Undepleted EC stimulate the allogeneic lymphocytes. IC (i.e. EC depleted of the 40 kD FcτR$^+$ cells) stimulate the allogeneic lymphocytes: in 3 experiments, this stimulation varies from 88 to 96% of the stimulation achieved by the undepleted EC; in 2 experiments this stimulation is two to three times greater than that of undepleted EC. It has previously been shown that after 2 to 3 days in culture, mouse LC lose several characteristic features such as Birbeck granules (BG) and Fc receptors, become DR^{+++} and show a greater stimulatory capacity for T cell proliferation. In the present study we show that in fresh human EC suspensions, IC (which are BG-, FcτR$^-$; DR^{+++}) markedly stimulate SLR and thus present many of the characteristics of the "in vitro aged" LC. Therefore, we suggest that IC could represent a more mature form of LC in vivo. In addition, our study suggests that LC could exert an inhibitory action on SLR.

Tuesday, 21 June, 1988

8.00-10.30

Cuncurrent session – Cuvilliés

J.H. SAURAT, A. VAHLQUIST, Chairpersons
Retinoids, Tumours (Abstracts 75-86)

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LOW DENSITY LIPOPROTEINS AS CARRIERS OF ETRETINATE. V. Simonin[1], P. Morlière[1], J.-C. Mazière[2], G. Hüppe[3], R. Santus[3] and L. Dubertret[1]. 1/ Laboratoire de Recherche Clinique en Dermatologie, INSERM U.12, Hôpital Henri Mondor, 94010 Créteil, France. 2/ Laboratoire de Biochimie, CNRS U.A.524, Faculté de Médecine Saint Antoine, 27 rue Chaligny, 75012 Paris, France. 3/ Laboratoire de Physico-Chimie de l'Adaptation Biologique, INSERM U312, Muséum National d'Histoire Naturelle, 43, rue Cuvier, 75231 Paris Cedex 05, France.

In vitro interaction of etretinate with human low density lipoproteins (LDL), human high density lipoproteins (HDL) and human albumin (HSA) were investigated. Etretinate was found to strongly interact with LDL and therefore LDL can be considered as potential extra- and intra-cellular carriers for this retinoid. Normal human serum doped with etretinate was submitted to gradient ultra centrifugation in order to isolate the lipoprotein fractions. With respect to their protein content, very low density lipoproteins and LDL were found to contain large amounts of etretinate. On the other hand, the ultracentrifugation bottom fraction, which contains proteins other than lipoproteins, was practically etretinate-free. This confirms that LDL, which undergo receptor-mediated endocytosis in most skin cells subject to proliferative growth, are interesting etretinate carriers. Delivery to human cultured fibroblasts, using LDL, HDL or HSA as carrier, indeed showed that etretinate uptake was efficiently achieved using LDL. This uptake is enhanced when, 24 hours prior incubation with LDL loaded with etretinate, cells are preincubated with LDL-deficient culture medium for maximal receptor expression. This illustrates that the uptake is, at least in part, taking place via the LDL-receptor endocytosis pathway. Further experiments are in progress to determine whether binding of LDL to their receptors, internalization of LDL-receptors and LDL degradation in the lysosomes are deregulated or not.

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LIGAND-SPECIFIC AND NON-SPECIFIIC IN-VIVO-MODULATION OF HUMAN EPIDERMAL CELLULAR RETINOIC ACID BINDING PROTEIN (CRABP). Suzanne Hirschel-Scholz, Georges Siegenthaler, Jean-Hilaire Saurat. Clinic of Dermatology, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland.

Recent reports about the successful cloning of a nuclear retinoic acid (RA) pertaining to the group of steroid receptors have refocused the interest on the role of the other well known, cellular binding proteins (CRABP).

We previously reported that topical application of the natural ligand of CRABP, retinoid acid (RA), as well as acitretin induced a rise in epidermal CRABP in healthy human volunteers (JID 1987, 89; 311). Using the same experimental model and techniques, we now investigated the time course of this phenomenon and compared it to the effects of retinoids that do not bind to CRABP (retinol, Ro15-0778), unrelated chemical agents (dithranol, steroids) and physical stimuli (stripping, UVB). We report also on the inhibition of the ligand-induced CRABP increase by topical steroids.

We found that CRABP was increased after daily application during 4 days of a 0,1% solution of RA * (T 8.5 ± 1-4/NT 2.5 ± 0.5 p< 0.05), acitretin (T 16.3 ± 3.9/NT 2.3 ± 0,7 p< 0,02), isotretinoin (T 18.5 ± 4.4/NT 4,9 ± 0,6 p< 0,05) and Ro13-7410 (T 12.0 ± 2.8/NT 2.3 ± 0,6 p< 0,05). Retinol also increased CRABP (T 20.5 ± 3.8/NT 3.7 ± 0.5 p< 0,08), while Ro15-0778 had no effect (T 4.4 ± 0.9/4.4 ± 0.5). No macro-or microscopically discernable changes could be observed in any of the tested substances. A rise of CRABP induced by RA and acitretin was seen as early as 24 h after one single application. An important increase of CRABP was also observed 96 h, but not 24 h after dithranol (T 9.2 ± 0.8/NT 2.6 ± 0.5 p< 0.001), UVB (T 13.6 ± 3.1/NT 4.4 ± 1.0 p< 0.001) and stripping (T 7.1 ± 1.0/NT 3.8 ± 0.9 p < 0.001), that caused evident erythema, swelling and later desquamation. Topical flucortolone decreased epidermal CRABP (T 0.8 ± 0,1/NT 2.2 ± 0,7 n.s.) and was able to diminish the rise of CRABP induced by etretin (E 22.1 ± 2.4/E+flu 9.3 ± 1.4 p< 0.01).

Thus we conclude that the increase of CRABP seems to be a biological phenomenon following processes of inflammation and proliferation with a lag of several days, while retinoids seem to be able to induce such a rise independently of their affinity to CRABP and of inflammatory processes as early as 24 h after their application.

* T = treated NT = solvent treated; values expressed in pmol/mg prot.

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ISOTRETINOIN DIFFERS FROM OTHER SYNTHETIC RETINOIDS IN ITS MODULATION OF CELLULAR RETINOIC ACID BINDING PROTEIN (CRABP) AFTER ORAL BUT NOT AFTER TOPICAL ADMINISTRATION. Jean-Hilaire Saurat, Suzanne Hirschel-Scholz. Clinic of Dermatology, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland.

Isotretinoin differs from acitretin and Ro13-7410 by its striking sebostatic effect in acne after oral but not topical administration.

We previously observed a modulation of epidermal cellular retinoic acid binding protein (CRABP) by oral and topical acitretin treatment. We now investigated if isotretinoin differs also from the other synthetic retinoids in its ability to influence epidermal CRABP levels after oral and topical treatment.

Patients that presented with different dermatological problems, but were otherwise in good health, were treated orally with either isotretinoin (0.5 mg/kg/d), acitretin (0.7 mg/kg/d) or Ro 13-7410 (0.5 µg/kg/d).

Before and after an average of 25 days of treatment an epidermal shave biopsy of non-lesional skin was taken under local anesthesia and CRABP levels determined by a polyacrylamide gel electrophoresis. 0.1% solutions of acitretin, isotretinoin and Ro 13-7410 and their solvents were applied as previously described (JID 1987,89;311) once a day during four days.

Oral treatment with acitretin and Ro 13-7410 lead to a striking increase of epidermal CRABP while isotretinoin failed to induce a comparable rise, although it displayed in all patients a striking sebostatic effect. After topical application, however, increase of CRABP was comparable between all of the three drugs (see table).

	isotretinoin			acitretin		Ro13-7410
	*T	NT	T	NT	T	NT
oral	3.2±0.7	3.7±0.7	12.8±2.6	2.7±1.3	21.7±4.4	2.5±1.6
No		5 (p<ns)		4 (p<0.02)		4 (p<0.02)
topical	18.5±4.4	4.9±0.6	16.3±3.3	2.3±0.7	12.0±2.8	2.3±0.6
No		4 (p<0.05)		5 (p<0.02)		5 (p<0.05)

Therefore when given topically isotretinoin does not have a sebostatic effect and has the same potential to act on epidermal CRABP as acitretin and Ro13-7410; it loses this capability after oral administration but is sebostatic. This observation might help to explain its particularity among synthetic retinoids.

*T=treated NT=solvent treated; values expressed in pmol/mg prot.

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VARIATION OF RETINOIC ACID BINDING CAPACITY OF CRABP IN PSORIASIS. Didier Junquero, Nicole Basset-Sequin, Guy Modat, Claude Bonne, Chantal Levy* and Jean-Jacques Guilhou*. Laboratoire de Physiologie Cellulaire, Université Montpellier 1, France. *Service de Dermatologie, Centre Hospitalier St Charles, Montpellier, France.

Retinoic acid (RA) is a modulator of growth/differentiation in epithelial cells and is a potent antagonist of tumor promoters. The mechanism of action of RA is not yet clear, but there is strong evidence that RA acts at least in part through a specific cytosolic binding protein, CRABP, characterized in various tissues including epidermis. The CRABP characterized in human epidermal cytosol exhibits an increased binding capacity in lesional psoriatic epidermis as compared to normal and uninvolved skin. On the other hand, it has been demonstrated that CRABP from calf uterus was a substrate for protein kinase C (PKC). This key enzyme, identified as phorbol ester receptor, is involved in the regulation of cellular differentiation and proliferation. Since the activity of this kinase is decreased in psoriatic epidermis, the aim of the present study was to confirm the increase in RA-binding capacity in psoriasis and determine whether the CRABP-binding capacity of normal and psoriatic epidermis could be modulated by PKC activation.

The present study confirms that CRABP binding capacity is three-fold in lesional psoriatic epidermis. It has subsequently been discovered that CRABP-binding capacity is significantly decreased by incubating the epidermal homogenate with a phorbol ester. This effect is probably due to the activation of PKC, the phorbol ester receptor, as it requires the presence of ATP.

Conversely, the CRABP-binding capacity in psoriatic epidermis was not modified by phorbol ester, in both involved and uninvolved skin. This lack of phorbol ester - induced CRABP modulation could be correlated with the recently reported defective PKC activity in both lesional and nonlesional psoriatic epidermis.

The decrease in PKC could be responsible for elevated RA-binding capacity which in turn could induce a stronger PKC inhibition. This speculation however is not valid for uninvolved skin in which low PKC activity is associated with normal CRABP levels. Further studies are needed to clarify the exact relationships between PKC and the retinoids' mechanism under physiological and pathological conditions.

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MODULATING EFFECTS OF RETINOIC ACID ON KERATIN EXPRESSION AND LIPID COMPOSITON OF NORMAL KERATINOCYTES CULTURED ON AIR-LIQUID INTERFACE. M. Ponec, J. Kempenaar, A. Weerheim, G.N.P. van Muijen*. Dept. of Dermatology, University Hospital Leiden, and *Dept. of Pathology, University of Nijmegen, The Netherlands.

Using a conventional (submerged) culture system normal keratinocytes undergo differentiation, although to a lower extent as compared with the in vivo situation. As shown in our earlier studies, under these conditions the keratinocytes express keratins (K4, K13, K19) that are found only in the early fetal skin. Furthermore, the low extent of keratinocyte differentiation was also confirmed by lipid analysis which revealed high phospholipid (PL) and low ceramide (CER) content and virtual absence of acylglucosylceramides (AGC) and acylceramides (AC).

A quite different situation was found in air-exposed culture in which the keratinocytes grown on de-epidermized dermis (DED) were found to show morphological features of differentiation and lipid composition close to those seen in vivo. The results of the present study show that also keratin expression is close to that seen under the in vivo situation. Namely, K13 and K19 were found not to be expressed and K4 to be expressed only in a few cells.

The addition of retinoic acid (RA) at 2 uM to medium of air-exposed cultures led to marked changes of morphology, resulting in an increase in the number of cell layers, terminal differentiation of individual cells and transformation of horny layer into a parakeratotic layer. This RA-induced modulation of differentiation was accompanied by marked changes in both the keratin expression and the lipid composition. In the presence of RA not only K4, K13 and K19 but also K18 were locally expressed in both the suprabasal and the superficial cells. Furthermore, K10 expression was lower as compared with cultures grown in the absence of RA. The analysis of lipid composition revealed marked increase in PL content and drastic decrease in CER, AGC, AC and lanosterol content. The results show that the extent of keratinocyte differentiation in the air-exposed culture in the presence of retinoic acid was markedly decreased and was close to that seen in cultures grown under the submerged conditions.

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SYNTHETIC RETINOIDS INHIBIT THE MIXED EPIDERMAL CELL-LYMPHOCYTE REACTION IN HUMAN. Patrick Dupuy, Martine Bagot, Michele Heslan and Louis Dubertret. Laboratoire de dermatologie, INSERM U.312, Hôpital Henri-Mondor, 94010 Créteil, France.

It has been suggested that the therapeutic effects of retinoids are not only related to their action on epidermal cell proliferation and differentiation, but might also be mediated by immune mechanisms. However, the possible immunologic action of retinoids on the epidermis is yet to be demonstrated.

For that purpose, the effects of retinoids on human T-cell activation were studied on phytohemagglutinin (PHA)-induced lymphocyte proliferation, in allogeneic mixed lymphocyte reactions (MLR) and mixed epidermal cell-lymphocyte reactions (MECLR), comparatively. The retinoids tested were as follows: retinol (RO 1-5488), isotretinoin (RO 04-3780), etretin (RO 10-1670) and arotinoid free acid (RO 13-7410). Since retinoids are light-sensitive, the drugs were prepared just before the addition in the culture wells and cultures were incubated in the dark. Cultures containing equivalent amounts of diluent (DMSO) were used as controls.

Results obtained with PHA-induced proliferation were highly variable, whatever the retinoids used. In contrast, in MECLR, the synthetic retinoids consistently induced a significant decrease of the lymphocyte proliferation, approximately 25%. That was found with drug concentrations equivalent to therapeutic levels in human serum (10^{-8} to 10^{-7}M). In MLR, the synthetic retinoids inhibited marginally but not significantly the lymphocyte proliferation, about 10%. Retinol induced no effect in both reactions. In MECLR, the action of etretin on lymphocyte activation was further dissected. Etretin diminished the proliferation in a dose-dependent manner. This diminution was also associated with a decrease of the cytotoxic T-lymphocyte (CTL) induction. Time-sequential additions of 10^{-6}M of etretin revealed that the presence of the drug at the initiation of the cultures was necessary for inducing a significant inhibition. Furthermore, as demonstrated by cell preincubations prior to MECLR, the drug exposure of epidermal cells was critical for reproducing the inhibition, suggesting a direct effect on the antigen presentation by epidermal cells.

In conclusion, synthetic retinoids inhibit specifically the lymphocyte proliferation and the CTL induction in MECLR. The modification of the epidermal cell-lymphocyte interactions with synthetics retinoids may explain in part their therapeutic effects in the skin.

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HYALURONATE ACCUMULATION IN HUMAN EPIDERMIS TREATED WITH RETINOIC ACID IN SKIN ORGAN CULTURE. Raija Tammi, James A. Ripellino, Richard U. Margolis, Howard I. Maibach and Markku Tammi. Department of Anatomy, University of Kuopio, Kuopio, Finland. Department of Dermatology, University of California, San Francisco, CA (HIM), and Department of Pharmacology, New York University Medical Center, New York, NY (JAR.RUM).

The effect of retinoic acid (RA) on the content and localization, as well as the synthesis and disappearance rates of hyaluronate (HA) in human epidermis cultured in organ culture was studied to test the idea that some of the known influences of RA on epidermal differentiation are associated to alterations of keratinocyte HA metabolism. A specific probe, the hyaluronate binding region complex of cartilage proteoglycan (HABC) (Ripellino et al. J Histochem Cytochem 33: 1060, 1985) was applied for the histological detection of HA.

After five days in culture, the chemically quantified HA was more than doubled in the RA-treated epidermis compared to control samples. The staining with HABC at light and electron microscopic level also revealed higher staining intensity in RA-treated epidermis than in control specimens. The difference was most marked in the upper vital cell layer, where the terminal differentiation into corneocytes normally takes place. In RA-treated skin a patchy, discontinuous staining with HABC was also seen in stratum granulosum and corneum which were not stained at all in control skin. RA stimulated the incorporation of ^{3}H-glucosamine into epidermal HA up to 50% at concentrations between 0.5 nM and 5 µM, whereas the pulse-chase experiments with ^{3}H-glucosamine revealed no change in the disappearance rate of epidermal HA due to RA-treatment. The present study demonstrates that RA accumulates HA in the superficial layers of epidermis by stimulating its synthesis in keratinocytes. This change in the composition of epidermal extracellular matrix may account for the weakened cohesion of the keratinocytes and the delay in terminal differentiation, observed previously both in vivo and in vitro.

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IMMUNOPHENOTYPIC CHARACTERISTICS OF THE INFLAMMATORY INFILTRATES ASSOCIATED WITH EPIDERMALLY - DERIVED TUMOURS. A.C. Markey, M.J. Tidman, D.M. MacDonald. Laboratory of Applied Dermatopathology, United Medical and Dental Schools (Guy's Campus), London, U.K.

The presence of an inflammatory infiltrate around cutaneous tumours is a common finding. The aim of this study was to immunophenotype the infiltrate associated with a wide variety of epidermally-derived tumours. Cryostat sections were cut from 10 squamous cell carcinomas, 10 basal cell carcinomas, 6 keratoacanthomas, 10 seborrhoeic keratoses, 10 actinic keratoses and 6 lesions of Bowen's disease. In addition, a range of inflammatory dermatoses (lichen planus, allergic contact dermatitis, psoriasis and discoid lupus erythematosus) was studied for comparison. A panel of monoclonal antibodies, recognising T-cells and their subsets, B cells, macrophages, natural killer cells and activation markers, was employed in a standard peroxidase-antiperoxidase reaction, and the cells staining with each antibody were quantitatively assessed.

The infiltrate around all neoplasms consisted principally of T-lymphocytes, predominantly of the helper subset (Leu 3+). Further dissection of this subset revealed the majority to be of the T helper inducer phenotype (Leu3+, Leu8-, Leu18-, UCHL1+), with relatively few T suppressor inducer cells. Lymphocytes were found in close association with macrophages (p15+). Activation markers (IL2 receptor, HLA-DR, OKT9) were expressed by variable numbers of T-lymphocytes around tumours and in inflammatory dermatoses. However, clusters of cells reactive with OKT10 (a putative marker of activated lymphocytes) were present in substantial numbers around tumours, but were consistently lacking from the infiltrates of inflammatory dermatoses. B-cells were absent in most specimens, with the exception of keratoacanthomas, in which they were generally present in small numbers. Leu11+ (natural killer) cells were confined to the infiltrate around squamous cell carcinomas and keratoacanthomas, although Leu7, a less specific marker for natural killer cells, was more widely distributed around tumours and within inflammatory dermatoses.

This study has revealed qualitative and quantitative differences in the composition of the infiltrate associated with epidermal tumours and inflammatory dermatoses, and in addition has highlighted variations between tumour types.

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AN ABSENCE OF CYTOKERATIN 8 AND AN INCONSISTENT EXPRESSION OF CYTOKERATINS 7 AND 19 IN HUMAN BASAL CELL CARCINOMA. Bhupandra Tank, Jan M.W. Habets, Vojislav D. Vuzevski* and Theodoor van Joost, Depts. of Dermato-Venereology and *Pathology, Erasmus Unversity, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands.

The expression of the low molecular weight cytokeratins (K) 7, 8, 18, 19 and the high molecular weight cytokeratin 10 was studied in 21 basal cell carcinoma (BCC) using an indirect immunoperoxidase technique and seven different monoclonal antibodies (MoAbs) with specific anti-cytokeratin activity. MoAbs RCK 105 (anti-K7), RPN 1164 (anti-low molecular weight cytokeratins of basic group), Ks 19.1 (anti-K19) and Cam 5.2 (anti-K8, K18, K19) reacted positively but inconsistently in the BCC that were examined. They did not react with normal epidermis. MoAbs 1166 (anti-K8) and RGE 53 (anti-K18) did not react at all. MoAb RKSE 60 (anti-K10) did not react with the tumor cells but reacted incidentally with a few tumor cells with a squamoid appearance. The inconsistency in the staining pattern of MoAbs RPN 1164, RCK 105 and Ks 19.1 may be due to a heterogeneity of the tumor cells. However, a masking effect cannot also be totally excluded. Since low molecular weight cytokeratins are expressed in early fetal skin, it is tempting to speculate that the expression of cytokeratins 7 and 19 in BCC may be the result of dedifferentiation or transformation. From these results, it can be concluded that only cytokeratins 7 and 19 are expressed in (43% and 71%, respectively) BCC, whereas cytokeratin 8 is not expressed.

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EPIDERMAL BASEMENT MEMBRANE ASSOCIATED C3d,g IS ABSENT FROM TUMOR NESTS OF PAPULONODULAR BASAL CELL CARCINOMAS. N. Basset-Séguin, P. Uhle, D. Emanuel, P. Henry, K.B. Yancey, Department of Dermatology, Montpellier, France and Department of Dermatology, USUHS, Bethesda, MD, USA.

Recent studies in our laboratory have shown that C3d,g, a specific cleavage fragment of the third component of complement, is present along the base of the lamina densa and in the sublamina densa region of normal human epidermal basement membrane (BM). Moreover, the demonstration that C*d,g is absent from the skin of a patient* with congenital C3 deficiency has confirmed the specifity of this finding. In studies of human skin, papulonodular basal cell carcinomas (PNBCCs) have served as a useful model for the investigation of various BM antigens and matrix proteins. To further investigate the presence of C3d,g within the epidermal BM as well as examine its relationship with other known BM constituents, we have analyzed serial sections of ten PNBCCs by light and immunofluorescence microscopy. In these experiments, tumor nest and adjacent normal epidermal BMs were studied for the presence of C3d,g, laminin, and type IV collagen as well as bullous pemphigoid (BP), epidermolysis bullosa acquisita (EBA), and KF-1 antigens. In PNBCC tumor nest BMs, C3d,g was either absent (N = 9) or minimally detectable (N = 1). While BP and KF-1 antigens were absent (N = 6 and N = 3, respectively) or markedly decreased (N = 4 and N = 7, respectively) in PNBCC tumor nest BMs, the EBA antigen was routinely present though somewhat (N = 3) or moderately decreased (N = 3) in selected samples. Laminin and type IV collagen were expressed normally in all PNBCC tumor nest BMs. All BM constituents including C3d,g were present in normal amount and distribution in adjacent normal epidermal BM of all PNBCC samples. This study has demonstrated unique alterations within each ultrastructural subregion of PNBCC tumor nest BMs by identifying the virtual absence of C3d,g (sublamina densa) as well as showing a significant reduction in KF-1 (lamina densa) and BP (lamina lucida) antigens. Moreover, the presence of laminin, type IV collagen, and the EBA antigen in tumor nest BMs suggests: 1) that these particular BM constituents are not responsible for low grade C3 conversion and 2) that they do not act as essential binding sites for C3 in epidermal BM. These studies give additional support to the hypothesis which suggests that this C3 fragment is a previously unrecognized constituent of epidermal BM and does not represent passive incorporation of circulating C3 at this site in human skin.

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AN IMMUNOHISTOCHEMICAL STUDY ON 13 CASES OF CHONDROID SYRINGOMA SUPPORTS ITS EPITHELIAL AND CONTRADICTS ITS MYOEPITHELIAL ORIGIN. René Lautier[1], Wolfram Sterry[2], Helmut H. Wolff[1], Department of Dermatology, Medical University of Lübeck, Klinikum der Christian-Albrechts Universität Kiel[2], Federal Republic of Germany.

The histogenetic differentiation of chondroid syringoma (CS) is still a matter of controversy. Some authors postulated by light and electron microscopical investigations epithelial, epithelial plus myoepithelial, or purely myoepithelial tumor cells in chondroid syringoma. Different opinions exist about as to an eccrine or apocrine differentiation of CS. To our knowledge up to now there is no systematic immunohistochemical investigation of chondroid syringoma.

We investigated formalin-fixed, paraffin-embedded tissue sections of 13 cases (11 with tubular, branching lumina and 2 with small tubular lumina) of chondroid syringoma by the peroxidase-antiperoxidase technique, using antibodies to actin, desmin, cytokeratin, vimentin, S100 protein, carcinoembryonic antigen (CEA) and neuron specific enolase (NSE). The majority of tumor cells in all cases reacted with antibodies to cytokeratin, S100 protein and NSE. There was no reaction of the tumor cells with actin and desmin antibodies, while the myoepithelial cells of normal sweat glands showed a strong staining with actin antibodies. The 2 cases with small tubular lumina expressed CEA in 50% and vimentin in 80% of the tumor cells, while only a few cells were stained with CEA and vimentin antibodies in the other 11 cases.

Our results strongly support an epithelial and contradict myoepithelial differentiation of chondroid syringoma. Since CEA is preferentially expressed in eccrine as compared to apocrine sweat glands, our results also hint to an eccrine differentiation of CS with small tubular lumina, while the more frequent type with tubular, branching lumina shows features of apocrine differentiation.

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AN ATTACHMENT ASSOCIATED CELL SURFACE GLYCOPROTEIN IS INCREASED IN TRANSFORMED MESENCHYMAL AND EPITHELIAL CELLS. C. Eberhard Klein, Brigitte Hartmann, Lutz Weber and Lloyd J. Old, Department of Dermatology, Univ. Ulm, FRG; Memorial Sloan Kettering Cancer Center, New York.

We have recently identified a 90 kD cell surface glycoprotein (gp 90) - defined by mouse monoclonal antibody F8 - that is highly increased in basal cell carcinomas and SV40 transformed human keratinocytes when compared to normal epidermal cells (J. Invest. Dermatol., in press). Here, we studied whether increased expression of gp 90 is a common characteristic of cancer cells also occuring in other cell systems as a consequence of malignant transformation. To investigate this question, we compared viral and chemical transformed fibroblasts and renal cells with the corresponding normal cell cultures. Gp_{90} expression was analyzed in radioimmunoprecipitation tests of $[^{35}S]$methionine labeled cell cultures and in indirect immunofluorescence assays using a fluorescence activated cell sorter. When the fetal fibroblast cell line Hs74 was compared with its SV40 virus transformed derivative SV/HF-5/39, gp 90 was expressed at increased levels on the SV40-transformed cells. Similar results were obtained, when other viral and chemical transformed fibroblasts (Wi 38 ⟶ Wi 38VA13, KD ⟶ Hut14) were compared with their normal parent cells. Furthermore, SV40 transformed renal cells (Cos) and 2 out of 6 human renal cancer cell lines expressed gp 90 at 8- to 12-fold higher levels than the corresponding nontransformed cell cultures. In addition, 2 squamous carcinoma cell lines derived from skin (SCL 1 and SCL 2) also showed increased expression of gp 90. Previous studies indicated that gp 90 is selectively expressed on cells adhering to substrate and is induced, when suspension growing tumor cells are selected for adhesion to plastic (Rettig et al., PNAS 81: 6437 (1984) and unpublished). The data indicate an interesting combination of properties: A cell surface glycoprotein is associated with attachment to substrates and is also significantly increased after cellular transformation. Based on these characteristics we postulate that gp 90 fits the concept of a molecule involved in tumor invasion mechanisms. We are currently investigating this hypothesis.

Tuesday, 21 June, 1988

8.00-10.30

Cuncurrent session – Effner

J. THIVOLET, G. STINGL, Chairpersons
Antigen Presenting Cells (Abstracts 87-98)

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REQUIREMENTS FOR T_h HELPER CELL (Th) ACTIVATION BY ANTIGEN PRESENTING CELLS. C. Hauser*†, and S.I. Katz*. *Dermatology branch, NCI, NIH Bethesda, Md, USA and †Department of Dermatology, Hôpital Cantonal Universitaire, Geneva, Switzerland.

We recently described a culture system in which hapten-modified cultured Langerhans cells (cLC) stimulated the primary proliferation of specific Th from nonsensitized animals. When thus actived Th were restimulated with hapten-modified cLC in repeated cycles hapten-specific Th lines were established. To investigate a possible down regulatory role of $Thy1^+$ dendritic epidermal T cells on primary Th activation, we added syngeneic $Thy1^+$-enriched (-50%) dendritic epidermal T cells or Thy1-depleted, MHC class II-depleted epidermal cells to cultures containing nonsensitized Th and TNP-modified cLC. Slight inhibiton was observed with either cell population added, suggesting that $Thy1^+$ dendritic epidermal T cells do not suppress the magnitude of the primary Th proliferation stimulated with TNP-modified cLC. Similarly, no inhibition of proliferation was seen when nonmodified cLC and allogeneic Th cells were used. To compare cLC with other antigen presenting cells, dendritic cell enriched spleen cells and cLC were simultaneously tested for their stimulatory capacity in primary hapten-dependent proliferation and primary allogeneic proliferation as well as restimulation assays of previously in vivo and in vitro activated, TNP-specific Th, cLC and dendritic cell enriched spleen cells performed well in all 4 assays, supporting the view of a functional similarity of cLC and dendritic cells. Th lines generated by repeated stimulation of Th with hapten-modified cLC responded in a hapten-specific manner to syngeneic and allogeneic cLC. The response to allogeneic cLC showed a greater dependence on the dose of hapten used to modify the cLC than the response to syngeneic cLC. Similar findings have been made for conventionally generated Th lines. Comparing the proliferative response of a FITC-specific Th line (7 cycles of stimulation with FITC-modified cLC) and its subline (23 cycles of stimulation with FITC-modified cLC) to FITC-modified cLC, spleen cells and M12 c cells, a MHC class II positive B cell lymphoma line, revealed responsiveness of the mother line to all 3 types of antigen presenting cells whereas the subline responded only to cLC but not to spleen cells and M12c. This observation demonstrates the existence of "skin-specific" Th as it has been shown for cytotoxic T cells.

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HUMAN FETAL SKIN DENDRITIC CELLS. Laura Fina, Alberto Brusasco, Emanuela Cecca, Fiametta Cappio, Simona Muratori, Emilio Berti. 1st Department of Dermatology, University of Milan, Italy.

In human fetal skin dendritic cells (DC) have been identified in the epidermis after 8 weeks gestational age employing OKT6 (CD1a) immunological marker.

The aim of this study was to characterize fetal skin dendritic cells employing several monoclonal antibodies (MAbs) specific for the myelo-monocytic lineage (CD1a, CD1b, CD1c, CD4, CD11a, CD11b, CD11c, CD14b, CD45 and HLA DR).

Fetal skin specimens from 8 weeks to 20 weeks estimated gestational age were snap frozen in liquid nitrogen and employed in this study. 4 micron cryostat sections were fixed 10' in acetone, dried and then incubated with MAbs diluted 1:400 from ascitis. An immunohistochemical Alkaline Posphatase (APAAP) method and immunofluorescence double labeling technique was used.

Our studies showed the presence of DC in the epidermis and near the dermoepidermal junction from 8 weeks estimated gestational age; these cells were strongly labeled by pan-leucocytes T200-CD45 MAbs (epitopes 1 and 2) while only a few DC in the epidermis also express CD1a positivity.

In addition in earlier gestational age the number of DC CD1c positive in the epidermis and particularly in the dermis was greater than that of CD1a positive DC. CD1a positive DC also showed CD4, CD11c and HLA DR staining. These quantitative data were confirmed by double labeling techniques.

In summary all DC in fetal skin express common leucocyte antigen T200-CD45, while in earlier gestational age only a minority of these DC shows CD1c, CD1a, CD4 and CD11c positivity. We suggest that CD1a (OKT6) is not a reliable marker to investigate DC in human fetal skin.

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FcγRECEPTOR AS A FUNCTIONAL MARKER ON LANGERHANS CELLS IN SITU IN HUMAN SKIN. Jens R. Bjerke, Maya Tigalonowa, and Roald Matre, Department of Dermatology and Broegelmann Research Laboratory for Microbiology, University of Bergen, Norway.

Langerhans cells (LC) in suspension express receptors for the Fc-part of the IgG-molecule (FcR), as described by Stingl et al (1977) who found that all LC bear FcR. However, the detection of FcR on LC in situ in human skin has been difficult. We have examined cryostat sections from normal skin and report the first in situ demonstration of functionally active FcR on LC. FcR were detected with soluble immune complexes of horseradish peroxidase (HRP) and rabbit IgG anti-HRP (HRP-anti-HRP). The binding of HRP-anti-HRP to LC was demonstrated using a double immunofluorescence staining in which LC were identified with a CD1 specific monoclonal antibody (Leu 6).

The immune complexes gave granular staining of CD1+ cells in sections of all specimens from normal skin. The staining with HRP-anti-HRP showed a clearly defined dendritic pattern of the CD1+ cells. However, not all dendrites detected with Leu 6 were stained with the immune complexes. We found immune complex binding on 48 ± 12% (n = 8) of the LC. The staining intensity of LC with HRP-anti-HRP was weaker as compared to the strong staining of CD1- macrophages in the dermis.

The results show that under normal conditions in human skin LC express FcR. The demonstration of functionally active FcR in situ may be useful also in the study of LC in pathological skin disorders.

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HIGH AND LOW AFFINITY FcγRECEPTORS ON LANGERHANS CELLS AND KERATINOCYTES FROM NORMAL SKIN. Maya Tigalonowa, Jens R. Bjerke, and Roald Matre. Department of Dermatology and Broegelmann Research Laboratory for Microbiology, University of Bergen, Norway.

Recently, 3 classes of receptors for the Fc-part of IgG (FcR) have been identified; the high affinity FcRI, and the low affinity receptors $FcRII$ and FcR_{LOW}. In the epidermis Langerhans cells (LC) have been considered to be the only cells expressing FcR (Stingl, Tamaki & Katz, 1980). We have studied FcR on human epidermal cells (EC) in suspension using 1) soluble immune complexes of horseradish peroxidase (HRP) and rabbit IgG-anti-HRP (HRP-anti-HRP) and 2) a monoclonal antibody (B1D6) against placental FcR. Previous data indicate that B1D6 reacts with low affinity FcR. LC were identified with the CD1 specific monoclonal antibody Leu6.

The results of double immunofluorescence staining showed that ≥95% of the CD1+ cells bound HRP-anti-HRP. None of the CD1+ cells were stained with B1D6 which gave granular membrane staining of 35% of the EC. 75% of the B1D6+ cells were stained with HRP-anti-HRP giving a weak granular fluorescence that apparently was located on the cell membrane.

The results could be explained by Lc expressing high affinity FcR, but lacking low affinity FcR reactive with B1D6. Keratinocytes express low affinity FcR. Many of the B1D6+/HRP-anti-HRP+ cells were relatively large cells indicating that they belong to the upper part of the epidermis.

The functional significance of keratinocyte FcR is unclear. The possible immune function of keratinocytes is still poorly understood.

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SEMI-QUANTITATIVE ANALYSIS OF THE EXPRESSION OF GLYCOPROTEINS OF THE LEUKOCYTE ADHESION-PROMOTING HETERODIMER FAMILY (CD11a,b,c/CD18) ON LANGERHANS CELLS. G. De Panfilis, G.C. Manara, C. Ferrari, D. Soligo*, C. Torresani, A. Zucchi, Dept. of Dermatology, Parma University, and *Inst. of Medical Sciences, Milan University, Italy.

The recently defined leukocyte adhesion-promoting glycoprotein family is comprised of the three heterodimers LFA-1, and gp150,95, each consisting of a unique alpha subunit of 180kd (CD11a), 165 kd (CD11b) or 150kd (CD11c) respectively, noncovalently associated with a common beta subunit of 95kd (CD18). Our preliminary findings provided qualitative evidence for both the CD11b and the CD11c expression by Langerhans cells (LC). The present study investigates the expression on LC of CD11a, CD11b, CD11c, and CD18, by a semi-quantitative approach.

An indirect immunogold method was performed, both in transmission-(ITEM) and in scanning-electronmicroscopy (ISEM), on epidermal cell suspensions freshly isolated from normal human skin, using eight monoclonal antibodies (moAb): anti-CD11a (Tsl/22.1.1.17; MHM24), anti-CD11b (D12; 60.1; Vim12; anti-C3bi), anti-CD11c (SHCL3), and anti-CD18 (60.3). The number of gold particles bound to the cell membrane of 40 Birbeck granule-bearing LC, per each moAb, was counted.

In ITEM the mean (±SD) gold particle-binding to the LC surface was as follows: CD11a: 0.3 ± 0.6 (n = 80); CD11b: 6.9 ± 10.0 (n = 160); CD11c: 35.0 ± 28.0 (n = 40); CD18: 7.5 ± 4.8 (n = 40). The results obtained by the highly sensitive ISEM system were consistent with the ITEM findings.

In ITEM a cell is considered positive when at least three gold particles are linked to its surface. This investigation therefore assesses the expression on LC membrane of two adhesion-promoting glycoproteins, namely gp150,95 (CD11c/CD18) and, presumably to a lesser extent, HMac-1 (CD11b/CD18). Since these glycoproteins display an important role in cell migration and localization and in cell-cell interactions, the present findings, although within the limits of merely immunostaining results, suggest that HMac-1 and gp150,95 might serve as adhesion molecules in migration, localization and cellular interactions of LC.

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FLOW CITOMETRICALLY-SORTED RESIDUAL HLA-DR+T6+ LANGERHANS CELLS IN TOPICAL STEROID-TREATED HUMAN SKIN EXPRESS NORMAL AMOUNTS OF HLA-DR AND T6/CD1a ANTIGENS AND EXHIBIT NORMAL ALLOANTIGEN-PRESENTING CAPACITY. J. Ashworth, J. Booker, M.C. Kahan* and S.M. Breathnach, Department of Medicine (Dermatology), Charing Cross and Westminster Medical School and *Charing Cross Sunley Research Centre, London, U.K.

We have reported that topical steroid therapy in man decreases not only the number of HLA-DR+T6+ Langerhans cells (LCs), which appear larger with longer dendrites, but also the antigen presenting capacity of epidermal cells (ECs) in the epidermal lymphocyte reaction (ELR). Inhibition of the ELR resulted partly from suppressed lymphocyte proliferation, due either to keratinocyte-derived inhibitory cytokines or to steroid carry-over into the culture system. To eliminate such effects as far as possible and investigate in isolation the properties of the morphologically abnormal residual HLA-DR+t6+ LCs, we obtained highly purified populations of HLA-DR+T6+ LCs from control and steroid-treated skin by flow cytometric sorting.

Normal volunteers applied clobetasol propionate 0,05% or inactive control ointment to forearm skin twice daily for 7 days. EC suspensions obtained by trypsinisation of suction blister-derived epidermal sheets were double stained with fluoresceinated OKT6 and phycoerythrin-conjugated anti-HLA-DR; aliquots were analysed and sorted into HLA-DR+T6+ -enriched (85-90% LCs) and HLA-DR-T6- (LC-depleted) populations using a FACStar flow cytometer (FACS), LC-dependent stimulation of T lymphocyte proliferation was measured by tritiated thymidine uptake in the ELR, in which EC were cultured with allogeneic peripheral blood mononuclear cells (PBMNs).

FACS analysis revealed that steroid therapy reduced the percentage of HLA-DR+T6+ LCs in unsorted EC suspensions to 46% of control (from a mean ± SEM of 2.49 ± 0.30 in control skin to 1.15 ± 0.22 in steroid-treated skin, n = 5), but did not significantly alter the relative amounts of HLA-DR and T6/CD1a antigens per individual HLA-DR+T6+ cell. HLA-DR+T6- and HLA-DR-T6+ cells were not detected in either group. Steroid therapy markedly decreased the allostimulatory capacity of unsorted ECs, as previously reported. By contrast, in parallel experiments in which the same EC suspensions were subjected to flow cytometric sorting (n = 4), the allostimulatory capacity of purified LCs from steroid-treated skin was not significantly different from that of equivalent numbers of control purified LCs (either $350_{,3}$ 500 or 750 sorted HLA-DR+T6+ -enriched ECs were added to 50 x 10^3 allogeneic PBMNs per well in triplicate). The inhibitory action of topical corticosteroids on the skin immune system, as evidenced by depressed allostimulation in the ELR, is associated with an overall reduction in HLA-DR+T6+ LCs. Residual HLA-DR+T6+ LCs, however, express normal amounts of HLA-DR and T6/CD1a antigens, and appear to exhibit normal alloantigen-presenting function.

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ROLE OF SUPEROXIDE RADICALS IN UV INDUCED DAMAGE OF LANGERHANS CELLS. A.C. Chu, J.F. Morris and P. Norris. Unit of Dermatology, Hammersmith Hospital, London W12.

We have studied the role of superoxide radicals in UV induced Langerhans cell damage by the use of the enzyme superoxide dismutase (SOD).

Blood and suction blisters of skin were taken from normal (n = 4) volunteers. Mononuclear cells were separated from blood using Ficoll Hypaque and T cells purified using E rosetting. Epidermal cell suspensions were produced by overnight trypsinisation of blister roofs. Epidermal cells were irradiated using a UVB Westinghouse FS/20 "sunlamp" at times from 0-600 seconds with or without the addition of SOD - 50-500 IU/ml. Unseparated blood mononuclear cells, T cells, T cells + irradiated or unirradiated epidermal cells and T cells + irradiated or unirradiated epidermal cells incubated with SOD, were set up in a Terasaki hanging droplet technique using PPD as a soluble recall antigen. T cells and epidermal cells were used at a ratio of 10:1. T cells + unirradiated epidermal cells gave similar or higher responses to PPD than blood mononuclear cells and this was not affected by the addition of SOD. UVB irradiation of epidermal cells caused a dose dependant reduction of Langerhans cell function, reducing this by 50% after 1.3 - 6 mJ/cm2 irradiation and abolishing response after 30 mJ/cm2. SOD at 50 IU/ml had no influence on the UV damage to Langerhans cell function but at higher concentrations (100 and 500 IU/ml) protected Langerhans cells from UV damage up to 30 mJ/cm2 but further UVB irradiation caused rapid and profound reduction of Langerhans cell function despite the presence of SOD.

Results of this study indicate that superoxide radicals are involved in Langerhans cell damage by low levels of UVB irradiation. At higher levels of irradiation, the loss of protection afforded by SOD may be due to inactivation or consumption of SOD or may suggest that other mechanisms are operative at these levels.

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PRIMARY CULTURE OF HUMAN EPIDERMAL LANGERHANS CELLS. J. Czernielewski, J. Cambrou, P. Vaigot. Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, 06565 Valbonne, France.

To further characterize a human Langerhans cell (LC) population we examined the possibility of long-term LC culturing. Single epidermal cell suspensions were prepared from surgical skin specimens by trypsinisation. LC-enriched suspensions were obtained either by FACS analysis and sterile cell sorting of OKT-6 (+) cells or by treatment of total cell suspensions with OKT-6 antibody, followed by incubation with anti-mouse IgG_1 coated immunomagnetic Dynabeads and subsequent isolation of rosetted cells using a magnetic particle concentrator.

Both methods resulted in highly enriched (more than 90%) LC suspensions. The latter method, however, was less satisfactory for LC culturing due to the strong, irreversible binding of Dynabeads to the cell surface. The FACS sorted cells were therefore used in all culture assays described.

Small culture chambers (Lab-Tec) were seeded at 10^4 cells/cm2. RPMI medium or semi-solid medium containing methyl cellulose, both supplemented with either Il-1, Il-2, Con A, different concentrations of foetal calf or human AB serum (0-20%) or with the supernatant from cultured keratinocytes, were used. We also varied culture substrates (plastic, glass, collagen or poly-L-lysine coated plastic dishes, mitomycine treated 3T3 fibroblasts, dried kearatinocyte cultures). Cultures were examined regularly for morphology, viability, alkaline phosphatase activity and capacity to induce T-cell allogeneic responses. Under the various conditions studied, LC remained rounded in shape, possessed microvilli but not dendrites. They partially attached to glass and ply-L-lysine pretreated dishes. They did not form colonies. The majority of LC remained viable for at least 1 month. They were able to stimulate allogeneic T-cells and presented alkaline phosphatase activity up to 14 days. The majority of them retained FITC-OKT-6 labelling in culture.

The presence of serum or keratinocyte supernatant and the type of media (liquid or semisolid) influenced morphological and functional parameters.

The experimental approaches used, therefore, were sufficient to keep LC alive for several weeks but failed to produce proliferating, colony-forming Langerhans cells.

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ADHESIVE PROTEIN RECEPTORS SHARED BY LANGERHANS CELLS AND BASAL KERATINOCYTES. Marie-Jeanne Staquet, Colette Dezutter-Dambuyant, Giovanna Zambruno. Daniel Schmitt and Jean Thivolet. INSERM U. 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôp. Ed. Herriot, Lyon, France and Clinica Dermatologica, Università di Modena, Italy.

K20 and 4B4 (CDw29) monoclonal antibodies (Mab) recognize glycoprotein complexes preferentially expressed on early haematopoietic cells, monocytes and T cells. In epidermis, they were shown to react with Langerhans cells (LC) and basal keratinocytes (BK) as demonstrated by double immunofluorescence labelling experiments (K20, 4B4/HLA-DR for LC, and K20, 4B4/Bullous Pemphigoid antibodies for BK). The aim of this study was to identify the antigen recognized by K20 and 4B4 on epidermal cells.

LC-enriched cell suspensions obtained by Ficoll-Hypaque sedimentation and BK-enriched suspensions obtained after specific adhesion to collagen, were surface labelled with ^{125}I and then 1% NP40 cell lysates were used for immunoprecipitation. The immunoprecipitates were analyzed by SDS-Page. Under reducing conditions, K20 and 4B4 immunoprecipitated from LC and BK, a broad Mr 105,000 band and proteins of Mr 145,000, 90,000 and 80,000. Under non-reducing conditions, each band was shifted approximately 5,000 Mr down. Immunoprecipitation of ^{35}S-methionine labelled lysates obtained from overnight labelled BK-enriched suspensions showed that the Mr 145,000 and 105,000 protein bands were synthesized by these cells. Cross-linking experiments indicated that this structure exists on the cell surface as a Mr 145,000 heavy chain in noncovalent association with a Mr 105,000 light chain. Digestion of these subunits with Endoglycosidase F revealed that each subunit contains one N-linked oligosaccharide residue.

Similar structures were recognized by K20 and 4B4 on lymphoid cells. It has recently been demonstrated that these antibodies recognize the common subunit of the VLA (very late activation antigen) protein family. The VLA belong to a superfamily of related cell surface receptors involved in cell-matrix and cell-cell adhesion.

The present investigation provides evidence that LC and BK share with lymphoid cells, antigens of the VLA family, that means the presence of adhesive protein receptors on epidermal cells.

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REAPPEARANCE OF CD1a ANTIGENIC SITES AFTER ENDOCYTOSIS ON HUMAN LANGERHANS CELLS EVIDENCED BY IMMUNOGOLDRELABELLING. A. Ray, D. Schmitt, C. Dezutter-Dambuyant and J. Thivolet. INSERM U.209, Pav.R, Hôp. E. Herriot, 69137 Lyon Cx 03.

It has been shown that CD1a antigen(Ag)/BL6 monoclonal antibody (MCA) complexes are internalized by receptor-mediated-endocytosis (Hanau et al., 1987). The present study has been conducted in order to investigate the intracellular pathway of the CD1a antigens after internalization.

Langerhans cell (LC) enriched epidermal cells (trypsinization and Ficoll-Hypaque density gradient centrifugation) were sensitized with BL6 at 4°C then BL6 was revealed with 15 nm gold granule-conjugated anti-mouse IgG at 4°C. The immunolabelled cells were incubated at 37°C for 1 h then a second BL6 immunolabelling was performed. In order to distinguish the second immunolabelling from the first one, some control experiments were undertaken (absence of second labelling). In some other experiments, cyclohexamide or monensin were added during the labelling and the one hour-incubation at the concentrations of 10 µg/ml and 50 nM, 100 nM, 25 µM, respectively.

Labelled endocytosis structures visualized internalization of CD1a Ag/BL6 MCA complexes. After a one hour-incubation of LC, CD1a antigenic sites were again evidenced on the cell membrane by a positive relabelling. The treatment of LC with cycloheximide, a synthesis inhibitor, modified neither the process of internalization nor the reappearance of CD1a antigenic sites. The cells treated with monensin, which has been found to raise the pH of the endocytic vesicles and the lysosomes, presented an accumulation of large vacuoles within the Golgi apparatus. Endocytosis and reappearance of CD1a antigenic sites were not altered by this drug.

These results suggest that the reappearance of CD1a sites after internalization is not due to a synthesis of these antigens. Since monensin does not affect this recovery of sites, it can be best explained by recycling of earlier internalized molecules or by expression of stored molecules not participating in recycling.

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BONE MARROW LANGERHANS CELL PRECURSORS: IN VITRO ULTRASTRUCTURAL AND PHENOTYPE ANALYSIS. A. de Fraissinette, C. Dezutter-Dambuyant, D. Schmitt, M.J. Staquet, J. Thivolet. Laboratoire de Recherche Dermatologique et Immunologie, INSERM U.209, Hôp. E. Herriot, Lyon, France.

The Langerhans cells (LC) are migratory bone marrow derived cells with distinctive intracytoplasmic Birbeck granules. In the epidermis, LC are antigen presenting cells and express CD1a, CD1c antigens and no CD1b antigen on the membrane. The human bone marrow origin of the LC was indirectly demonstrated after allogeneic bone marrow transplantation.

We have used a method of CFU-granulocyte macrophage cell culture in 0,8% methylcellulose in order to obtain an enrichment for T6 (CD1) positive cell subpopulation. After 8 days of culture, the bone marrow cells were analyzed by indirect immunofluorescence test, cytofluorometric analysis and quantitative immunogold labelling. The percentage of CD1 (T6) positive bone marrow cells increased from 1 % before culture to 8% after 8 days of culture. Cultured cells analyzed by cytofluorometry presented the following stained cell subpopulations: 9.4% CD1a (BL6), 13.4% CD1c (L161), 4.3% CD1b (NuT2), 4.6% CD2 (T11) and 25.5% CD33 (My9). Using 14 monoclonal antibodies, the phenotype of cultured cells was defined by electron microscopy on immunogold labelled cells. A cell subpopulation was shown to express surface markers of LC and promonocyte/monocytes. These cells expressed the following phenotype: CD1a+, CD1c+, CD14+, CD33+, CD4+, HLA-DR+, HLA-DP+, HLA-DQ- and showed the typical promonocyte/monocyte ultrastructural aspect. These data indicate that these bone marrow promocyte/monocyte progenitors express a phenotype similar to that of epidermal Langerhans cells but the density of each antigen is much lower than that observed on mature skin dendritic cells. T lymphocyte and thymocyte differentiation antigens (CD1b, CD2 and OKT10 antigens) were not expressed by such cells. Birbeck granules were not observed in all these CD1 positive cells.

This model of cultured bone marrow cells provides a useful tool for testing the role of the epidermal microenvironment on the CD1+ precursor differentiation.

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INCREASED EXPRESSION OF Fc RECEPTORS FOR MOUSE IgG1 (FcRII) ON LANGERHANS CELLS IN LESIONAL SKIN OF ATOPIC ECZEMA. Thomas Bieber, Daniel Hanau, Bernard Dannenberg, and Johannes Ring. Department of Dermatology, Ludwig-Maximilians University, Munich, West Germany, and INSERM U311, Strasbourg, France.

Recently, we have demonstrated that the 40 kD Fc receptor for mouse IgG1 (FcR11) is expressed on 50 to 55% of CD1a positive epidermal dendritic cells i.e. Langerhans cells (LC) and not on the so called indeterminate cells. Because of the low expression of this receptor on LC in normal skin, it is only detectable by a rosette assay with human red blood cells (HRBC) sensitized by a mouse IgG1 anti-glycophorin A MAB on epidermal cell suspension but not immunohistochemically on cryosections. Since the attention has been focused on a FcεR (receptor for IgE)-bearing sub-population of CD1a+ cells (i.e. LC) in atopic eczema suggesting that this disease could be a delayed-type hypersensitivity reaction and regarding to the major role of this type of receptor in the mechanism of CD3-induced T cell activation by monocytes, we investigated its expression by epidermal dendritic cells in this disease. Therefore, biopsies from normal skin of non atopic healthy volunteers and uninvolved as well as lesional skin of atopic eczema were studied immunohistochemically with CIKM5 (anti-FcRII MAB), IOT6 (anti-CD1a MAb) and M-E567 (anti-IgE MAb). In normal skin and uninvolved skin no reactivity could be detected with CIKM5. In lesional skin of some patients, numerous cells with dendritic morphology showed a strong positive pattern in the epidermis. On serial sections as well as on double labelling with anti-IgE or anti-CD1a, these cells corresponded to a subpopulation of CD1a+ cells i.e. LC. Furthermore, in the dermal compartment FcRII receptors seemed to be expressed by a high amount of infiltrating cells. Thus, these results suggest that like FcεR, FcRII expression on LC and infiltrating cells is increased in lesional skin of atopic eczema. Since the presumed physiological ligands of these receptors are human IgG-molecules, a possible role of the latter in the regulation of the pathophysiology of atopic eczema is of major interest. Furthermore, clinical and biological investigations of those patients in whom this increase has been observed seems to be important in order to better understand the mechanism leading to this phenomenon.

Tuesday, 21 June, 1988

8.00-10.30

Concurrent session – Holl

T. KRIEG, G. PIERARD, Chairpersons

Collagen (Abstracts 99-110)

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EFFECTS OF KETANSERIN, A SEROTONIN ANTAGONIST, ON HUMAN DERMAL FIBROBLASTS. Patrick Kellens, Charles Lambert, Marc Paye, Betty Nusgens and Charles M. Lapière. Laboratory of Experimental Dermatology, University of Liège, Belgium.

Ketanserin is a specific antagonist of serotonin at its S2 receptors. Besides its hypotensive properties it has a beneficial effect on wound healing in vivo. Our study was aimed at estimating the effects of Ketanserin on some cellular functions of human dermal fibroblasts in vitro as adhesion, multiplication and biosynthetic activity. 3H--thymidine incorporation assays and fluorimetric measurement of DNA demonstrated that, in presence of 10% fetal calf serum, Ketanserin had no effect on cell multiplication. Similarly, the attachment of fibroblasts to type I collagen and to fibronectin was not modified by Ketanserin. By metabolic labelling of the cells with 3H proline, a specific stimulation of collagen synthesis (160% of the control at a concentration of 10^{-5} M) but not of the non-collagen proteins was observed. The steady-state level of specific procollagen mRNAs was measured in human dermal fibroblasts cultured in monolayer in presence of various concentration of Ketanserin (10^{-7} to 10^{-5} M) for 72 h. Total RNA was extracted from the cells and purified according to the method of Chirgwin et al. (Biochemistry 18, 5294, 1979). RNA in equivalent amounts (from 0.25 to 2 µg) from non treated cells (three different strains of normal human skin fibroblasts) were slotted on nitrocellulose membranes and hybridized with ^{32}P labelled cDNA probes for COL1A1, COL1A2 and COL3A1 human genes. At 10^{-6} M Ketanserin, an increase in the steady-state level of the procollagen mRNAs of collagen types I and III was already detectable as early as after 5 hours of treatment. At 10^{-5} M, a two to seven fold increase was observed when compared to non treated cultures. Under similar conditions of culture serotonin (10^{-8} to 10^{-5} M) had no significant effect on the steady-state level of COL1A1. Our data indicate that Ketanserin acts at a pretranslational step by increasing the steady-state level of collagen m-RNA. The beneficial effect of Ketanserin in wound healing in vivo might be related to its stimulating activity on the differentiated phenotype of the fibroblasts.

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REGULATION OF PROTEIN SYNTHESIS IN FIBROBLASTS WITHIN A THREE-DIMENSIONAL COLLAGEN GEL. C. Mauch, A. Hatamochi, K. Scharffetter, Th. Krieg , Dermatologische Klinik der LMU München, FRG.

Fibroblasts in monolayer cultures represent a valuable model to study molecular defects in disorders of connective tissue. However, some of these studies are hampered by the fact that several biosynthetic capacities of fibroblasts are adapted to the artificial conditions and do not resemble the in vivo situation. We therefore cultivated fibroblasts within three-dimensional collagen gels which simulate more closely the in vivo situation. Fibroblasts embedded into these collagen gels stop dividing and adapt their morphology to a typical spindle-like shape. After a few hours cells start to contract the gel to a very dense tissue and modify their metabolic activity. Measurement of protein and mRNA levels revealed a marked reduction of protein and collagen synthesis. Quantification of mRNA levels using radioactively labeled cDNA probes specific for different proteins showed that in the collagen gel system fibroblasts may regulate several proteins differently. So, synthesis of type I and III collagen is concomitantly reduced to 5% of values found in monolayer culture whereas synthesis of type VI collagen is induced. Also for collagenase, a protease specific for interstitial collagens, a strong increase of synthesis could be detected. In addition cytoskeleton proteins displayed a differential regulation. Whereas actin mRNA was reduced during contraction of the gels, tubulin mRNA was not modified and could be found in equal amounts after contraction. These data suggest a specific reprogramming of various cellular activities in response to contact with the reconstituted extracellular matrix. Cultivation of fibroblasts within a threedimensional collagen gel therefore represents a valuable model to investigate inborn and acquired disorders of connective tissue which are due to disturbed control in the regulation of synthesis of extracellular matrix proteins.

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ISOMETRIC TRACTION DEVELOPED BY FIBROBLASTS IN A COLLAGEN GEL: A MODEL FOR PHARMACOLOGICAL STUDIES? Pierre Delvoye, Alain Colige and Charles M. Lapière Laboratory of Experimental Dermatology, University of Liège, Belgium.

Fibroblasts (F) seeded in a floating collagen gel immobilized at their extremities progressively develop forces on their supports (isometric traction). The force was measured by connecting the supports to strain gauges. The tension developed by increasing numbers (1 to 12.10^6) of skin F was measured for cells from normal human volunteers (3 strains), patients with Ehlers-Danlos type I (4 strains) and systemic scleroderma (5 strains). No significant difference was observed between the 3 groups and the intensity of the force varied from 0.2 to 1 g/10^6 cells and was highly reproducible for each strain. Cheloid F (1 strain) developed a significantly increased traction compared to normal F from the same patient, while dermatosparactic F (2 strains) displayed a reduced capacity to exert a mechanical traction compared to normal calf F. In all cases the force was directly related to the cell number and to the collagen concentration. Colchicine (2.10^{-6}, 6.10^{-6}, 10^{-5} M) and betamethasone dipropionate (5.10^{-6}, 10^{-5} M) inhibited in a dose related manner the force developed by normal human F. Epidermal growth factor (EGF ~50 ng/ml) inhibited by a factor 1.4 the maximal force with a lag time of 6 hours. Preincubation for 24 and 48 hours of F with EGF (50 ng/ml) in monolayer inhibited the initial traction respectively by a factor 1.3 and 4.3 and the maximal force by a factor 2.2 in both cases. These results suggest that isometric traction might be a useful technique for screening drugs that modify the interactions of cells with their connective support.

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INFLUENCE OF VARIOUS HUMAN TUMOR CELL LINES ON COLLAGEN SYNTHESIS BY NORMAL FIBROBLASTS. Agnès Noel *, Betty Nusgens *, Jean-Michel Foidart ** and Charles M. Lapière *. * Laboratory of experimental dermatology and ** Laboratory of foetoplacental physiopathology, University of Liège, Belgium.

During the process of primary or metastatic malignant growth, the interaction occuring between the tumor cells and the stroma cells can modulate their respective behaviour and functions. In this study, we have tested the effects of various tumor cell lines on collagen synthesis by normal human skin fibroblasts. Fibroblasts and tumor cells were cultured either separately (monoculture) or cocultured on plastic at a cell ratio 1:1 or cultured in the presence of conditioned medium. Collagen synthesis was determined by radiolabeling with 3H proline for 24 h and measurement of 3H-hydroxyproline. The level of transcription of the type I procollagen gene was estimated by measuring the steady-state level of mRNA for α1 and α2 polypeptides. Tumor cells of various origins were tested: B16 (melanoma cells), A431 cells (human epidermoid carcinoma), Bewo (human choriocarcinoma) and various human breast carcinoma cell lines either oestrogen-dependent (MCF 7, T47D) or independent (MDA, BT20, SA52). Among the strains of cells tested only three (MCF 7,, T47D and SA52) stimulated collagen biosynthesis by fibroblasts in coculture. In monoculture in the presence of conditioned medium by these tumoral cells, fibroblasts synthesized about 2 times more collagen than in presence of medium conditioned by other fibroblasts or of inactive epithelial cells. This enhancement of collagen synthesis was not paralleled by an increase in the steady-state level of procollagen type I mRNAs neither by a modification of the prolyl-hydroxylation. No correlation between oestrogen-dependency and collagen stimulation was observed. Treatment of tumor cells with 17-β oestradiol or an antioestrogen (Tamoxifen) did not influence collagen stimulation. These results demonstrated that tumoral epithelial cells are able to modulate the biosynthetic activity of the mesenchymal cells in the host tissue by a posttranscriptional mechanism.

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EFFECTS OF CYTOKINES ON SKIN FIBROBLAST PROLIFERATION. Ahmad M.E. Nouri, Irène M. Leigh, Jamiela Mangera and Hilliard Festenstein. Department of Dermatology and Immunology. The London Hospital. London. UK.

We have investigated the effects of a number of biological mediators on skin fibroblast proliferation in isolation or in concert. The cell proliferation was studied by measuring the degree of tritiated thymidine (3H-TdR) incorporation into cellular DNA using scintillation counter. The results (mean± 1 SD of three replicates from fibroblasts of 4 to 10 individuals) of our findings are as follows: a) IL-1 (1/2000 dilution of Genzyme purified), TNF (0.25 ng/ml) and EGF (10 ng/ml) increased the uptake of 3H-TdR from 2,022 ± 115, 1,639 ± 199 (p< 0.001), 2,500 ± 1,582 (p< 0.001) AND 2,873 ± 1,1417 (p< 0.006) cpm respectively. b) δ IF (500 u/ml) decreased the uptake from 2,020 ± 1,158 to 860 ± 630 (p< 0.002) cpm. Similar results were also obtained using α IF. c) EGF added to either IL-1- or TNF-activated fibroblasts increased the uptake from 2,106 ± 1,383 and 3,549 ± 1,439 to 3,241 ± 2,167 (p< 0.3) and 4,834 ± 2,051 (p< 0.25) cpm respectively. d) δ IF suppressed the uptake of both TNF- and IL-1-activated cells from 3,549 ± 1,401 and 2,518 ± 645 to 1,225 ± 934 (p< 0.08) and 1,719 ± 1,404 (p< 0.06) cpm respectively. e) supernatant from mitogen-activated human mononuclear cells, known to contain IL-1, suppressed fibroblasts proliferation from 2,051 ± 1,345 to 539 ± 318 (p< 0.06) cpm and this suppression could not be reversed by indomethacin, excluding the possibility of prostaglandin involvement as a suppressive agent in this system.

These results demonstrate that: a) many biological mediators influence fibroblast proliferation and b) some of these mediators which might be present at site of immunological reactions may act in concert, as seen in the case of our active supernatant. The ability of these biological mediators to stimulate fibroblast proliferation may have important implications in the process of wound healing and tissue remodeling.

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REGULATION OF COLLAGEN VI SYNTHESIS: DEPENDENCE ON CELL DENSITY AND FOLLOWING CHEMICAL TRANSFORMATION. A. Hatamochi[1], M. Aumailley[2], M.L. Chu[3], R. Timpl[2] and T. Krieg[1]. Dermatologische Klinik der LMU München[1] and MPI für Biochemie, Martinsried, FRG[2]; Jefferson Medical College, Philadelphia, USA[3].

Human skin fibroblasts synthesize mainly the interstitial collagens I and III, but produce also collagen VI, which serves as a cell attachment protein. Several in vitro studies have indicated that fibroblast proliferation and regulation of synthesis of the interstitial collagen are closely linked. In order to obtain more information concerning the biological role of collagen VI we present here data demonstrating the dependence of gene expression of various collagen chains on cellular proliferation in two different in vitro systems. cDNA clones were used to measure the amount of mRNA specific for the various collagen chains in skin fibroblasts grown in monolayer culture either in different densities or after transformation by treatment with TPA. Total RNAs were prepared by the guanidinium isothiocyanate method and used the dot blot and northern blot analysis. The blots were hybridized with 32P-labeled α1 (I), α1 (III), α1 (VI), α2 (VI) and α3 (VI) collagen cDNAs. β-actin cDNA was used as control. Synthesized proteins were quantified by dot blotting the medium and extracts from the cell layer on nitrocellulose filters, which were then incubated with specific antibodies. Collagen VI synthesis was found to be induced both on the mRNA and protein levels when the cells reached confluency and stopped dividing. All different collagen VI chains were simultaneously regulated. TPA treatment of fibroblasts resulted in a sharp decrease in both collagen I and III mRNA levels. However, no changes were observed for type VI collagen mRNA. These data support previous results indicating that the gene expression of collagen I and III are concomitantly regulated, whereas type VI collagen chains underlie other control mechanisms, which is in agreement with the assumption that the biological function of these collagens varies considerably.

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DIFFERENTIAL EXPRESSION OF COLLAGEN I AND VI FOLOWING GAMMA-INTERFERON TREATMENT OF HUMAN SKIN FIBROBLASTS. M. Heckmann[1], A. Hatamochi[1], M. Aumailley[2], M.L. Chu[3], R. Timpl[2], T. Krieg[1]. Dermatologische Klinik der LMU München[1] and MPI für Biochemie[2], Martinsried, FRG; Jefferson Medical College[3], Philadelphia, USA.

Collagen, the major structural glycoprotein, is present in seven different types in the dermis. An exact regulation of gene expression of the various collagens is therefore required to guarantee the physiological functions of the dermis during remodelling, inflammation and repair processes. Several mediators are known to specifically influence the biosynthesis of the interstitial collagens I and III. Recently, however, a new collagenous protein has been isolated and biochemically characterized (Collagen VI) which is composed of three different chains (α1, α2, α3). Since gamma-interferon has been shown to interfere with the metabolism of fibroblasts and to reduce mRNA levels of collagen I, we here report on its influence on the expression of collagen VI. Human fibroblasts were incubated for 24, 48 and 72 hours with recombinant gamma-interferon, ranging from 0.01-1000 ng/ml. Total RNAs were isolated from the cells and transferred to nitrocellulose paper, followed by hybridization with 32P-labelled cDNAs. The results of dot blot analysis and Northern blotting were quantified by densitometry. In addition the amount of synthesized proteins in the medium and in extracts from the cell layers were estimated using specific antibodies against collagen VI in serial dilutions. For the α3 chain of collagen VI as well as for α1 (I) and α1 (III) a dose dependent decrease in mRNA was found, starting at concentrations of 10 ng/ml. There was no change, however, in the expression of the α1 and α2 chain of collagen VI, nor β-Actin at concentrations up to 1000 ng/ml. These results demonstrate a selective effect of gamma-interferon on the mRNA expression of the different collagens and further indicate that even the various collagen VI chains are regulated individually.

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DETECTION OF VITRONECTIN (S-PROTEIN OF COMPLEMENT) ON DERMAL ELASTIC FIBRES IN ADULTS BUT NOT IN THE YOUNG. ITS PRESENCE IN ABNORMAL PROTEIN DEPOSITS IN SELECTED SKIN DISEASES. Karin Dahlbäck, Helge Löfberg, and Björn Dahlbäck, Departments of Dermatology and Pathology, Lund Hospital, and Department of Clinical Chemistry, Malmö General Hospital, University of Lund, Lund, Sweden.

Vitronectin, identical to serum spreading factor, epibolin and S-protein of complement, is a multifunctional glycoprotein, present both in plasma and tissue. It functions as an inhibitor to the membrane attack complex of complement and has regulatory effects in the coagulation cascade. In addition, it has potent stimulatory effects on cell adhesion and spreading, like the more well-known matrix protein, fibronectin. Specimens of non-lesional skin from persons of different ages were investigated with monoclonal and polyclonal antivitronectin antibodies with an avidin-biotin-peroxidase complex technique. In addition, skin biopsies from patients with solar elastosis, pseudoxanthoma elasticum, primary localised cutaneous amyloidosis, lichen ruber planus, erythropoietic porphyria and porphyria cutanea tarda were studied. Strong vitronectin immunoreactivity was found in conjunction with the elastic fibre network, including the oxytalan fibres, in normal adult skin but not in skin specimens from young cases. It was also found in association with the pathological deposits in cases with elastosis and pseudoxanthoma elasticum and in conjunction with cutaneous amyloid, globular bodies in lichen ruber and with PAS-positive deposits of dermal vessel walls in porphyria. The pattern of vitronectin immunoreactivity in adult normal skin corresponded to that of serum amyloid P component and fibrillin, suggesting association of vitronectin with elastin-associated microfibrils. The findings indicate an age-related deposition of vitronectin on dermal elastic fibres, providing a substratum for migrating cells with vitronectin surface receptors. Furthermore, the findings of vitronectin immunoreactivity in association with abnormal protein deposits in several cutaneous diseases is a basis for further studies on its role in the pathogenesis of these diseases.

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AMYLOID P COMPONENT IS NON-COVALENTLY ASSOCIATED WITH ELASTIC TISSUE MICROFIBRILS IN NORMAL HUMAN DERMIS. Stephen M. Breathnach, Julie Booker, John Ashworth, Mark B. Pepys* and Helmut Hintner. Department of Medicine (Dermatology), Charing Cross and Westminster Medical School, London, U.K. and *Immunological Medicine Unit, Royal Postgraduate Medical School, London, U.K.

Tissue amyloid P component (TAP), a glycoprotein which crossreacts immuno-histochemically with the normal plasma protein serum amyloid P component (SAP), is invariably associated with elastic fiber microfibrils in normal adult humans. TAP on elastic fibers, through its binding affinity for fibronectin and glycosaminoglycans, may play a role in the maintenance of connective tissue architecture and dermo-epidermal adhesion. We have investigated the biochemical nature of the association between TAP and elastic fiber microfibrils in normal adult human dermis. Aliquots of minced, homogenised dermis, obtained following ethylenediamine tetraacetic acid (EDTA) separation of whole skin, were extracted with different reagents, and the presence or absence of SAP in the residual pellet and in the supernatant following centrifugation was determined by SDS-PAGE and immunoblotting using anti-SAP antibodies.

TAP was extractable from dermis using reagents which disrupt non-covalent bonds, including 0.1% sodium dodecyl sulfate (SDS) and 6M guanidine hydrochloride. TAP was not extracted by 2M NaCl solution, 2M hydroxylammonium chloride, the non-ionic detergents 1% Triton X-100 and 1% Nonidet P-40, phosphate buffered saline, or the reducing agents 0.01M dithiothreitol and 5% 2-mercaptoethanol. 0.01M EDTA solution was similarly unsuccessful at eluting TAP from the dermal preparation, indicating that the association of TAP with elastic fiber microfibrils is not simply the result of Ca^{++}-dependent binding. Although 1% collagenase alone resulted in solubilisation of some TAP, this fact does not prove covalent linkage to elastic tissue of part of the TAP, since the apparent M_r of TAP extracted, when run in reduced SDS-PAGE, was the same as that of normal SAP subunits. Although we cannot completely exclude the possibility that a few of the subunits in each multimeric TAP molecule are covalently attached, release of most of the TAP by SDS, and virtually all of it by 6M guanidine, indicates that the majority of the TAP molecules in the dermis are non-covalently bound to the microfibrils. Thus TAP would appear not to be an integral constituent of elastic fiber microfibrils.

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CHARACTERIZATION OF THE CHANGES IN MATRIX MOLECULES AT THE DERMOEPIDERMAL JUNCTION IN LUPUS ERYTHEMATOSUS. E. Mooney, W.R. Gammon and J.C. Jennette, Department of Dermatology, Faculty of Medicine, University of Iceland, Reykjavik, Iceland and Departments of Dermatology and Pathology, University of North Carolina School of Medicine, Chapel Hill, NC, USA.

Electron microscopy has revealed that the deposition of immunoglobulin in the skin of lupus erythematosus (LE) patients occurs on and below the basal lamina of the basement membrane (BM). Furthermore, a reduplication of the basal lamina is seen in the lesional skin. Since the composition of the BM is now, to some extent, known and antibodies have been developed against its individual components, a study was performed to elucidate the status of matrix molecules at the dermoepidermal junction in LE. Lesional and nonlesional skin of 37 LE patients, diagnosed according to clinical, histological and immunohistological criteria, was examined using indirect immunofluorescence with monoclonal antibodies against type VII collagen, type IV collagen, laminin, type V collagen and fibronectin. A polyclonal antibody was used against bullous pemphigoid antigen. Immunoelectron microscopy (IEM), using monoclonal antibodies was used to discern changes in type IV collagen and type VII collagen in lesional and nonlesional skin of 4 LE patients. In the 37 patients whose skin was examined by indirect immunofluorescence, type IV collagen and type VII collagen, as well as fibronectin, were altered in the BM of lesional skin (statistical significance at p 0.001 - p 0.02 by chi square analysis). There was also a statistically significant correlation between the presence of immunoglobulin and alteration of type IV collagen and type VII collagen in lesional skin (p less than 0.001 by chi square analysis). The changes in type IV collagen and type VII collagen were confirmed by IEM which showed nonlinearity of staining in the form of fragmentation of both antigenic components as well as reduplication of type VII collagen. In some instances stained fragments were seen below the BM. The results show that there is disruption of type IV collagen and type VII collagen in and below the basal lamina of lesional skin of LE patients, which is in the area corresponding to that of immune complex deposition.

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QUALITATIVE AND QUANTITATIVE EVALUATION OF COLLAGEN mRNA DURING PRIMARY CUTANEOUS WOUND HEALING BY IN SITU HYBRIDIZATION AND IMAGE ANALYSIS. K. Scharffetter, W. Stolz, B. Lankat-Buttgereit, M. Kulozik, F. Lobkowicz, Th. Krieg. Dermatologische Klinik der LMU München, FRG.

In situ hybridization has proved to be a useful technique to study collagen biosynthesis on a cellular level. However, a quantitative evaluation of specifically localized grains by visual estimation often appears to be rather unprecise. For this reason we have combined application of in situ hybridization technique using riboprobes of specific cDNA clones for α_1 (I) and α_1 (III) and image analysis system in order to evaluate intensity and localization of collagen gene expression during primary wound healing. For this purpose melanoma reexcision biopsies at different time intervals after first surgical intervention have been used. Following in situ hybridization and autoradiography the number of grains within 100 cells per section have been determined by image analysis (IPS, Kontron) and after substraction of background labelling have been plotted as histograms. Our results clearly indicate that synthesis for type I and type III collagen was concomitantly regulated on a pretranslational level. In early phases of primary wound healing restoration processes start within the reticular layers of the dermis while in later phases biosynthetic activity of cells within the papillary dermis was dramatically increased. In addition cells within the media and adventitia of small vessels participate in repair processes producing large amounts of both collagens. Since smooth muscle cells are known to produce mainly type III collagen, wound healing represents a physiological condition which potentially modulates gene expression pattern of these cells. In this study combination of in situ hybridization with image analysis has turned out to be valuable in quantitating different grain densities.

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LEVELS OF EXPRESSION OF α 2 (I) GENE IN HUMAN TRISOMY 7. A. Delvig *, Charles Lambert **, Marc Lacroix **, Alain Colige **, Betty Nusgens ** and Charles M. Lapière **. * Institute of Medical Enzymology, Moscow, USSR and ** Laboratory of experimental Dermatology, University of Liège, Belgium.

The gene coding for the human α 2 (I) collagen polypeptide is located on chromosome 7 while the gene coding for α 1 (I) is on chromosome 17. Trisomy 7 is a human genetic aberration resulting in spontaneous abortion. Our study aimed at analyzing the expression of the type I collagen genes at the level of their m-RNAs and their translation products in human fibroblasts derived from spontaneously aborted embryos carrying an extrachromosome 7 (Tri 7) by comparison with normal diploid fetal fibroblasts (N). The presence of an extragene for α 2 (I) chain was checked by karyotype and by slot blot and Southern blot performed on purified DNA from 2 N and 1 Tri 7 strains and hybridized with specific c-DNA probes. The Tri 7 contained 1.6 times more DNA coding for α 2 (I) than for α 1 (I) as determined by slot blot and 2 times more from the Southern blot data. The RNAs were extracted according to the technique of Chirgwin et al. (1979), blotted on nitrocellulose, hybridized with the specific c-DNA probes for α 1 (I) and α 2 (I) and the autoradiogramms were scanned. When the cells were cultured in presence of 10% FCS the ratios α 1 (I) m-RNA/ α 2 (I) m-RNA were 1.84 and 1.81 for the N strains and 1.84 for the Tri 7 fibroblasts. Similar informations were obtained at the protein synthesis level in which the α 1 (I) chains were two times more abundant than the α 2 (I) polypeptide in the three strains. In a medium at low concentration of FCS (0.1%) collagen synthesis of Tri 7 was repressed (-72%) and the steady-state level of m-RNAs for procollagen type I was parallely decreased as compared to N strains. The ratio of the α 1 (I) m-RNA/α 2 (I) m-RNA was 0.8 for the Tri 7 and 3.8 for the N strain. The addition of EGF to Tri 7 cells maintained in low FCS resulted in an increase of the steady-state level of procollagen m-RNAs. No such stimulation was observed for N strains. These results suggest that in Tri 7 the control of the expression of α 2 (I) chain by EGF is different from that in N fibroblasts.

Tuesday, 21 June, 1988

10.45-11.45

Plenary session – Cuvilliés

O. BRAUN-FALCO, Chairperson (Abstracts 111-114)

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IDENTIFICATION OF A NOVEL FAMILY OF HIGHLY POTENT NEUTROPHIL CHEMOTACTIC PEPTIDES IN PSORIATIC SCALES. Jens-M. Schröder, and Enno Christophers, Department of Dermatology, University of Kiel, FRG.

We recently found an anionic neutrophil activating peptide (ANAP) in psoriatic scale extracts, which demonstrated some biochemical similarities to IL-1 or ETAF. More recent studies, however, revealed that IL-1 is not a chemotaxin for neutrophils in vitro. Therefore we tried to purify ANAP to apparent homogeneity: Acidic extracts of psoriatic scales were applied to cation-exchange HPLC and fractions tested for biological activities. Two broad peaks of neutrophil stimulating activity (chemotaxis, degranulation) were detected. Both crude chemotactic factors (α- and β-ANAP) were further purified by subsequently performed wide pore RP-8-HPLC, size exclusion-HPLC, wide pore RP-18-HPLC, reversed phase Poly-F-HPLC and finally by narrow pore RP-18-HPLC. As a result we obtained at least four distinct and apparently homogeneous polypeptides we termed α_1-ANAP, α_2-ANAP, α_3-ANAP and β-ANAP, all demonstrating a single line in sodium dodecylsulfate electrophoresis at 7 kD, 6.5 kD, 10 kD, and 15 kD, respectively. All of these polypeptides exhibited chemotactic as well as degranulating activity in human PMN. Moreover, none of these peptides demonstrated IL-1 activity indicating nonidentity with these IL-1 related cytokines. In addition the ANAP fractions showed cross-reactivity in the densitization-assay using human neutrophils with the novel monocyte derived neutrophil activating peptide MONAP, we recently purified to homogeneity (J. Immunol. 129, 3474, 1987), not however with well characterized chemotaxins like C5a, FMLP, PAF or LTB_4. Moreover one of these newly detected peptides, α_3-ANAPm which is one of the major ANAP-peptides, shows strong similarities to MONAP by retention time in RP-18-HPLC as well as SDS-PAGE. The presence of large amounts of these novel chemotaxins in psoriatic scale material makes it attractive to speculate about its role in neutrophil accumulation in the psoriatic epidermis.

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ENDOTHELIAL CELLS OF UNINVOLVED AND INVOLVED PSORIATIC SKIN DISPLAY AN ANGIOGENIC PHENOTYPE NOT INDUCED BY b FGF, ANGIOGENIN, HAF OR HEPARIN OR HUVE CELLS. H.H. Hagemeier, S. Goerdt, C. Sorg and E. Macher, Center of Dermatology, University of Münster, D-4400 Münster, FRG.

Psorasis is an example of an inflammatory disease in which the microvessels play an active role. The capillary loops in the dermal papillae have been observed to become dilated and tortuous, before epidermal hyperplasia has been detected morphologically. The purpose of our study was to investigate angiogenic properties of psoriatic endothelial cells. Therefore, monoclonal antibody EN7/44 (1) reacting with endothelial cells of budding vessels in tumors, inflammatory tissues and wound healing was used. Immunohistological studies with EN7/44 were performed on: a) cryostat sections of uninvolved and involved psoriatic skin lesions before and after Dithranol treatment, b) human umbilical vein endothelial cells (HUVEC) stimulated with either basic fibroblast growth factor (b FGF), angiogenin, human angiogenic factor (HAF), heparin or medium as control over 24 h. In contrast to normal skin of control persons which are negative for the EN7/44 antigen, capillary loops and larger vessels of uninvolved psoriatic skin continuously display the EN7/44 phenotype. Furthermore, in the psoriatic plaque the capillary loops of the upper plexus elongate and their endothelial cells become strongly positive for the EN7/44 phenotype. Endothelial buds and new vessel formation like in tumor angiogenesis have never been detected. After Dithranol treatment the capillary loops shorten and the endothelial cells become nearly negative for the EN7/44 antigen. Neither b FGF, angiogenin, HAF nor heparin could stimulate the expression of the EN7/44 phenotype on HUVEC. Recently, we could demonstrate that expression of the EN7/44 antigen reflects a transient feature in the multistep process of angiogenesis in tumors and wound healing. In contrast, psoriatic EC continuously display the EN7/44 phenotype. Expression of this phenotype could not be stimulated by well known angiogenic factors on HUVEC but could be down-regulated by the calmodulin antagonist Dithranol. It is emphasized that psoriasis could be an altered or prolonged form of wound healing with loss of any angiostatic properties leading to hyperproliferation of keratinocytes.

1) Hagemeier HH., Vollmer E., Goerdt S., Schulze Osthoff K., Sorg C. Int.J.Cancer 38, 481-488 (1986).

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EXTRATHYMIC MIGRATION OF THY-1+DEC-PRECURSORS TO THE EPIDERMIS. Michio Honjo, Adelheid Elbe, Georg Steiner, Irene Assmann, Klaus Wolff, Georg Stingl. Division of Immunobiology, Department of Dermatology I., University of Vienna, Vienna, Austria.

Thy-1+dendritic epidermal cells (Thy-1+DEC) and immature thymocytes share several phenotypic features: CD45+, Thy-1+, asialo-GM1+, CD3+, CD4-, CD8-. In view of this similarity, it has been suggested that the epidermis may be a site of either postthymic or extrathymic T cell development. In order to address this issue, we used AKR (Thy-1.1) --- C3H/He (Thy-1.2) radiation bone marrow chimeras. Animals were first either thymectomized or sham-thymectomized, then lethally irradiated (700 R) and, finally, reconstituted with allogeneic bone marrow cells which had been previously depleted of Thy-1-bearing cells.

Six weeks after bone marrow transplantation, peripheral lymphoid organs in sham-treated animals, but not in thymectomized animals, contained large numbers of CD3+ donor-type Thy-1+ cells. Importantly, however, the ear epidermis of both thymectomized and sham-treated animals contained not only many recipient-type CD3+, Thy-1+DEC, but also small numbers of CD3-, donor-type Thy-1+ cells. After 4 months, the frequency of donor cells had greatly increased, but they still lacked CD3 antigens. Most of the donor cells had a rounded shape, but some exhibited a dendritic configuration.

These results demonstrate that Thy-1- bone marrow-derived precursors of Thy-1+ DEC can migrate to the epidermis without thymic influence and yet acquire Thy-1 antigens during their journey. The failure of donor-type Thy-1+ cells to express CD3 antigens even in sham-treated animals with CD3+ donor-type Thy-1+ cells in peripheral lymph node organs may be due to radiation-induced injury of the epidermis and supports the concept that signals inducing maturational events in Thy-1+DEC are derived from non-thymic epithelia.

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EXPRESSION OF THE LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN 1 (LFA-1) GLYCOPROTEIN FAMILY ON HUMAN EPIDERMAL LANGERHANS CELLS. Yael Gothelf, Daniel Hanau, Didier A. Schmitt, Jean-Pierre Cazenave and Ephraim Gazit. Tissue Typing Laboratory, Chaim Sheba Medical Center, Tel-Hashomer, Israel; INSERM U.311, Centre Régional de Transfusion Sanguine, Strasbourg, France.

The LFA-1 family is a group of three glycoproteins (LFA-1, Mac-1, p150,95) which function in cell-substrate and cell-cell adhesion reactions of myeloid and lymphoid cells. These glycoproteins are noncovalently linked αβ heterodimers that have distinct α chains but share a common β chain of 95 kD. These glycoproteins can be defined by monoclonal antibodies (MAb) specific either for the unique α subunits or for the common β chain. The expression of these three glycoproteins on the surface of human epidermal Langerhans cells (LC) was studied using MAb of the 3rd International Workshop on Human Leukocyte Differentiation Antigens. CD18 MAb MHM23 and 60.3 identify the common β chain of the LFA-1 family, CD11a MAb MHM24 and CIMT define the LFA-1 α chain (p180,95), CD11b MAb Mol and 44 define the Mac-1 α chain (p165,95) and the CD11c MAb KB23, 3.9, Ki-M1 and Bu15 define the "X" α chain (p150,95). Double labelling immunofluorescence studies of human epidermal cell suspensions indicated that 35.3±4.9% of T6 positive cells react with the MHM23 MAb and 23.9±5.5% of T6 positive cells react with the 60.3 MAb, which both identify the common β subunit of the LFA-1 family. None of the MAb of the CD11 group specific for the α subunits of Mac-1, LFA-1 and p150,95 stained T6 positive cells. Electron microscope studies show that gold-labeled CD18 MAb binds to LC. Taken together, these results show that epidermal LC express the common β subunit of the LFA-1 glycoprotein family and do not possess any of the unique α subunits of this group, at least the epitopes recognized by the MAb used in this study. The common β chain seems to be expressed on all leukocyte types, while the expression of the LFA-1 antigen precedes that of Mac-1 and p150,95 along myeloid differentiation pathways. The absence of these glycoproteins from the surface of epidermal LC might add further evidence to the fact that freshly isolated LC are in fact immature cells.

Tuesday, 21 June, 1988

15.00-16.00

Concurrent session – Cuvilliés

A. GIANNETTI, Chairperson
Atopic Dermatitis (Abstracts 115-119)

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IN VITRO IgE ELUATION AND HISTAMINE RELEASABILITY FROM PERIPHERAL LEUKOCYTES OF ATOPICS AND NORMALS. Franz Sedlmeier, Walter Dorsch and Johannes Ring. Dermatologische Klinik, Kinderpoliklinik, Ludwig-Maximilian University, Munich, W. Germany.

Releasability has been described as non-immunological parameter influencing the quantity of mediator substances released after specific or non-specific stimulation. Altered patterns of histamine releasability have been reported in patients with atopic eczema. Here we investigated the correlation between the amount of cell-bound IgE and histamine releasability. Peripheral leukocytes from 28 patients with atopic diseases and 16 non-atopic controls were stimulated in vitro with anti-IgE and histamine release (HR) was determined spectrofluorometrically. Concomitantly the cell-bound IgE eluted in acid buffer was measured in a separate experiment and the number of IgE molecules per basophil as well as the amount of HR by acid treatment was calculated. Acetate buffer (pH 3.7) induced significantly higher HR (32 net %) in atopics compared to 18% in controls. The amount of IgE eluted by acid treatment was significantly higher (6.9 ng/10^6 cells) compared to normals (1.0 ng/10^6 cells). The calculated number of IgE molecules/basophil was 332.000 in atopics compared to 177.000 in controls. There was a significant positive correlation between plasma IgE and in vitro eluable IgE in atopics ($r = 0.73$) with values of $r = 0.24$ in controls. After careful washing procedures it was tried to "resensitize" the basophils through incubation with autologous plasma. When resensitization was possible, there was no correlation between histamine releasability after "resensitization" and original IgE-content of basophils. It is concluded that the increased histamine releasability from leukocytes of atopics after stimulation with anti-IgE may partly be due to the increased number of IgE molecules per basophil surface. Apart from this specific factor a non-specific increased releasability can be demonstrated by increased HR after acid buffer treatment. The data support the concept of "altered releasability" being one possible factor in the pathogenesis of atopic eczema.

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IN VITRO HISTAMINE INDUCED ELEVATION OF PHOSPHODIESTERASE ACTIVITY IN MONONUCLEAR LEUCOCYTES FROM ATOPIC AND NON ATOPIC SUBJECTS. C.A. Holden, I.H. Coulson, C.T. Yuen. St Helier Hospital, Wrythe Lane, Carshalton, Surrey, SM5 1AA England.

Increased phosphodiesterase activity (PDE) is present in mononuclear leucocytes (MNL) from patients with atopic dermatitis (AD). A similar elevation of MNL PDE activity can be induced in vitro by exposing leucocytes to 1µM histamine. It is unknown whether atopic MNL PDE activity responds to histamine stimulation in a similar way to normal MNL.

We prepared MNL from 21 patients with AD who had used emollients only for one week, 18 patients treated with topical fluorinated steroids and 16 non atopic normal subjects. The MNL were suspended at 4×10^6 cells/ml Geys balanced salt solution (GBSS) and 1µM histamine was added to half the cells. The MNL were incubated for one hour at 37°C with 5% CO_2 in a humidity chamber. Subsequently the cells were washed, resuspended in GBSS at 1×10^6 cells/ml and PDE activity was measured using a radioenzyme assay. Results are expressed as pmol/min/mg protein ± SEM. Statistical analysis was performed using Student's t-test for paired or unpaired means.

	Effect of 1µM Histamine on MNL PDE activity		
	Normal (16)	Untreated AD (21)	Treated AD (18)
Unstimulated	7.2 ± 0.34	8.3 ± 0.33	6.6 ± 0.37
Histamine (1µM)	7.6 ± 0.38	8.9 ± 0.40	6.7 ± 0.40

Unstimulated PDE activity was significantly higher in untreated AD patients than in normal subjects or treated patients ($p < 0.01$). A small but significant increase was induced by histamine in normal subjects and untreated patients ($p < 0.01$ and $p < 0.003$ respectively). No change was seen upon histamine exposure of treated patients'MNL.

The data implies that PDE activity shows a similar sensitivity to histamine exposure in both AD patients and normal subjects. However, the response to histamine may be blocked by the use of topical steroids and suggests that elevated PDE activity seen in AD may be secondary to cutaneous inflammation.

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EXPRESSION OF HLA-DR, CD1a AND IGE-RECEPTOR-MOLECULES BY KERATINOCYTES IN LESIONAL SKIN OF ATOPIC ECZEMA. Bernard Dannenberg, Thomas Bieber and Johannes Ring, Department of Dermatology, Ludwig-Maximillians University, Munich, West Germany.

Recently, positive intercellular staining patterns with anti-CD1a-antibodies (T6) have been observed in various diseases but so far not in atopic eczema. In order to investigate this topic, serial cryosections from normal appearing and lesional skin from patients with atopic eczema were studied immunohistochemically with anti-CD1a, anti-HLA-DR, anti-IgE antisera and the APAAP-method as well as by double immunofluorescence. The patients were selected on the basis of a positive staining with anti-IgE on dendritic epidermal cells in lesional skin. Thus, a weak CD1a+ intercellular pattern was observed in uninvolved skin in the majority of the patients. Intercellular HLA-DR+/CD1a+ reactivity was mainly observed around the basal keratinocytes in acanthotic areas whereas a HLA-DR+/CD1a+/IgE+ network was shown in lesional skin only in some of the patients. This last keratinocyte-pattern was predominantly localized in those areas characterized by lymphocytic exocytosis, spongiosis or vesicle formation. The pattern remained after serial dilution of the primary antibody and the control of binding-specificity for IgE was obtained by prior removal of cell-bound IgE with glycine HCl buffer followed by incubation with autologous serum. There was no correlation between the presence of an intercellular pattern positive for IgE and IgE-serum levels. The results suggest that the intercellular pattern is due either to a shedding of CD1a and IgE molecules or to an expression of CD1a and IgE-receptor (FcεR) by keratinocytes in this condition. It was proven that HLA-DR-expression can, in certain circumstances, be induced on keratinocytes through the action of gamma-interferon. On the other hand, it has been demonstrated that IL-1 induces CD1a-expression on certain epithelial cells and IL-4 induces FcεR on B cells. Therefore, a possible synthesis of CD1a and/or FcεR-molecules by keratinocytes in lesional skin of atopic eczema or in other inflammatory conditions cannot be totally excluded. Further investigations using epidermal cell cultures under various conditions may give more informations to answer this question.

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CUTANEOUS REACTIONS TO INTRADERMAL SUBSTANCE P AND HISTAMINE IN ATOPIC DERMATITIS. I.H. Coulson and C.A. Holden, Skin Laboratory, St Helier Hospital, Carshalton, Surrey, SM5 1AA England.

Patients with atopic dermatitis (AD) may develop stress related immediate cutaneous erythema and itching possibly due to local release of neurotransmitters. Substance P (SP) is present in dermal nerve fibres and in free nerve endings in the epidermis; its intradermal injection causes similar immediate reactions due to the release of histamine from dermal mast cells. To investigate the possible role of SP in exacerbations of AD we compared the flare and wheal reactions in a group of 13 AD patients and 12 non atopic controls.

Flare area and wheal volume were measured after 50 µl intradermal injections of SP and histamine into non eczematous forearm skin. All injections were made up in phosphate buffered saline containing 0.5 gl^{-1} bovine serum albumin as a carrier for the SP. Dose response curves were determined with 10, 100 and 200 pmols SP and 0.08, 0.16 and 1.6 nmols of histamine.

Three AD patients developed reaginic reaction wheals to the bovine serum albumin in the injection diluent and were excluded from further study. No difference in flare area was found between AD patients and controls with both SP and histamine. Wheal volumes with both SP and histamine in the AD patients were almost double those of the control group at all doses tested (p less than 0.05 at all doses - see table).

These results show that the increased wheal volumes produced by SP may be entirely due to an increased vascular permeability coused by SP induced histamine release.

SP dose pmol	10	100	200
Wheal vol µl			
AD	96.1	382	562
Control	45.7	132	299
P	0.05	0.01	0.01

H dose nmol	0.08	0.16	1.6
Wheal vol µl			
AD	113	256	551
Control	53.3	125	375
p	0.02	0.02	0.02

Tuesday, 21 June, 1988

15.00-16.00

Concurrent session – Effner

H. WOLFF, T. REUNALA, Chairpersons
Bullous Diseases (Abstracts 120-124)

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STRATUM CORNEUM LIPIDS IN PATIENTS WITH ATOPIC DERMATITIS, Sjan Lavrijsen, Maria Ponec, Johan Kuyvenhoven, Don de Winter, Arij Weerheim and Jan Gerrit van der Schroeff, Department of Dermatology, University Hospital Leiden, The Netherlands.

Patients with atopic dermatitis (AD) usually have dry skin combined with a defect in the water barrier function of the stratum corneum. This is associated with a reduced water binding capacity and a higher transepidermal water loss. In AD very little is known about the intercellular stratum corneum lipids (SCL) which are thought to be essential for the water barrier function.

SCL were analyzed in non-eczematous skin of 11 patients with AD and the lipid composition was compared with SCL of 10 atopic persons without eczema and of 30 healthy volunteers, aged 20-85 years (control group). The sebaceous lipids were extracted by a 5 minute application of 1:1 acetone/ether solution in a metal cylinder placed on the flexure side of the forearm. Subsequently, SCL fractions were collected in a similar way during 25 min. The extracted lipids were separated using a one-dimensional thin-layer chromatography system (TLC). Two developing systems were used which enabled the separation of lipids into 15 different fractions: cholesterol sulfate (CSO_4), 5 different ceramides (CER) including acylceramide (AC), monoglycerides (MG), free fatty acids (FFA), free cholesterol (FC), 1,2- and 1,3-diglycerides (DG), lanosterol (LAN), triglycerides (TG) and cholesterol esters (CE). For quantitative determination the separated lipids were charred and the chromatograms were scanned using a photodensitometer.

Significantly higher values for 1,2-DG, CSO_4, all CER and lower values for CE, FFA and FC were found in the AD group compared with the group of atopic individuals without eczema and the age matched control group. In the control group we found with increasing age a significant decrease of one of the CER, AC and FC and an increase in LAN which was quantitatively not related to the observed decrease of FC.

These data reveal differences between SCL composition of non-eczematous AD skin and normal skin, which might play a role in the dry appearance and reduced water binding capacity of AD skin.

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IgA SUB-CLASS IN CHRONIC BULLOUS DERMATOSIS OF CHILDHOOD. B.S. Bhogal*, F. Wojnarowska +, S. Kelly*, M.M. Black*, * Institute of Dermatology, London, WC2, + The Slade Hospital, Oxford.

Chronic bullous dermatosis of childhood (CBDC) is a rare blistering condition characterised by linear IgA deposits in the cutaneous basement membrane zone (BMZ) and the presence of circulating IgA anti-BMZ antibodies in the serum. The origin of the IgA in CBDC is unknown. In adult linear IgA disease (LAD) the IgA deposits are predominently IgA1 and lack J-chains and secretory piece (1).

Two major sub-classes of IgA have been identified. IgA1, which comprises 80-90% of total serum IgA is of bone marrow origin and exists in a monomeric form. IgA2 is derived from the gut associated lymphoid tissue and is mainly polymeric (2).

Employing monoclonal antibodies against IgA1 and IgA2 (NORDIC) in both indirect and direct immunofluorescence technique we studied the distribution of the IgA sub-classes in the skin and the sera of 23 patients with CBDC. Biopsies and sera from five adult LAD patients were used as positive controls. Skin and sera from five normal volunteers were used as negative controls.

Linear deposits of IgA were present at the BMZ in skin of all 23 patients studied. Sub-class analysis showed this to be universally IgA1. IgA2 was not detected. Similarly circulating IgA anti-BMZ antibodies which were present in 18/23 sera examined were also of IgA1 class and IgA2 was not detected.

Thus the IgA in skin and sera in CBDC is IgA1 as it is in adult LAD. This is further evidence that CBDC and adult LAD may be the same disease.

1. Leonard et al., (1984), B.J.D., 110,315.
2. Hall et al., (1985), J. Immuno., 135,3,1760.

121

KERATINOCYTE BINDING AND INTERNALIZATION OF AUTOANTIBODIES TO THE EXTRACTABLE NUCLEAR ANTIGENS Ro, La AND RNP FOLLOWING ULTRAVIOLET IRRADIATION IN VITRO AND IN VIVO. David A. Norris, Mari Sawami, and Fukumi Furukawa. Departments of Dermatology, University of Colorado School of Medicine, Denver, USA and Kyoto University, Kyoto, Japan.

Since autoantibodies to Ro, La and RNP are highly associated with photosensitive lupus, we postulate that ultraviolet light may induce the expression of these antigens on keratinocyte cell membranes, leading to autoantibody binding to keratinocytes and cytotoxicity. We have verified that UVL induces keratinocyte cell surface expression of Ro, La, RNP and (to a lesser extent) Sm using three different techniques:

1. Immunofluorescence of irradiated human keratinocyte cultures,
2. FACS analysis of irradiated cultured human keratinocytes, and
3. Immunofluorescence of intact epidermal sheets irradiated in vivo, harvested as suction blister roofs and incubated in vitro with specific antibody.

In cultured human keratinocytes, membrane augmentation of these extractable nuclear antigens was seen following UVB but not UVA irradiation. This cell membrane antigen expression was completely blocked by inhibitors of protein synthesis and glycosylation. It was not seen in dead cells, and was not associated with any one phase of the cell cycle.

Epidermal sheets harvested 24 hours after UVB irradiation, and then incubated in vitro with specific antibody probes also showed similar keratinocyte binding of anti-Ro, La, RNP and Sm. As was occasionally observed in cell culture experiments, antibody to each antigen was also internalized and concentrated in keratinocyte cell nuclei in intact epidermis, but only after UVL irradiation. Such internalization has been previously reported in cell culture with anti-RNP, but not in intact human epidermis post-irradiation.

UVL induces the synthesis, glycosylation and membrane expression of the extractable nuclear antigens Ro, La, and RNP in human keratinocytes in cell culture and in intact epidermis. Might this trigger antibody-mediated keratinocyte cytotoxicity in photosensitive lupus?

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WESTERN BLOT ANALYSIS OF EPIDERMAL EXTRACT IN THE CHARACTERISATION OF THE ANTIGEN IN PEMPHIGOID GESTATIONIS. Susan E. Kelly, B.S. Bhogal, *F. Wojnarowska, ** P. Whitehead and M.M. Black. Institute of Dermatology, Lisle Street, London, *Slade Hospital, Oxford and The London Hospital, London.

In pemphigoid gestationis (PG) tissue damage appears to be mediated by an IgG antibody directed against a constituent of the cutaneous basement membrane zone (BMZ). This antibody is of the IgG1 subclass. IgG deposition and subsequent activation of complement is integral in bulla formation. The antigen against which the antibodies are directed awaits full characterisation. Ultrastructural studies demonstrate immunoreagent deposition within the lamina lucida of the BMZ. Indirect immunofluorescence using 1M NaCl separated tissue as a substrate places the antigen on the epidermal aspect of the basal keratinocyte, a position comparable to bullous pemphigoid antigen (BPA). Bullous pemphigoid (BP) and PG share many histological and immunopathological features and it has previously been considered that BPA may be a major antigenic determinant in PG. BPA is a glycoprotein macromolecule with Mr 220 kD.

We have employed a Western blotting technique in the analysis of the PG antigen. Human skin was chemically separated by incubation with 1M NaCl. Proteins extracted from the epidermal preparation by heat and chemical denaturation were then separated by SDS PAGE and after transfer to nitrocellulose paper probed by 21 PG sera and visualised in an immunoperoxidase technique. Control experiments were performed with 10 sera from patients with BP.

17/21 PG sera reacted with a 180 kD epidermal protein which was also observed to be a minor antigenic determinant in 25% patients with BP. 10/21 PG sera reacted, in addition, with a 220 kD epidermal protein which was the major antigenic determinant in BP, identified by all 10 BP sera.

PG antigen is, therefore, an 180 kD molecule which is also of importance in the immunopathology of BP. PG and BP appear to share antigenic determinants but BPA is not the major antigen in PG.

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RECOGNITION OF THE MAJOR BULLOUS PEMPHIGOID ANTIGEN BY HETEROGENEOUS ANTIBODIES: A STUDY USING THE IMMUNOBLOTTING TECHNIQUE. A.L. Cook, R.B. Mallett, *T.H.P. Hanahoe and R.J. Pye. Dermatology Research Unit E5, Addenbrooke's Hospital, Cambridge and *School of Natural Sciences, The Hatfield Polytechnic, Hatfield, England.

The human squamous carcinoma cell line, SCaBER, has been used as a substrate in an ELISA to study the sera of patients with bullous pemphigoid. In order to determine if antigens recognised in the cell line were similar to those in normal cultured human epidermal cells and epidermis, proteins from cell extracts were compared using an immunoblotting technique. Proteins were extracted using a hypotonic solution containing SDS, EDTA, 2-mercaptoethanol and five protease inhibitors, prior to separation by SDS-PAGE. Following transfer to nitrocellulose, protein extracts were incubated with patient serum. Binding of antibody was detected using peroxidase-conjugated anti-human IgG and the substrates diaminobenzidine and hydrogen peroxide. Sera from seventeen patients reacted with a range of proteins of M_r 240kD, 230kD, 220kD, 190kD, 180kD, 120kD, 100kD and 80kD. All bullous pemphigoid sera reacted with proteins of M_r 190-180kD, or both groups of proteins. Antibodies to proteins of M_r 120-80kD were only present in five bullous pemphigoid sera. All sera, including sixteen control sera, reacted with bands of less than M_r 80kD. Individual sera showed identical binding patterns with extracts from all the cell types. Previous work using an immunoblotting technique has shown that the majority of bullous pemphigoid sera recognise a similar range of proteins, although not all the sera identified proteins of M_r 240-220kD or M_r 190-180kD [1]. In contrast, using an immunoprecipitation technique, a single antigen of M_r 240-220kD was identified by bullous pemphigoid sera [2]. There may be two or more epitopes on one major antigen of at least M_r 240-220kD which are recognised by all the bullous pemphigoid sera in this study. The immunoblotting technique allows identification of two of these epitopes on protein fragments of M_r 240-220kD or M_r 190-180kD. Antibodies against either or both of these epitopes may be important in bullous pemphigoid.

[1] Labib et al. (1986) J. Immunol. 136;1231-1235.
[2] Stanley et al. (1981) Cell 24;897-908.

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BULLOUS PEMPHIGOID AND CICATRICIAL PEMPHIGOID: IMMUNOBLOTTING DETECTION OF INVOLVED AUTOANTIGENS. Yves Sarret, Alain Reano, Hong Su, Jean-François Nicolas, and Jean Thivolet. INSERM U 209, CNRS UA 601. Laboratoire de Recherche Dermatologique et Immunologie, Hop. E. Herriot. Lyon France.

Bullous pemphigoid (BP) and cicatricial pemphigoid (CP) are subepidermal bullous autoimmune diseases which have distinct clinical features but identical immunological status. In order to determine whether these diseases could be dissociated on the basis of qualitative differences in serum antibodies to basement membrane zone (BMZ) antigens, the reactivity of sera from 7 CP and 29 BP patients with proteins extracted from normal human epidermis was analysed using immunoblotting and compared to that of 10 normal sera.

Epidermal tissue obtained by heat treatment of skin from normal subjects was homogeneized in 10 mM Tris-HCl pH 7.8 containing 2% SDS, 5% B-mercaptoethanol and protease inhibitors. Soluble proteins were subjected to SDS-PAGE then transferred onto nitrocellulose membrane. For immunodetection of the antigens, strips were incubated with the test sera, then the visualization of antigen-antibody complexes was achieved using antihuman peroxydase conjugate.

20 out of the 29 BP sera contained antibodies recognizing one or several protein(s) of 240, 200, 180 and 165 kD molecular weight (Mr). Antibodies in 4 out of 7 CP sera specifically reacted with one or two polypeptides of Mr 240 and 120 kD. These data confirm the heterogeneity of BP antigens and show the presence in CP of a novel 120 kD Mr polypeptide which is found only in CP but not in BP.

Taken together these findings demonstrate that in BP and CP, autoantibodies are directed to both common and specific BMZ antigens, their physiopathological significance need to be understood.

Tuesday, 21 June, 1988

15.00-16.00

Concurrent session – Holl

R. CAMP, B. CZARNETZKI, Chairpersons
Cyclosporins (Abstracts 125-129)

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EFFECTS OF CYCLOSPORIN ON PHYSIOLOGICAL AND PHARMACOLOGICAL REACTIONS IN SKIN
E.M. Higgins, C.M. Munro, J. Rees, F. Humphreys, P.M. Farr, B. Ramsay, J.M. Marks P.S. Friedmann, S. Shuster, Dermatology Department, Royal Victoria Infirmary, Newcastle upon Tyne, UK.

Cyclosporin A (CsA) suppresses a number of inflammatory skin diseases, but its mode of action is not established. Although it inhibits IL-2 release it may also prevent release of other soluble mediators. We have examined the effects of CsA on a variety of physiologic and inflammatory response. 11 patients (6 psoriasis, 3 alopecia, 2 atopic eczema) were studied before and again having been on CsA (5mg/kg/day) for at least 4 weeks. Physiologic functions (sweating induced by pilocarpine iontophoresis and sebum excretion) were measured by standard gravimetric methods. There was a small reduction (not significant) in both. Responses to intradermal histamine (0.5-8µg range), 48/80 (1-100µg range), topical anthralin (10µg in chloroform) and UVB radiation - quantitative MED - were measured. Changes in thickness were measured with Harpenden callipers, UVB erythema with a reflectance instrument. A significantly increased response to histamine was found during treatment but the other responses were unchanged.

	Number	Mean of paired differences	P value
Histamine 2µg	9	0.42mm (SE 0.33)	<0.05
48/80 1µg	9	0.16mm (SE 0.05)	>0.05
Anthralin 10µg	10	-0.5mm (SE 0.27)	>0.05
UVB (calculated MED)	9	9.06mJ/cm^2 (SE 7.9)	>0.05

Effect on induction of the immune response to DNCB was measured in 10 psoriatic and 6 atopic subjects. After at least 2 weeks on CsA, 30µg of DNCB was applied to the thigh on a 1cm patch test felt. 4 weeks after withdrawal of CsA, sensitivity was assessed by measurement of 48 h responses to challenge with 3 doses of DNCB (8.75-17.5µg). Compared with untreated control subjects there was a highly significant and virtually total abrogation of the induction of sensitisation.

	Untreated controls	CsA Treated	X^2	P value
Psoriatic	19/20*	0/10	20.4	<0.001
Atopic	12/16	0/6	9.24	<0.005

* proportion showing sensitivity

Thus CsA has little effect on wide range processes in skin but we have confirmed its powerful inhibition of the afferent limb of the immune response.

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CYCLOSPORIN A INHIBITS PROLIFERATION OF NUDE MOUSE EPIDERMAL CELLS IN VIVO. A. Urabe, J. Kanitakis, J. Viac, J. Thivolet. INSERM U 209 Hôp. Ed. Herriot, 69437 Lyon, France.

Cyclosporin A(CyA), an inhibitor of activation and proliferation of T lymphocytes, has been reported to lack a direct effect on the proliferation of epidermal keratinocytes (EK). However, CyA is effective in the treatment of psoriasis, a hyperproliferative skin disease. In this work, we evaluated the in vivo effect of CyA on nude mouse epidermal cells, using bromodeoxyuridine (BrdU) which is incorporated into DNA-synthesizing cells. Nude mice were injected subcutaneously with CyA diluted in olive oil at a dosage of 10, 50 and 100 mg/kg/day for 3 weeks. Control mice received a daily injection of olive oil. At the end of the study, mice were injected with a solution of BrdU every 6 h intraperitoneally. Blood levels of CyA were also evaluated by a radioimmunologic assay; they varied from 74 ng/ml (10 mg/kg, n = 2) to 650 ng/ml (100 mg/kg, n = 2). Incorporation of BrdU was revealed immunohistochemically on deparaffinized tissue sections, obtained at various time intervals, with an anti-BrdU monoclonal antibody, using an avidin-biotin-alkaline phosphatase technique. The percentage of labelled cells (labelling index, LI%)was evaluated by counting at least 1000 basal EK on each section. The results are listed below:

		24 h	48 h
Control	(N = 2)	12.0±2.1	31.9±4.8
10 mg/kg	(N = 3)	9.8±2.7	22.6±6.7
50 mg/kg	(N = 2)	9.7±2.2	15.3±5.4
100 mg/kg	(N = 3)	8.7±3.4	15.2±4.1

These results show that CyA has a direct effect on nude mouse epidermal cells in vivo, by decreasing their proliferation rate (evaluated through BrdU incorporation). This effect was obtained even with blood levels of CyA that are effective therapeutically in humans. Further studies are now in progress in order to confirm these results.

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EFFECTS OF CYCLOSPORINE (CS) ON INITIATION AND PROMOTION OF GRANULOMAS IN SKIN OF MICE. A. Fujioka, C. Pincelli, A. Hashimoto, and W.L. Epstein, Department of Dermatology, University of California, San Francisco, USA, University of Modena, Italy.

We have reported that although Cs inhibits T helper/inducer cell function, it does not prevent initiation of granuloma formation in mice (J Invest Derm 90: No4, 1988). To further study the role of Cs immunosuppression in granulomatous inflammation, daily Cs injections were delayed until 10 days after grafting of naive mice with schistosome egg granulomas, at the time when new granulomas just begin to form. As controls a group of mice was pre-treated with Cs for 2 wks before grafts and another group was grafted without Cs injection. The dose of Cs IM injection was 150 mg/kg 5x/wk. At 3 wks after grafting mice were sacrificed. Lymph nodes were examined for cell numbers, % Thy 1.2^+ lymphocytes by the avidin biotin peroxidase technique and T cell function by mitogenic response to phytohemagglutinin. Morphology of granulomas was assessed by light microscopy and morphometry. We found:

Cs therapy	Cell number ($x10^5$) Total lymphocytes	T-cells	Mitogen response (stimulation index)
2 wks pre- (n=15)	14.2	3.8	12.3
10 days post- (n=4)	12.5	3.5	13.2
none (n=4)	48.0	12.3	57.9

Histologically the skin granulomas in Cs pre-treated and controls were almost identical, but in the group given Cs 10 days after grafting granulomas were smaller and poorly organized despite a strong inflammatory response outside the graft. The results suggest that prolonged Cs treatment allows emergence of Cs-resistant T-cells, while in the short terms Cs prevents T cell amplification and organization of granulomatous inflammation.

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CUTANEOUS RESPONSE TO HOUSE DUST MITE ANTIGEN IN ATOPIC ECZEMA TREATED WITH CYCLOSPORIN A (CsA) C.S. Munro, J.M. Marks, P.S. Friedmann, S. Shuster. Dermatology Department, Royal Victoria Infirmary, Newcastle upon Tyne, UK.

Cyclosporin A (Sandimmun) is a potent orally-active immunosuppressant. We wished to examine its effects in atopic eczema, in which both immediate type and cell-mediated hypersensitivity may play a role. 10 patients (5 M; age 17-43) with severe chronic atopic eczema were treated with 5 mg/kg/day CsA. Clinical improvement occurred within 3 weeks in all cases. 2 subjects relapsed on treatment, deteriorating further when CsA was stopped after 7 & 8 weeks respectively. In the other cases, improvement was maintained and 7 were able to do without topical corticosteroids during treatment. When CsA was stopped after 8 - 16 weeks, eczema of varying severity recurred. There were no major adverse effects.

Despite the good clinical response to CsA, no consistent changes were observed in prick test reactions to standard antigens or in total serum IgE levels on treatment. In 6 cases, callipers were used to measure oedema following intradermal challenge with 1, 5 & 25 units of house dust mite (HDM) antigen (Pharmacia) before and after 4 - 6 weeks on CsA. Responses were not reduced by CsA; indeed, there was a trend to greater responses on treatment, more marked at higher antigen doses, in both early (15 - 45 min) and late (6 hr) phases of the reaction:

		Increase in skinfold thickness (mm); mean ± s.e.m.			
HDM	Group	15 min	30 min	45 min	6 hr
1u	Control	4.1 ± 0.5	4.6 ± 0.6	3.5 ± 0.5	1.3 ± 0.3
	CsA	5.0 ± 0.7	5.0 ± 1.0	4.3 ± 1.0	1.4 ± 0.3
5u	Control	5.5 ± 0.9	5.3 ± 0.8	4.6 ± 0.7	1.4 ± 0.3
	CsA	6.1 ± 0.5	6.2 ± 0.6	5.1 ± 0.8	2.3 ± 0.5
25u	Control	6.1 ± 0.9	6.5 ± 0.9	6.1 ± 0.7	1.5 ± 0.2
	CsA	7.2 ± 0.5	7.5 ± 0.6	6.0 ± 0.8	3.7 ± 0.3

We conclude that CsA is an effective oral agent for the short term control of atopic eczema, but that its effects are not mediated by a reduction in IgE-dependent hypersensitivity reactions.

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RESPONSE AND RELAPSE OF MODERATE PSORIASIS TREATED WITH CYCLOSPORIN A. Janet Marks, Peter Friedmann, Sam Shuster, Colin Munro, Elisabeth Higgins. University Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne, UK.

We and others have previously reported the clearance of severe long standing psoriasis with low dose cyclosporin A (CsA) (Sandimmun). Such patients have relapsed rapidly when the CsA has been stopped, while continuing it long term has resulted in hypertension and a rise in serum creatinine. The object of the present study was to look at the effect of CsA used short term on the clearance and subsequent relapse of psoriasis that was less severe particularly with respect to previous history of relapse. Seventeen patients of mean age 33 y, range 23-60, and mean disease duration 15 y, range 4-28, with chronic plaque psoriasis who had not in the past needed more than two courses of treatment per year were treated with oral CsA 5 mg/kg/d and time to clearance was measured. Blood trough levels of CsA were measured monthly and serum creatinine and blood pressure were monitored throughout. 11 patients cleared within 12 weeks (mean 9, range 5-12) and 6 failed to clear after 13-21 weeks. There was no significant difference between the ages of the two groups, the duration of their disease or the CsA blood level at the end of 12 weeks: the mean area involved before treatment was greater in the group who failed to clear (31%) than in those who cleared (16%). The 11 patients who cleared were allocated at random to one of three groups, (a) CsA stopped at point of clearance (n=4), (b) CsA stopped 2 weeks after clearance (n=4), (c) CsA stopped 4 weeks after clearance (n=3) and time to appearance of new lesions was measured. These started to appear in a mean of 6 days (range 2-25) and there was no detectable difference between the three groups. Side effects included mild hirsutism (6), dyspepsia (3), folliculitis (2), gum hypertrophy (1), amenorrhoea (1), paresthesia (1). There was no significant rise in blood pressure or serum creatinine. We conclude that low dose CsA is effective in clearing moderately severe psoriasis in the majority of cases but that the rash starts to relapse within days of stopping CsA. Serious side effects did not occur. The usefulness of CsA in this type of psoriasis will ultimately depend upon the rate at which the rash having started to recur spreads to an extent unacceptable to the patient. If this were as long as 6 months two courses of CsA per year would be needed and this would seem a reasonable way of managing the disease.

Wednesday, 22 June, 1988

8.00-10.30

Concurrent session – Cuvilliés

M. FAURE, P. FRIEDMANN, Chairpersons
Immunology (Abstracts 130-141)

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ALTERED PRODUCTION OF IMMUNO-MODULATING CYTOKINES IN PATIENTS WITH ATOPIC DERMATITIS. Alexander Kapp, *Reinhard Kirnbauer, *Thomas A. Luger and Erwin Schöpf, Dept. of Dermatology, Univ. of Freiburg, FRG, and *2nd Dept. of Dermatology, Univ. of Vienna, Austria.

Atopic Dermatitis (AD) represents an inflammatory skin disorder which is characterized by many signs of immunodeficiency. Particularly, decreased lymphoproliferative responses upon stimulation with mitogens as well as bacterial antigens were reported repeatedly. In the present study we investigated whether an altered production of immuno-modulating cytokines is responsible. For this purpose the 24-h supernatants (SUP) of LPS- and PHA-stimulated or unstimulated mononuclear cells (MNC) from patients with AD of a moderate to severe disease activity and from nonatopic healthy controls were tested for Interleukin-1 (IL-1) and Interleukin-2 (IL-2) activity. Furthermore, in the sera of patients and controls the concentration of IL-2 receptor was determinded by an ELISA. Whereas SUP of unstimulated MNC did not exhibit significant differences between AD patients and controls, LPS-stimulated MNC of AD patients released significantly less IL-1 in the SUP. Similarly, the production of IL-2 by PHA-stimulated MNC of AD patients was significantly decreased in comparison to the controls. There was a strong correlation between IL-1 and IL-2 levels. The concentration of circulating IL-2 receptor in the sera of the patients was significantly increased. The findings of diminished lymphoproliferative responses in AD may be caused by a decreased release of immuno-modulating cytokines upon appropriate stimulation. However, these results are in contrast to the enhanced concentration of IL-2 receptor in the patients' sera. We, therefore, suggest that the decreased production of cytokines by blood MNC could be due to down-regulation induced by cytokines released from activated cells in the inflamed dermis. Alternatively, the hyporesponsiveness of MNC in vitro could be a sign of "exhaustion" following excessive stimulation in vivo.

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IMMUNOPRECIPITATION ASSAY OF SERA FROM PSORIASIS PATIENTS: CORRELATION OF A NEW AUTOANTIBODY SPECIFICITY (Pso-44) WITH DISEASE ACTIVITY. Sabine Scheublein, Edward K.L. Chan, Michael Meurer*, Roger C. Cornell and Eng M. Tan. Scripps Clinic and Research Foundation, La Jolla, CA, and *Dept. Dermatology, Ludwig-Maximilians University, Munich, FRG.

Anti-nuclear antibodies (ANA) have been reported inconsistently in sera of patients with psoriasis and there has been no molecular characterization of the nuclear autoantigen. In this study, sera from 60 patients (30 males, 27 females; mean age 49.7 yrs.) with psoriasis, ranging from mild to severe disease activity, were tested with an immunoprecipitation technique using extracts from ^{35}S-methionine labeled HeLa cells. Fourteen sera (23%) specifically precipitated a 44KD protein antigen that was different from any known autoantibody specificity previously described for lupus or other connective tissue diseases. It was clearly distinct from antigens of related molecular weight such as the 48KD SS-B/La antigen or 45KD actin. This new autoantibody specificity (Pso-44) was not detected in normal human sera but was found in 1 out of 25 SLE sera and 2 out of 23 sera from patients with photosensitivity. These positive sera were further examined by indirect immunofluorescence using HEp-2 and HeLa cell substrates. The pattern of immunolocalization which appeared to correlate with Pso-44 antibody was visualized as dispersed punctate staining in nucleus and cytoplasm. On HeLa cells 13 of 17 Pso-44 positive sera also stained the nucleolus in most cells. The disease activity of patients was determined by the degree of skin involvement and by the presence of recent exacerbations. Among the 14 psoriatics positive for Pso-44 antibody, 13 (93%) had generalized psoriasis and 12 (86%) had a recent aggravation of their skin manifestations. In contrast, among 46 Pso-44 negative psoriatics, only 13 (28%) had generalized psoriasis and 10 (22%) presented with recent exacerbations. The molecular identification of the 44KD antigen should lead to elucidation of its structure and, by way of its function, perhaps lead to understanding of its role as an autoantigen in psoriasis.

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BACTERIAL ANTIGEN DETECTION IN ANTIGEN PRESENTING CELLS IN SITU. W.R. Faber, B. Naafs, J.D. Bos, A. Waterdrinker, S.R. Krieg, A.H.J. Kolk. Departments of Dermatology and Pathology, University of Amsterdam and Royal Tropical Institute, Amsterdam and Department of Dermatology, Erasmus University, Rotterdam, The Netherlands.

By means of histochemical techniques M. leprae organisms have been described to be present in all types of antigen presenting cells (APCs) investigated in the dermal infiltrates in multibacillary leprosy.

The aim of this study was to find out if M. leprae specific determinant could be demonstrated in the different APCs in the dermal infiltrates in untreated paucibacillary as well as multibacillary leprosy.

A biotinylated monoclonal antibody F47-10 directed against a specific epitope on the 65 kD M. leprae protein was used in a double staining technique. Cells of the mononuclear phagocyte system (MPS) were detected by the following Moabs: OKM1 (monocytes and granulocytes), RFD2 (all monocytes-macrophages), RFD7 (mature tissue macrophages), RFD9 (epitheloid cells); dendritic cells (DC) were detected by RFD1 (interdigitating cells), OKT6 (Langerhans cells), M241 (indeterminate cells). No M. leprae specific antigen could be demonstrated in APCs in three biopsies from paucibacillary leprosy patients; on the other hand in two biopsies from multibacillary leprosy patients M. leprae specific antigen was found in the different APCs of the MPS as well as in RFD1 positive cells but not in OKT6 and M241 positive cells. In the cell types in which the antigen was found it was always present in only part of these cells.

It appears that this M. leprae specific antigen is present in all MPS cell types. The possible participation of DCs in antigen processing in leprosy needs further clarification.

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HUMAN STRATUM CORNEUM ACID PHOSPHATASE: CHARACTERIZATION AND COMPARISON TO DERMIS AND LEUKOCYTES. Jürg Ch. Meyer, Department of Dermatology, University Hospital, Zürich, Switzerland.

Epidermal acid phosphatase (AcP) which shows its highest activity in the upper granular layer and stratum corneum was suggested to play a role in the degradation of phospholipids, protein phosphates like profilaggrin or phosphate groups in the stratum corneum. In the present paper we present further characteristics of this enzyme. Stratum corneum and epidermis were prepared by a trypsinization procedure from skin samples obtained from the department of plastic surgery. Whole skin samples as well as epidermis and stratum corneum sheets were homogenized in a freeze press. AcP activities were measured with 4-methylumbelliferone phosphate as substrate or naphtol AS-BI phosphate after isoelectric focusing (IEF). IEP of Triton X-100 extracts was performed on an ampholine gradient of 3.5-9.5 in polyacrylamide and gelpermeation HPLC on a TSK 3000SW column.

AcP which preferentially remained bound to the 50'000g sediment if fractionation was performed at pH 4.5, was found to be tightly bound to the pellet. Attempts to solubilize this activity by resuspension in water or detergents failed. Comparison of AcP activity in Triton X-100 extracts from whole skin, epidermis, stratum corneum, cultured skin fibroblasts and leucocytes by IEP yielded one identical prominent band in all skin fractions at a pI of 5.65, whereas three different bands were found in cultured skin fibroblasts at pI values of 5.8, 6.1 and 6.25, the pI of 6.1 also occurring in leucocytes. Gelpermeation HPLC of stratum corneum extracts yielded a minor AcP peak at MG 300'000 and a major peak of MG 25'000.

From this we conclude that different AcP's exist between epidermis and dermis. The major activity found in epidermal and stratum corneum extracts with a MG of 25'000 probably represents the soluble enzyme.

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CHARACTERIZATION OF A HUMAN BM-40 cDNA CLONE AND ITS EXPRESSION IN EPIDERMAL CELLS AND FIBROBLASTS. B. Lankat Buttgereit, M. Kulozik, R. Timpl, and T. Krieg, Dermatologische Klinik und Poliklinik der Universität München.

The Ca^{2+}-binding protein BM-40 was first isolated from the Engelbreth-Holm-Swarm (EHS) sarcoma but then found in a variety of other tissues. Recently we isolated a murine cDNA clone coding for BM-40 and demonstrated its identity to the Ca^{2+}-binding proteins SPARC and osteonectin. We now report on the isolation and characterization of a human cDNA clone and investigated the expression of BM-40 in epidermal cells and fibroblasts in vivo and in vitro.

A lambda *gt11 gene expression* library constructed from human placenta was screened with the ^{32}P-labeled nicktranslated cDNA clones for murine BM-40. Positive clones were subcloned into pUC19 and further characterized. Northern hybridisation against RNA isolated from human fibroblasts showed a hybridisation signal with RNA of 2200 nucleotides. Moreover a minor band at 3000 nucleotides was found. Sequencing of the human cDNA clone comprising the 3`-half of the coding part, revealed 90% homology to the murine cDNA clone in the coding region and nearly no homology in the non-coding part. Base exchanges were mostly found in the 3rd position of the codons and caused no change in the deduced amino acid sequence. The high conservation between murine and human BM-40 in the 3`- half may be important considering the fact that this region contains a putative Ca^{2+}-binding domain.

The biological functions of BM-40 are further elucidated by the expression of BM-40 in epidermal cells and fibroblasts detected by northern blot analysis and in situ hybridisation. In addition its modulation by tissue mediators indicate an important role of this protein during development and tissue repair.

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EFFECT OF TRANSPLANTATION OF NONVIABLE GRANULOMAS ON LOCAL AND SYSTEMIC IMMUNITY IN MICE. C. Pincelli, A. Hashimoto, A. Fujioka, K. Fukuyama, and W.L. Epstein, Department of Dermatology, Univ. of Modena, Italy and University of California, San Francisco, Calif.

Organized granulomas develop in the skin of euthymic mice by transplantation of egg granulomas isolated from livers of mice infected with Schistosoma mansoni. The granulomatous skin reactions are identical between grafting freshly isolated granulomas (FGR) and freeze-dried granulomas (FDGR), indicating that viable cells are not required in eliciting host cells to form the skin granuloma. In this study the effects of the inocula on lymphocytes in regional lymph nodes (LN) and spleen (SP) were evaluated. At 10 and 21 days after transplantation LN and SP were removed, weighed, and total cell number counted. In addition the percent of cells positively stained with anti-Thyl.2 monoclonal antibody was analyzed by a fluorescence-activated cell sorter and total T-cell number calculated. The weight, number of total cells and T-cells of LN increased in both groups of recipients, but were greater in mice that received FGR than FDGR. Light and electron microscopy confirmed that the well-organized granulomas which developed in skin of mice from FGR and FDGR were indistinguishable.

Days post graft	Weight (mg)		Cell number ($x10^6$)			
	FGR	FDGR	FGR		FDGR	
			Total	T-cells	Total	T-cells
0	6.8	6.8	0.77	0.46	0.77	0.46
10	26.7	18.0	8.75	2.73	3.20	1.42
21	25.0	23.6	5.40	2.82	4.00	2.30

In contrast those changes were not detected in SP from mice in either group. The results indicate that granuloma components stimulate local but not systemic immunity. Granulomas also contain a factor(s) to promote skin granuloma formation which is stable after freeze-drying, but LN-stimulating ability is partially destroyed by the process.

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THE APPLICATION OF POSTEMBEDDING IMMUNOELECTRONMICROSCOPY TO VISUALIZE THE LDL RECEPTOR ON NORMAL AND DISEASED SKIN. J. Tada*, A.M. Mommaas-Kienhuis and B.J. Vermeer, * Department of Dermatology, Okayama University Medical School, Okayama, Japan and Department of Dermatology, University Hospital, Leiden, The Netherlands.

Both biochemical and morphological studies have demonstrated a reciprocal relationship between the stage of epidermal cell differentiation and the binding capacity of low density lipoproteins (LDL). It was unclear however, whether this loss in ability to bind LDL was caused by disfunction or by absence of the LDL receptor. To investigate this we used a postembedding IEM technique in which whole pieces of skin were fixed and embedded in Lowicryl K4M. A monoclonal antibody against the LDL receptor was applied on ultrasections and visualized with colloidal gold particles, in order to locate the receptor from the basal cells of the epidermis up to the stratum corneum. In normal skin we found a moderate amount of labeling in basal cells, which reduced gradually in more upwards located cell layers. However in psoriasis skin the labeling in the basal cells was more pronounced. This labeling pattern remained constant throughout the stratum spinosum and only diminished in the stratum corneum. Furthermore, in cells of the suprabasal layers of psoriasis skin, LDL receptors were predominantly located along the plasmamembrane, whereas in normal skin they were found more inside the cells. Finally, a strong reduction of labeling, compared to psoriasis and normal skin was found in Ichthyosis skin. These results suggest:

1) synthesis of LDL receptors in epidermal keratinocytes reduces in the process of differentiation.
2) in normal skin the transport of LDL receptors to the cell surface is inhibited, whereas in psoriasis skin the receptor can be inserted in the plasmamembrane.
3) the reduction of labeling in Ichthyosis skin might be a reflection of a disturbance in differentiation.

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MORPHOLOGIC PATTERNS OF ENDOTHELIAL CELL ACTIVATION AFTER INTRACUTANEOUS INJECTION OF MONAP AND $C5a_{des\ arg}$. Christoph Schubert, Jens-M. Schröder, Taiki Isai, Ole Swensson, and Enno Christophers, Department of Dermatology, University of Kiel, FRG.

Previously we have shown, that after intracutaneous injection of $C5a_{des\ arg}$ mast cell degranulation together with endothelial cell alteration signaling secretory activity take place. These changes are followed by a perivascular infiltration of inflammatory cells. MONAP (monocyte derived neutrophil activating peptide) is a newly discovered monokine specifically activating neutrophils in vivo and in vitro. Using this cytokine we now investigated by sequential ultrastructural analysis the sequence of morphological alterations after intracutaneous injection in healthy volunteers. Sequentially biopsied skin samples were prepared for light and electron microscopy.

In contrast to $C5a_{des\ arg}$ mast cell degranulation was absent. Instead the first structural changes occured in the endothelial cells of postcapillary venules and consisted in the presence of cytoplasmic microfilament bundles as well as numerous protrusions of the luminal plasma membrane. As a striking feature multiple electron lucent vesicles could be observed in the cytoplasm underneath the luminal and abluminal plasma membrane. These structures differed from micropinocytotic vesicles and suggest high secretory activity. When neutrophils and monocytes appeared in the vascular lumen the electron lucent vesicles disappeared. Following these events neutrophils became adherent.

Interestingly neutrophils (PMN) appeared to subsequently migrate into the perivascular space by traversing the endothelial cells. By comparing a greater number of specimens PMN seemed more often to migrate transcellularly as compared to the intercellular route. These findings demonstrate that contrasting response patterns can be produced by proinflammatory mediators injected into healthy skin.

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METALLOTHIONEIN INDUCTION IN A HUMAN B CELL LINE BY STIMULATED IMMUNE CELL PRODUCTS. Jürgen Oberbarnscheid, Peter Kind, Josef Abel, and Ernst Gleichmann, Medical Institute of Environmental Hygiene, Department of Dermatology, University of Duesseldorf.

Metallothionein (Mt) is a protein inducible by heavy metals, various chemicals, immunomodulatory substances, and interleukins. It is probably important for metal homeostasis and protection against environmental hazards. Previously we could demonstrate that Mt can be specifically induced in mouse skin (J Invest Dermatol 52: 89-94). Now we have asked the question whether immunostimulatory substances, such as lipopolysaccharide (LPS) or Concanavalin A (Con A), or supernatants obtained from human peripheral mononuclear cells (HPMC), which had been stimulated with LPS or Con A, are able to induce increased Mt concentrations in cells of the immune system. HPMC, separated on a Ficoll-Hypaque gradient, and murine spleen cells were stimulated with LPS and Con A for 48 hours. Afterwards supernatants were incubated for 24 - 96 hours with the human B Cell line RPMI 1788. After various time intervals Mt content was measured by the Cd saturation assay. Furthermore the cells of the human B Cell line RPMI 1788 were incubated with different concentrations of Zn^{++}, dexamethasone and τ-interferon (τ-IFN). In all experiments cell viability was determined by trypan blue exclusion.

Supernatants obtained from HPMC that were stimulated by LPS or Con A increased the Mt concentration in RPMI 1788 cells. However, LPS or Con A failed directly to increase Mt concentrations in RPMI 1788 cells, whereas they increased Mt concentrations in HPMC. In conclusion, activated HPMC and murine spleen cells produce an inducer of Mt that appears to differ from τ-IFN and Il-1. The exact cell type among HPMC that produce this mediator remains to be determined. Our data show that Mt is induced during immunostimulation. This is consistent with the hypothesis that Mt is a cell protecting protein produced during inflammation.

Wednesday, 22 June, 1988

8.00-10.30

Concurrent session – Effner

M. GREAVES, T. LUGER, Chairpersons
Cytokines, Mediators (Abstracts 142-153)

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ULTRASTRUCTURAL LOCALIZATION OF CHROMOGRANIN A-LIKE IMMUNOREACTIVITY AND COEXISTENCE WITH MULTIPLE PEPTIDES (VIP, PHI, SUBSTANCE P and CGRP) IN MERKEL CELLS OF PIG SKIN. Wolfgang Hartschuh, Eberhard Weihe, Ursula Egner and Bernd Gauweiler, Dermatological Clinic, University of Heidelberg, Institut of Anatomy, University of Mainz.

The Merkel cell is regarded as a member of the diffuse neuroendocrine or APUD cell systems. Recently it has been shown that most of their members contain, besides neuropeptides and biogenic amines, also chromogranin A (CG-A) which is the main representative of the CG family. It is known that Merkel cells of pig contain VIP and the VIP-related peptide PHI. However, the presence of CG-A in Merkel cells has been denied. Therefore the objective of this study was to proof by immunohistochemistry whether or not Merkel cells contain CG-like immunoreactivity (ir) and to provide evidence for coexistence of peptides and CG-A in Merkel cells.

For light microscopic immunohistochemistry adult and fetal skin of pig snouts (n = 7) was fixed by immersion in Bouin's solution and processed using biotinylated species-specific secondary antisera and streptavidin-biotin-horseradish peroxidase complexes on paraffin sections. For electron microscopic immunohistochemistry, the pre-embedding technique was employed. Primary polyclonal antisera against porcine or bovine CG-A (Immunonuclear), calcitonin gene-related peptide (CGRP), substance P (Peninsula, Serotec), VIP and PHI (N. Yanaihara, Japan) were used. In order to determine coexistence patterns of CG-A and peptides, paired adjacent sections were alternately stained with the different primary antisera.

In this study we were able to demonstrate that Merkel cells of adult pig in addition to VIP and PHI contain SP and CGRP-like immunoreactivities mutually coexisting in identical Merkel cells. However, fetal Merkel cells expressed only SP- and CGRP-ir but not VIP- and PHI-ir suggesting differences in peptide expression in various developmental stages. Furthermore, we provided evidence for the occurance of CG-A in adult and fetal Merkel cells and coexistence of peptides and CG-A as ascertained by paired consecutive sections. Electron microscopical immunohistochemistry revealed that CG-A is mainly localized in variable degrees in Merkel cell granules.

It is suggested that multiple peptides present in cutaneous Merkel cells may fulfill a transmitter or modulatory role in mechanoperception besides other speculative functions (e.g. paracrine, endocrine). The role of CG-A remains unclear. It may function in the storage and packaging of peptides or it may be released and act as a messenger on its own or in concert with neuropeptides.

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INHIBITION OF HUMAN NEUTROPHIL 5-LIPOXYGENASE BY GALLATES. Gottfried Wozel, Jens-M. Schröder*, Enno Christophers* and Jochen Barth, Department of Dermatology, Medical Academy "Carl Gustav Carus" Dresden, GDR and Department of Dermatology, University of Kiel, FRG*.

Propyl gallate has been shown to possess distinct biological properties including light protection as well as antiinflammatory activities in animals. In addition, propyl gallate is known as an inhibitor of soybean and pea lipoxigenases as well as has some antipsoriatic properties.

The purpose of this study was to investigate possible inhibitory effects upon leukotriene production by human granulocytes as well as to study the role of the length of the carbonhydrogen-chain in the gallate.

Human granulocytes were preincubated with different gallates (gallic acid, methyl-, ethyl-, propyl-, butyl-, octyl- as well as dodecyl-gallate) for 20 min followed by adding arachidonic acid (10^{-5} M), and Ca-Ionophore A 23187 (10^{-5} M) and subsequent incubation for 5 min. After centrifugation supernatants were analyzed by RP-HPLC, whereby leukotriene-production, 5-HETE-production and 15-HETE-production were determined.

As a result we found no inhibition of granulocytic lipoxigenases by gallic acid, tetradecyl- and heptadecyl-gallate below 10^{-2} M.

All the other gallates investigated demonstrated inhibition of cellular lipoxigenase, whereby octyl-gallate appeared to be the most potent gallate with lipoxigenase inhibiting properties (IC_{50}: 10^{-6} M).

In addition, octyl-gallate demonstrated the highest potency to inhibit inflammation in the carageenan edema model.

Preliminary data revealed, that some of the lipoxigenase inhibiting gallates show some antipsoriatic properties possibly pointing towards a novel class of antipsoriatic drugs with potent arachidonate 5-lipoxigenase inhibiting properties.

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CATALYTIC, ELECTROPHORETIC, AND IMMUNOCHEMICAL CHARACTERISATION OF HUMAN SKIN CYTOCHROME P-450. H.F. Merk, F. Jugert, I. Kaufmann, W.A. Khan. Depp. of Dermatology, University of Cologne, West-Germany and Case Western Reserve University, Cleveland, OH, USA.

Cytochrome P-450 (P450) is the terminal oxidase for the microsomal metabolism of numerous endogenous and exogenous compounds including steroid hormones, arachidonic acid derivatives, multiple drugs and carcinogens. This enzyme exists in multiple forms dependent on at least 21 gene families which belong to the P-450 supergenefamily. The expression of these genes is dependent on multiple compounds such as their substrates, several cytokines -e.g. interferons or interleukin 1- and tissue specific factors. Therefore the distribution of P-450 isoenzymes varies among different tissues and among different individuals which may determine the toxic potential of certain xenobiotics.

SDS-PAGE and 2-D electrophoresis of human skin microsomes prepared from surgical specimens exhibited coomassie blue stainable protein in Mr ~ 50 Kd. In opposite to skin from mouse or rat there was no immunoprecipitin band with monoclonal antibodies MAb 1-7-1 which is specific for P-450 c/d in Western Blot analysis. However the P-450 dependent catalytic activity aryl hydrocarbon hydroxylase (AHH) in human skin was dose dependent inhibited by imidazoles, 7,8-benzoflavone and MAb 1-7-1. There was no inhibition of AHH-activity in human skin by MAb 2-66-3 (binding to P450b/e) and by MAb 1-98-1 (binding to the ethanol inducible P-450j). According to these observations, there was also no p-nitrophenol-hydroxylase in skin which is highly dependent on cytochrome P-450j.

In conclusion these studies indicate that human skin possesses P-450c. This P-450 isoenzyme is known to participate in the malignant transformation evoked by certain classes of chemical carcinogens. Furthermore since there is one gene family for rat P-450c it is possible that it is similarly expressed in human skin.

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DO EPIDERMAL CELLS PRODUCE THYMIC HORMONES IN VIVO? J.F. Nicolas*, C. Auger*, M. Dardenne+, J. Thivolet*. *INSERM U.209, *Faculté Alexis Carrel, Lyon, +INSERM U.25, Paris, France.

Cultured epidermal cells have been shown to produce soluble factors endowed with T cell differentiating activities. In addition, the presence of thymopoietin and FTS/thymulin-like factors has been reported in normal human and mouse epidermis using immunohistochemical methods and antithymic hormone antisera. However, the latter studies were not correlated with a functional analysis of epidermal cell products, and a possibility still remained that the 2 antisera were cross-reacting with an epidermal antigenic structure unrelated to thymic hormones. The present study was conducted to re-evaluate the presence of thymic hormones in normal epidermal cells using a panel of monoclonal antibodies (mAbs) and rabbit antisera to several well characterized thymic factors. The reactivity of the following antibodies was tested by indirect immunofluorescence on human and mouse tissue sections: a) two anti-FTS/thymulin mAbs, b) one anti-FTS/thymulin rabbit antiserum, c) one anti-thymopoietin rabbit antiserum, d) one anti-thymosin alpha 1 mAb. Our results show that: 1) all antibodies reacted with human and/or mouse thymic epithelial cells; 2) only 2 out of 5 antibodies, namely the anti-FTS and anti-thymopoietin antisera cross-reacted with mouse and human epidermis and labeled keratinocytes, as previously reported (CHU et al., KATO et al.); however, these latter 2 antibodies also decorated nude mouse epidermal cells and labeled non-thymic, non-epidermal normal mouse epithelial tissues, suggesting that the cross-reactive epitope is common to a number of epithelial cells; 3) none of the 3 anti-thymic hormone mAbs reacted with normal skin; 4) the antigen defined by the anti-FTS and anti-thymopoietin antisera was not related to keratins, since absorption experiments using purified human epidermal keratins failed to abolish staining of the epidermis. We conclude from this study that epidermal cells do not produce in vivo the well characterized thymic hormones: FTS/thymulin, thymopoietin and thymosin alpha 1. The precise nature of the antigenic structure recognized within epidermis by the anti-FTS and antithymopoietin antisera remains to be defined.

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LIDOCAINE-INDUCED INHIBITION OF THE PRODUCTION OF INTERLEUKIN-1 LIKE EPIDERMAL CELL THYMOCYTE-ACTIVATING FACTOR (ETAF). C. Enk, Department of Dermatology, Hadassah University Hospital, Jerusalem, Israel.

With the purpose of investigating the influence of lidocaine on the production of epidermal cell thymocyte activating factor (ETAF), murine keratinocytes were incubated for 1 hour with 0.02 - 6 mg/ml lidocaine, washed, and incubated for additional 23 hours. ETAF activity was assayed by the comitogenic activity of the crude keratinocyte supernatants on PHA-stimulated murine thymocytes. Lidocaine reduced the ETAF activity significantly, most markedly at a concentration of 2 mg/ml. The reduction was not caused by cytotoxicity, by co-production of inhibitory factors, or by modification of the ETAF molecule. Although the murine thymocyte assay was highly sensitive to lidocaine, the reduction of ETAF-activity was not due to carry-over of lidocaine to the thymocyte assay. To investigate the immunomodulating influence of lidocaine in vivo, lidocaine was injected intradermally in one ear and saline in the other ear of mice, followed by isolation and incubation of the keratinocytes. Four of six murine lidocaine-processed biopsies showed less ETAF activity than their matched pairs. Our results indicate that lidocaine in vitro and in vivo inhibits ETAF production, and demonstrate that conventional manipulations, acceptable for morphological techniques, might not be pertinent in functional studies of the cellular components of the skin.

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INTERLEUKIN 1. IDENTIFICATION OF ALPHA AND BETA FORMS IN HUMAN SKIN ELUATES. Liliane Didierjean, Oliver Wiedow*, Catherine Fournier, Eno Christophers*, Jean-Hilaire Saurat. Clinic of Dermatology, Hôpital Cantonal Universitaire, 1211 Geneva 4 - Switzerland. *Département of Dermatology, University of Kiel, Kiel, FRG.

Bath eluates of psoriatic patients in 27% NaCl but not in tap water have been found to contain chemotactic activity for PMN; further factors that costimulated CH3 mouse thymocytes, IL-1 like, were also detected in the two types of bath eluates of both normal and psoriatic patients (JID 89, 337, 1987). We studied if this IL-1-like activity was associated to or correlated with the presence of IL-1 immunoreactive species. Skin eluates water with or without 27% NaCl were obtained from normal or psoriatic patients concentrated x 10 000 fold, separated by chromatography (twelve fractions per eluate). The 12 samples from each experiment were submitted to 5 - 20% SDS-PAGE. After transfer, the western blots were incubated with rabbit anti human IL-1α and IL-1β sera.

Both antisera bound to 17 kD proteins. Stronger reactions were observed with the anti IL-1β serum. IL-1α and β immunoreactivities were found in psoriatic eluates chromatography fractions which contained chemotactic activity for PMN and IL-1 biological activity, but also in normal eluates which contained IL-1 like biological activity but no chemotactic activity.

The results show that (i) bathing induces elimination of large amounts of IL-1 immunoreactivity from the skin surface in normal as well as in psoriatic patients (ii) the IL-1 like biological activity recovered from the skin surface correlates with the presence of both IL-1 α and β species and (iii) the IL-1 species detected correspond to the extra cellular secreted IL-1 that might in part originate from sweat.

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HUMAN EPIDERMOID CARCINOMA CELLS EXPRESS AND RELEASE INTERLEUKIN 6 (IL 6). A. Köck[1], R. Kirnbauer[1], T. Schwarz[2], J. Ansel[3], T.A. Luger[1]. [1]Dept.Derm. II and LBI for Dermven. Lab. for Cellbiol., Univ. of Vienna, [2]Dept.Derm., Hospital Lainz-Vienna, Austria, [3]Vet.Adm.Med. Center, Derm.Serv., Portland, USA.

Interleukin 6 (IL 6) is a cytokine with multiple biological activities and was previously known as interferon β2 (IFN β2), hybridoma growth factor (HGF), hepatocyte stimulating factor (HSF) and B cell differentiation factor (BSF2). Accordingly, IL 6 has antiviral activity, lymphocyte activating properties and may mediate many acute phase responses including fever. IL 6 is most commonly detected in fibroblast and monocyte cell culture supernatants. Since epidermal cells (EC) are known to release many immunoregulatory cytokines the present study was performed to investigate whether EC and epidermoid carcinoma cell lines (A431, KB) produce IL 6. Using a human IL 6 dependent cell line (B9) supernatants derived from freshly isolated EC and carcinoma cell lines contained significant levels of IL 6 like activity. Although EC released IL 6 spontaneously, factor production was significantly enhanced in the presence of PMA (50 ng/ml), UVB (20 mJ/cm2), LPS (50 ug/ml) and IL 1α (5 U/ml). An antiserum directed against IL 6 was able to block EC derived IL 6 in a dose dependent manner, indicating that both factors are closely related. Furthermore, 1.3 kb IL 6 mRNA expression was investigated. EC express little IL 6 mRNA spontaneously, but after induction with either IL 1α (5 U/ml) or IL 1β (5 U/ml) or PMA (50 ng/ml) abundant amounts of IL 6 mRNA were detected. Maximum IL 6 expression was observed within 6 hr and persisted over 24 hr. These findings indicate that EC in addition to other mediators of immunity and inflammation are also capable of synthesizing and releasing IL 6.

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EPIDERMAL CELL NATURAL KILLER CELL ACTIVITY AUGMENTING FACTOR (ENKAF) IS CLOSELY RELATED TO INTERLEUKIN 6 (IL 6). R. Kirnbauer[1], A. Köck[1], T. Schwarz[2], M. Micksche[3], T.A. Luger[1], [1]Dept.Derm.II and LBI for Dermven. Lab. for Cellbiol., Univ. of Vienna, [2]Dept.Derm., Hospital Lainz-Vienna, [3]Inst. Appl.Exp.Oncol., Univ. of Vienna, Austria.

Human epidermal cells (EC) and epidermoid carcinoma cell lines (A431, KB) have recently been reported to release a cytokine, which augments the activity of natural killer (NK) cells. In addition to its NK cell activity enhancing effects ENKAF also induces oxygen radical release by granulocytes and stimulates the proliferation of murine IL 3 dependent cell lines. ENKAF appears to be distinct from other known EC cytokines such as Interleukin, 2, 4, Interferon-gamma and colony stimulating factors. However, the biochemical properties of ENKAF were found to be similar to those of IL 6, a recently cloned multifunctional cytokine. Thus the present study was performed to investigate whether IL 6 is related to ENKAF. EC and A431 supernatants as well as purified ENKAF were found to stimulate the proliferation of an IL 6 dependent cell line (B9). Like ENKAF recombinant human IL 6 stimulated NK cell activity, oxygen radical release by granulocytes and the proliferation of murine IL 3 dependent cell lines. Moreover, an antiserum directed against IL 6 was able to block these biological activities of recombinant human IL 6 or ENKAF. In addition it was possible to demonstrate that human EC express 1.3 kb IL 6 mRNA. These findings strongly suggest that ENKAF is closely related if not identical with EC-IL 6.

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EICOSANOIDS IN EARLY PSORIATIC LESIONS. LEUKOTRIENE B_4, BUT NOT 12-HYDROXY-EICOSATETRAENOIC ACID IS PRESENT IN BIOLOGICALLY ACTIVE AMOUNTS IN ACUTE GUTTATE LESIONS. Karsten Fogh, Troels Herlin, and Knud Kragballe. Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark.

The biochemical events leading to the early clinical manifestations of psoriasis are unknown. Certain chemotactic eicosanoids derived from arachidonic acid metabolism have been suggested to play a pathogenic role in psoriasis, because of their presence in lesional psoriatic skin and their ability to elicite skin inflammation and to stimulate epidermal proliferation. The purpose of the present study was to elucidate which eicosanoids might be involved in the early phases of the development of psoriatic lesions. Eicosanoid levels were determined in acute guttate and chronic plaque psoriatic lesions. Eicosanoids were analysed both in scale and in the lesional skin below the scale. Methods for identification of eicosanoids included reversed-phase high-performance liquid chromatography combined with radioimmunoassay. Leukotriene B_4 (LTB_4) was present in acute guttate skin lesions in biologically active amounts (18.7 ± 7.1 ng/g wet tissue in scale, 3.2 ± 1.5 ng/g wet tissue in skin biopsy specimens). 12- and 15-hydroxy-eicosatetraenoic acid (HETE) only attained biologically active concentrations in scale of chronic plaque lesions (1,512 ± 282 and 1,441 ± 411 ng/g wet tissue, respectively). The level of prostaglandin E_2 in chronic plaque skin was similar to the level in normal skin, while the level was increased 2 fold in keratomed skin biopsy specimens of acute guttate lesions (78.0 ± 14.8 ng/g wet tissue). Furthermore, we found that the levels of LTB_4, 12- and 15-HETE were significantly higher in scale than in keratomed skin biopsy specimens. These results demonstrate that lipoxygenase products concentrate in scale, and that LTB_4 is present in acute guttate psoriatic skin lesions at concentrations able to exert biologic effects. LTB_4 may therefore be involved in the early inflammatory changes leading to the development of psoriasis.

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CHOLINERGIC STIMULATION ENHANCES ANTI-IgE-INDUCED IN VITRO HISTAMINE RELEASE FROM HUMAN PERIPHERAL LEUKOCYTES. Johannes Ring, Peter Thomas, and Helmut Ibel, Dermatologische Klinik, Ludwig-Maximilian University, Munich, W-Germany.

Altered reactivity towards autonomic nervous system transmitters as adrenergic and cholinergic stimuli is a well-known clinical feature of atopic diseases. Here we studied the influence of cholinergic stimulation upon in vitro histamine releasability of peripheral leukocytes from 41 atopics and 36 non-atopic controls. Direct stimulation with carbamylcholine (CC) showed little histamine liberating effect; however, when added simultaneously, it enhanced anti-IgE-induced histamine release (HR) in leukocytes of atopic donors. When anti-IgE was added after a ten minute preincubation with CC, a significant enhancement of anti-IgE-induced HR was observed both in atopics and controls. The CC-induced increase in HR was more pronounced in atopics. After addition of the muscarinic anticholinergic substance atropin in equimolar concentration the HR enhancing effect of CC was abolished. CC also enhanced HR induced by specific allergen stimulation, while preincubation with CC did not alter HR induced by non-immunological stimuli as Ca-ionophore. The data support the concept of the possible existence of a cholinergic muscarinic receptor on vasoactive mediator secreting leukocytes. They further stress the close interrelationship between autonomic nervous system and allergic inflammation, especially with regard to the pathophysiology of atopic diseases.

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HISTAMINE RELEASE AFTER PHYSICO-CHEMICAL STIMULATION OF HUMAN BASOPHIL GRANULOCYTES IN VITRO. Petra Spornraft, Bernhard Przybilla, Johannes Ring, Otto Braun-Falco, Department of Dermatology, Ludwig-Maximilians-University, Munich, FRG.

Altered releasability patterns of histamine have been described in atopic diseases. Mostly anti-IgE was used as stimulus. Here we investigated the effect of physico-chemical stimuli upon histamine release (HR) from human leucocytes of atopics and normals. Stimuli used were cold (+ 4°C), heat (40 - 43°C), mechanical trauma (Pasteur-pipetting), hypo- and hyperosmolar media (Aqua dest., NaCl 4.1%) and irradiation with UVA (10 J, 20 J/cm2). Spontaneous HR was higher in atopics (12.8%) compared to controls (7.6% of total release). Neither cold nor heat induced a significant change of HR except for one patient with localized heat urticaria who showed significantly rising HR with increased temperature. Mechanical trauma caused a dose dependent HR in all groups of patients. Stimulation with NaCl 4.1% induced a marked rise of HR more pronounced in atopics. There was no clear cut effect of hypoosmolar stimulation. Irradiation with UVA had no effect in polystyrol tubes, however, in macrotiter plates there was a significant increase of HR over the already elevated spontaneous release rates under these conditions. We conclude that physico-chemical alterations of leucocyte cell suspensions can affect histamine releasability. Suspensions of these cells should be handled carefully because of possible mechanical irritation. The increased spontaneous HR in atopics could reflect a non-specific increased releasability of mediators in these patients.

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EOSINOPHIL-CHEMOTACTIC ARACHIDONATE METABOLITES FROM HUMAN EOSINOPHILS. Eishin Morita, Jens-M. Schröder and Enno Christophers, Department of Dermatology, University of Kiel, FRG.

Whereas eosinophils (EOs) represent only a small fraction of peripheral blood cells, tissue eosinophilia is a characteristic feature of certain inflammatory skin conditions. Principles regulating influx of EOs into skin are not well understood. In contrast to neutrophils (PMNs), where production of as well as responsiveness to certain eicosanoids is well known, it is not clear whether EOs similarly produce eicosanoids with preferential activity for EOs.

In this study we report about arachidonate metabolites with apparent EO specific chemotactic activity derived from EOs.

Human EOs and PMNs were purified by Ficoll- and Percoll-gradient centrifugation. EOs were incubated with exogeneous arachidonic acid (10^{-4}-10^{-5} M) in the presence of Ca-Ionophore A 23187 (10^{-5} M) for 30-60 min. Metabolites were separated by reverse phase HPLC and peaks were identified by authentic samples and UV spectrometry. Aliquots of each fraction were tested for EO chemotactic activity as well as PMN chemotactic activity.

As a result we found two peaks with EO chemotactic activity: The first one eluted at the position of diastereometric 8,15-DiHETES and 5,15-DiHETE. The second peak with EO chemotactic activity eluted near the position of authentic LTB_4, the UV spectrum, however, was different from that to LTB_4. Apparently this second EO chemotactic activity is not derived exclusively from LTB_4. Using authentic 8,15-DiHETE and 5,15-DiHETE, we found potent EO chemotactic activity at a concentration range of 100-1000 ng/ml. In addition, these compounds showed only weak chemotactic activity for PMNs. We conclude that Ca-Ionophore stimulated EOs are able to produce potent chemotactic factors with apparent EO specificity.

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A BASOPHIL ACTIVATING FACTOR FROM LPS STIMULATED HUMAN BLOOD MONOCYTES. Hans-J. Hessler, Ulrich Mrowietz, Jens-M. Schröder, Enno Christophers, Department of Dermatology, University of Kiel, FRG.

Histamine release from basophil leukocytes can be induced by a variety of stimuli. Recently basophil activation by cell derived factors has been described. In the present study we isolated a basophil activating factor from human blood monocytes which is different from recently described cell derived basophil activating factors.

Human blood monocytes were isolated by adhesion to fibronectin coated culture flasks and incubated with LPS. After incubation, the cell free supernatant was concentrated on a YM 5 Amicon Diaflo membrane. The concentrated material was fractionated by Sephadex G-75 column chromatography. Fractions were collected and assayed for histamine releasing activity. The active fractions were pooled, concentrated and further purified by gel-filtration HPLC on a TSK 2000 column. The active fractions were pooled, concentrated and further separated by reversed-phase HPLC on a Nucleosil C-18 column. For assaying histamine releasing activity, basophils were isolated from peripheral blood by Ficoll-Paque density centrifugation. Cells were incubated with the column fractions in the presence of 5 ug/ml Cytochalasin B. Released histamine was determined fluorometrically after derivation with ophthalaldehyde and separation of the fluorescent products by HPLC. Total histamine content of the cells was determined after lysis with perchloric acid. LPS stimulated purified human blood monocytes produce a histamine releasing factor for human basophil leukocytes. The molecular weight was approximately 10 kD by HPLC gel filtration on TSK 2000. The factor induced dose-dependent histamine release up to 35% of the total histamine content of the cells. The retention times on TSK 2000 and RP-18 HPLC differed from IL-1 and $C5a_{des\ arg}$. Further, by deactivation experiments no cross deactivation with C5a/IL-1 was observed.

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PAF-ACETHER RELEASE DURING IN VIVO SKIN ANAPHYLACTIC REACTION. *L. Dubertret, *L. Michel, °Y. Denizot, *F. Jean-Louis, "C. de Vos, °Y. Thomas, °J. Benveniste, *INSERM U 312, Dermatologie, Hôp. Henri Mondor, F-Créteil, France, *INSERM U 200, rue des Carnets, F-92140 Clamart, France, "UCB, Pharmaceutical Sector, B-1420 Braine-l'Alleud, Belgium.

Using a skin chamber technique, we measured the in vivo release of the inflammatory phospholipid mediator paf-acether (paf) during the immediate (30 min) and the late (6 and 24h) phases of allergen-induced anaphylactic reaction. Six pollen-sensitive patients suffering from seasonal allergic rhinitis/conjunctivitis/asthma were investigated. Chambers overlaying skin blister base sites were filled for 6 h with either pollen (at a concentration that, when intradermally injected, induced a wheal-and-flare reaction), histamine (1 µg) or control buffer. All the chamber media were then replaced by buffer alone until the 24th h of experiment. At that time, media were collected, skin chambers removed and in order to study the recruitement of inflammatory cells, Rebuck's glass overslips were applied on the superficial dermis and hourly changed during 6 h.30 min-, 6 h-and 24 h-collected media were bioassayed for paf and its precursor content using rabbit platelet aggregation.

At 30 min, no significant amount of paf was detected in pollen-, histamine- and buffer-containing chambers. At 6 h, paf levels reached 2.8 ± 0.6 and 2.2 ± 0.4 ng/ml/cm2 (mean ± SEM) in chambers containing pollen and histamine respectively, as compared to 1.1 ± 0.4 ng/ml/cm2 of paf in control ones. At 24 h, despite replacement of the agonists by control solution since the 6th h, 3.8 ± 1.4 and 1.9 ± 0.6 ng/ml/cm2 were measured in media collected after pollen- and histamine-challenge respectively. At that time, very high levels of the paf precursors lyso-paf and (1-alkyl-2-acyl-glycero-3-phosphocholine (1-alkyl-2-acyl-GPC) were detected after pollen (490.3 ± 138.4 and 9808.5 ± 1857 ng/ml/cm2 respectively), whereas no significantly different levels of paf precursors were found in histamine and control chambers.

Moreover, 24 h after pollen challenge, numerous eosinophils (4.5 ± 2.1% of attracted inflammatory cells) were recruited on the superficial dermis whereas almost none were observed after histamine or control medium. Pollen-included eosinophil recruitement might be associated with the large paf amounts detected 24 h after allergen-induced reaction.

In conclusion, intense and long-lasting release of paf, lyso-paf and 1-alkyl-2-acyl-GPC as well as eosinophil recruitement are two specific events of allergen-induced cutaneous reaction in vivo.

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BIOSYNTHESIS OF PAF-ACETHER BY HUMAN SKIN FIBROBLASTS. *L. Michel, °Y. Denizot, *F. Jean-Louis, °Y. Thomas, °J. Benveniste, *L. Dubertret, *INSERM U 312, Dermatologie, Hôp. Henri Mondor, F-94010 Créteil, France, *INSERM U 200, 32 rue des Carnets, F-92140 Clamart, France.

We investigated the synthesis and release of the inflammatory mediator paf-acether (paf) by fibroblasts from normal human skin in vitro. Cultured fibroblasts were suspended in Tyrode's buffer containing 0.25% fatty acid-free bovine serum albumin at 37°C in the presence of 2 µM calcium ionophore A23187 (Io). Paf content in ethanolic extracts of both cell pellets and supernatants was measured using the usual bioassay of rabbit platelet aggregation.

As soon as the first min of Io-stimulation, fibroblasts produced paf (287 ± 92 pg/1x10^6 cells, mean ± SEM, N = 3). This production reached a maximum at 5 min (369 ± 85 pg/1x10^6 cells) and decreased thereafter. Half of the paf amount produced by fibroblasts was released in supernatants. No paf was detected in unstimulated samples. When fibroblasts were preincubated and stimulated in the presence of either 0.1 µM lyso-paf (the non-acetylated paf precursor) or 0.1 mM acetyl-coenzyme A, paf production was enhanced by 2-fold and 3-fold respectively. The paf molecule generated by fibroblasts was identical to synthetic paf since (1) it exhibited the retention time of synthetic paf during standard and reverse phase HPLC; (2) the platelet aggregation it induced was inhibited by the paf antagonist BN 52021 and by pretreatment of the molecule with phospolipase A_2 but not by lipase A_1.

The paf precursors lyso-paf and 1-alkyl-2-acyl-glycero-3-phosphocholine (1-alkyl-2-acyl-GPC) were detected in unstimulated fibroblasts. Under Io-stimulation, the level of 1-alkyl-2-acyl-GPC decreased whereas that of lyso-paf increased. As established by reverse phase HPLC and gas chromatography analysis, these precursors exhibited for 80% an hexadecyl and for 20% an octadecyl chain at the first carbon of the glycerol.

Our results demonstrate that human skin fibroblasts in vitro contain paf prescursors and, under Io-stimulation, produce paf. This may account for the pathogenesis of many acute and chronic inflammatory skin disorders.

Wednesday, 22 June, 1988

8.00-10.30

Concurrent session - Holl

J. KARVONEN, R. WILLEMZE, Chairpersons
Clinical Studies and Infetious Diseases
(Abstracts 154-165)

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CLASSIFICATION OF NON-PUSTULAR PSORIASIS: RISK OF DISEASE TRANSMISSION ACCORDING TO HLA-TYPE. Tilo Henseler, Enno Christophers, Department of Dermatology, University of Kiel, FRG.

Recent studies revealed the existence of two distinct subsets of non-pustular psoriasis which differ by HLA-antigens, heredity, age of onset as well as clinical course.

We now evaluated 256 psoriatic patients, their parents and their offsprings not studied before with respect to age of onset, family history of psoriasis, and the presence of psoriasis-releated HLA-antigens. In patients showing psoriasis of early onset and Cw6 (type I) the prevalence of psoriasis of parents is 14% and in siblings 24%. In late onset patients with psoriasis and negative Cw6 (type II) 5% parents or siblings are affected. Further, in type I patients 13.9% of the children were psoriatic in contrast to 2.5% in type II patients with psoriasis.

In patients presenting homozygous HLA-Cw6 antigen 50% of offsprings showed psoriasis.

Complete HLA identification in 256 psoriasis patients revealed that the combination HLA-Cw6, -A2, and -Bw57 is most often found in hereditary psoriasis. This supratype was identified in 18.6% of psoriasis patients of type I in contrast to less than 1.4% in normal population. HLA-B27 often associated with psoriasis arthritis was reduced in psoriasis vulgaris patients.

Though a multifactorial mode of inheritance of psoriasis is well established the risk of having psoriatic children can now be predicted more accurately by classification psoriasis subtypes together with analysis of the HLA antigens.

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ABNORMAL PHARMACOKINETICS OF THE CULPRIT DRUG IN TOXIC EPIDERMAL NECROLYSIS. P. Saiag, C. Capesius, J.C. Roujeau, R. Touraine, Department of Dermatology, University Paris XII, Créteil, France.

The pathophysiological mechanisms leading to toxic epidermal necrolysis (TEN) are still unknown. To look for abnormal drug-clearances in this situation, we measured the blood concentration of the culprit drug in 18 patients with TEN, including patients with AIDS-associated TEN.

Patients were 41 ± 22 years old. The mean body surface area was 40 ± 24%. The studied drugs were co-trimoxazole (9 patients, microbiological assay), isoxicam (5 patients, HPLC), phenobarbital (1 patient, fluorescence polarisation), carbamazepine (2 patients, HPLC) and salazosulfapyridine (1 patient, HPLC). In 3 patients, another potentially responsible drug was present. Blood samples were collected 9 ± 10 days after discontinuation of treatment. Results were compared to expected values, according to published pharmacokinetic data of the studied drugs and to the lag-time between discontinuation of treatment and sample collection.

Isoxicam plasma concentration was clearly abnormally elevated in 3 patients, normal in 1 (another potentially responsible drug was present in this patient) and undetectable in the remaining patient (blood sample was collected 1.5 month after discontinuation of treatment). Carbamazepine plasma concentration was elevated in 1 patient and undetectable in the remaining one. Phenobarbital plasma concentration was normal. Salazosulfapyridine plasma concentration was clearly abnormally elevated. When co-trimoxazole was the culprit drug, sulfamethoxazole (SMZ) serum concentration was assayed in 3 patients and was found abnormally elevated in 2 patients. Serum concentration of trimethoprim (a non responsible drug in inducing TEN) was assayed in 1 patients and was elevated in only 2 patients. The half-life of isoxicam, calculated in 2 patients, and the half-life of SMZ, calculated in one patient, were normal. The mean creatinine plasma concentration was 110 ± 37 µM/l. The mean aspartate amidotransferase level was elevated: 144 ± 185 U/l.

Blood concentrations of the culprit drug are often abnormally elevated in patients with TEN after discontinuation of treatment. These results were unexpected since fluid-loss in patients with TEN lead to elevated drug-clearances. The mechanisms leading to this drug accumulation are unknown.

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IMMUNE REACTIVITY OF PATIENTS WITH TOXIC EPIDERMAL NECROLYSIS, Martine Bagot, Michèle Heslan, Jean-Claude Roujeau, Patrick Dupuy, René Touraine and Louis Dubertret, INSERM U 312, Hôpital Henri Mondor, F-94010 Créteil, France.

The pathogenesis of toxic epidermal necrolysis (TEN) remains unknown. We have previously shown that a lymphopenia is observed in most patients and that the number of circulating pan-T and helper T cells are decreased whereas suppressor/cytotoxic cell counts remain unchanged.

In the present study, we tested the immune reactivity of 12 patients with drug-induced TEN. Patient peripheral blood lymphocytes (PBL) were collected in acute phase, early after the onset of cutaneous lesions and after recovery, one to three months later. Results were compared with an age and sex-matched group of healthy individuals. Lymphoid cells were studied for their proliferative response to phytohemagglutinin and to allogeneic lymphoid cells in mixed lymphocyte cultures, using patient PBL either as effector cells or as irradiated stimulator cells. Natural killer activity was measured as the percentage cytotoxicity in chromium-51 release assays with K562 target cells. Allogeneic cytotoxic T cell induction was measured after six days of culture in the chromium release test on PHA-blast targets autologous to stimulating PBL. Statistical analysis was performed using the nonparametric Wilcoxon paired test.

Results show that proliferative responses of patient PBL to phyto-hemagglutinin and allogeneic lymphoid cells, although slightly decreased, did not significantly differ from patient PBL after recovery and controls. In contrast, patient PBL in acute phase used as stimulator cells in mixed lymphocyte reactions consistently induced decreased proliferations of healthy PBL, when compared with patient PBL after recovery ($p < 0.05$) or controls ($p < 0.01$). Natural killing and allogeneic cytotoxic T cell activity were also significantly decreased, when compared with patient PBL after recovery ($p < 0.05$) or controls ($p < 0.05$). The immune reactivity of patient PBL after recovery did not differ from the controls.

We conclude that the reactivity of patient PBL in the early phase of drug-induced TEN does not strictly resemble the profound depression of all immunological functions observed in thermally injured patients. Cytotoxic activity is more depressed than proliferation, although the helper T cell subset was previously shown to be more decreased than the suppressor/cytotoxic T-cell subset. In addition, the most striking feature is the consistently impaired ability of patient PBL to induce proliferation of allogeneic lymphoid cells. This might be due to a reduced number of circulating activated mononuclear cells, trapped in the skin. Alternatively, it might be related to the specific activation of suppressor cells, as already described in burn patients.

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SEROLOGICAL ABNORMALITIES AND CLINICAL FEATURES OF SILICA ASSOCIATED SYSTEMIC SCLEROSIS. M.H.A. Rustin, Helen A. Bull, *V. Ziegler, *U.-F. Haustein, **P.J. Maddison and Pauline M. Dowd, Departments of Dermatology, The Middlesex Hospital, London and *Karl-Marx-Universität, Leipzig, GDR and **Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath.

Systemic sclerosis-like disorders have been reported to occur following exposure to vinyl chloride, aromatic hydrocarbon solvents, epoxy resins and silica[1]. We have studied 17 patients with systemic sclerosis (SS) associated with silica exposure to determine whether these patients have any characteristic findings which might distinguish them as a subgroup.

Seventeen male patients (mean age 58 years, range 47-70) with SS of a mean duration of 6 years (range 1-20 years) were studied. All the patients suffered from Raynaud's phenomenon (RP) and the mean interval between the onset of the RP and dermal sclerosis was 3 years. 14 patients had dermal sclerosis affecting the face, neck, forearms and fingers indistinguishable from that seen in acrosclerosis. 3 patients had generalised dermal sclerosis. 16 patients had pulmonary involvement, 10 had necrosis of the finger tips, 9 had oesophageal and 1 patient had renal involvement.

Nine patients had elevated levels of IgG, 7 of IgA and 3 patients had elevated levels of IgM. Circulating immune complexes were detected by PEG precipitation in 9 and lowered C3 levels were found in 2 patients. Using HEp-2 cells as substrate, antinuclear antibodies were detected in 16 patients with anti-centromere pattern in 2. 8 patients had the anti-Scl-70 antibody. Elevated levels of Factor VIII RAg were found in 10 patients.

Incubation of serum with cultured human umbilical vein endothelial cells for 24 hours caused a significant inhibition (with 20% serum $p < 0.001$) in calcium ionophore induced release of prostacyclin (measured by radioimmunoassay as the stable metabolic 6-keto-$PGF_{1\alpha}$) compared to control serum (CS). The mean release of 6-keto-$PGF_{1\alpha}$, decreased from 2.278 to 1.3647 ng/10^4 cells (SC) and from 1.9809 to 0.6439 (SS) with 1 and 20% serum respectively.

This study has shown that the majority of patients with silica associated systemic sclerosis have cutaneous features indistinguishable from acrosclerosis together with a high incidence of pulmonary involvement and the anti-Scl-70 antibody. In common with patients having idopathic systemic sclerosis they have evidence suggestive of endothelial cell injury and exhibit the serological factor which has the capacity to inhibit prostacyclin release from endothelial cells[2].

1. Haustein UF, Ziegler V, Int J Dermatol 1985, 24: 147-151.
2. Rustin MGA, Bull HA, Machin SJ, Koro O, Dowd PM, J Invest Dermatol 1987; 89: 555.

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ENZYMATIC DIAGNOSIS OF CONGENITAL DISORDERS OF KERATINIZATION, Mieke Bergers, P.D. Mier, R. van Dooren-Grebe, H. Traupe* and R. Happle. Department of Dermatology, University of Nijmegen, NL; *Department of Dermatology, University of Münster, FRG.

The membrane of Malpighian cells contain much phospholipid and glycolipid. Enzymes from the lamellar body remove the polar groups from these molecules to leave strongly hydrophobic lipids such as ceramide and free fatty acids. Since the lipid profile of the stratum corneum is altered in certain disorders of keratinization, we speculate that the enzymes contained in the lamellar body may be defective. We have therefore begun a screening programme to quantify the activities of these enzymes in the scales of patients with keratinization disturbances.

Results include the following (a) Six enzymes have been characterized; these seem to be distinct from the hydrolases occuring in the lysosome. (b) Two patients with chondrodysplasia punctata showed almost zero cathepsin B-like activity. (c) Six patients with erythrodermic autosomal recessive lamellar ichthyosis (EARLI) had relatively low β-glucosidase activities. (d) Ten patients with the non-erythrodermic form (NEARLI) showed low butyrase activities. The butyrase : glucosidase ratio gave a clear separation between these last two diseases (EARLI > 10, NEARLI < 3).

Enzymology has already become an accepted tool in the diagnosis of X-linked recessive ichthyosis. Here we show that this approach is of much more general applicability, and may help to clarify the nosology of the congenital disorders of keratinization.

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MULTILAYERED EPITHELIA RECONSTRUCTED BY CULTURE WITH KERATINOCYTES FROM JUNCTIONAL EPIDERMOLYSIS BULLOSA LETHALIS EXPRESS THE MAIN PHENOTYPIC CHARACTERISTICS OF THIS GENODERMATOSIS, *Luc Thomas, *Michel Faure, *Jean Kanitakis, *Frédéric Cambazard, **Jean-Paul Ortonne, *Jean Thivolet, * INSERM U 209 Hôpital Ed. Herriot, Lyon, France, ** Hôpital Pasteur, Nice, France.

Keratinocytes from a one-week-old male infant with junctional epidermolysis bullosa lethalis (HERLITZ type) were grown "in vitro" in absence of human dermal influence, then grafted as multilayered epithelia onto nude mice, in order to investigate the possibility that the defect in dermal-epidermal cohesiveness in the disease is of epithelial and not mesodermal origin. Cells in suspension were obtained through standard trypsinization of blister roof specimens and grown on irradiated 3T3 feeder layers, in immersed conditions in DMEM + Ham's F12 medium supplemented with 10% FCS, EGF, choleragen, hydrocortisone, insulin and adenin. The epidermal terminal differentiation was achieved by grafting the multilayered reconstructed epithelia onto nude mice, using a grafting chamber to stop the reepithelialization of the grafting bed by adjacent murine cells. In culture, keratinocytes did not behave like normal human ones: we noted a birefringent ring of cells at the edge of colonies with, at places, some cells as "ejected" from the periphery of the islets. After confluence was obtained, the multilayered epithelia were easily detachable from the culture flasks not only, as usual, with Dispase II, but also with mechanical disturbance only, which cultured epithelia from normal skin were not. By conventional microscopy the fully differentiated epithelium day 15, 20 or 30 after grafting showed occasional blistering at the dermal-epidermal junction. No labelling was noted with GB3 monoclonal antibody, which usually reacts with human normal keratinocytes in culture and with the basement membrane zone in normal skin, either "in vitro" before or after grafting. Our data indicate that the defect may be reproduced in culture and after grafting the cultured epithelia on a deepidermized wound and suggest a possible role for the epithelial structure recognized by GB3 in the dermal-epidermal cohesiveness.

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DETECTION AND ANALYSIS OF PHOSPOLIPIDS IN 3 SIBLINGS WITH HEREDITARY SENSORY AND AUTONOMIC NEUROPATHY TYPE II (HSAN-II), K. Bork, M. Böckers, R. Schopf and P. Benes, Department of Dermatology, University of Mainz, Mainz, FRG.

Skin symptoms are early and predominating features of the ulcero-mutilating sensory neuropathies. Three children in a turkish family were observed who developed from early childhood increasingly deformations of the feet and torpid ulcers with subfocal osteomyelitis and osteolysis, which subsequently led to amputations. The fingers showed ainhum-like constriction bands and spontaneous amputations. Neurologic studies revealed nearly complete sensory and autonomic loss affecting all modalities and in one patient a slight involvement of the motor fibres. The clinical symptoms fulfill many of the hallmarks of HSAN-II with autosomal recessive inheritance and onset of symptoms in childhood and mutilating acropathy. Blood group analysis showed that the two severely affected patients exhibit the blood-group O_{Bombay} which is extremely rare. The finding of birefringent material in urinary specimen led to analysis of urinary phospholipid excretion. The total amount of phospholipids was determined using an enzymatic assay (Bio-Merieux, Paris, France). There was a considerable urinary excretion of total phospholipids in the affected family members (5.98 mg/L; 0.20 mg/ml; 0.16 mg/L; normal values: 0 mg/L), and no excretion in the parents and in 4 healthy persons. In order to analyse the excreted phospholipids, thin layer silica gel chromatography was used. The concentrations of the separated fractions of sphingomyelin and lecithin were determined using the same enzymatic assay. In the affected subjects a considerable degree of sphingomyelin and lecithin was found. In the patient with the most severe clinical symptoms the concentration of lecithin was extremely high (5.2 mg/L). Gas chromatographic analysis of fatty acids within the two fractions confirmed analytically (by the ratio of palmitic to stearic acid), that the investigated substances were sphingomyelin and lecithin. Further gas chromatographic investigations were negative for sugar, so that the presence of oligosaccharides and therefore of ceramides could be ruled out. The findings suggest that the pathogenic mechanism may be a disorder in phospholipid metabolism.

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MACROLIDE-LINCOSAMIDE-STREPTOGRAMIN B (MLS) RESISTANT PROPIONIBACTERIA FROM ANTIBIOTIC-TREATED ACNE PATIENTS, E. Anne Eady, J.I. Ross, J.H. Cove, K.T. Holland and *W.J. Cunliffe, Dept. of Microbiology, University of Leeds and *Dept. of Dermatology, Leeds General Infirmary.

Erythromycin resistant (E^R) propionibacteria arise in some acne patients during oral erythromycin therapy and may be associated with therapeutic failure. In most bacterial genera erythromycin resistance is mediated by dimethylation of 23S ribosomal RNA resulting in reduced binding of three classes of antibiotics (macrolide e.g. erythromycin; lincosamide and streptogramin B). In order to ascertain whether propionibacteria possess a novel mode of resistance or have acquired mobile MLS resistance genes from other organisms, forty E^R strains isolated from the skin of acne patients were screened for the phenotypic expression of MLS resistance by determining their sensitivity to eight MLS antibiotics including clindamycin (a lincosamide) and virginiamycin S (a streptogramin B). Most of the isolates (21, 53%) were constitutively resistant to all eight antibodies, although individual MICs varied considerably. Two strains of P.granulosum exhibited inducible MLS resistance. Five isolates of P.acnes biotype I were phenotypically similar to the inducible strains but were not inducible by erythromycin. Eight isolates demonstrated high level resistance to macrolides (MIC ≥ 512 $\mu g.ml^{-1}$) but were sensitive to virginiamycin S. This latter phenotype is not compatible with the expression of MLS genes. Four isolates gave unique resistance patterns. Therefore, the majority of E^R propionibacteria express resistance which is phenotypically similar to that coded for by several well characterised methylase genes. E^R propionibacteria are now isolated from > 1 in 3 acne patients attending this Dermatology Clinic. Of 34 randomly selected erythromycin treated patients (including 13 non-responders) 46% carried E^R propionibacteria compared with 3% of 67 untreated controls ($p < 0.001$). Similarly 48% of patients treated with 1.5% clindamycin lotion also carried resistant propionibacteria ($p < 0.001$). Of 10 subjects shown not to carry E^R propionibacteria pre-treatment, four developed such organisms after 12-24 weeks oral erythromycin therapy. At 24 weeks resistant organisms remained a minor component (< 10%) of the total propionibacterial flora. Therefore it appears that, despite the high incidence of E^R propionibacteria, most patients do not experience therapeutic problems because resistance takes so long to develop. The future usefulness of erythromycin and clindamycin in acne management depends on avoiding prolonged (> 6 months) treatment regimes.

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SEROLOGICAL EVIDENCE FOR BORRELIA BURGDORFERI AS A POSSIBLE ETIOLOGIC AGENT IN MORPHEA, Uwe Neubert, Marianne Kauzmann, Michael Meurer, Johannes Gerstmeier and Thomas Krieg, Department of Dermatology, Ludwig-Maximilian-University, Munich, FRG.

The aim of our study was to find further serological evidence for the involvement of Borrelia burgdorferi infections in the initiation of morphea lesions.

Sera of 80 patients with different types of morphea of 2 months to 54 years (median 3 years) duration were examined by an indirect immunofluorescence assay using B.burgdorferi strain B 31 as antigen. Sera of sex-age-matched healthy persons and patients with other dermatoses (n = 50) were included as controls. Cross-reactive antibodies and false positive results caused by rheumatoid factor (RF) were eliminated by preabsorption of the sera with Treponema phagedenis and RF-absorbent.

Elevated antibody titers against B.burgdorferi were found in sera of 24 (30%) of the morphea patients (IgG n = 12, IgM n = 8, IgG and IgM n = 2). In contrast only 3 (6%) of the control sera showed elevated titers (IgG n = 2, IgM n = 1; p = 0.0008). Antibodies against B.burgdorferi were most frequently detected in atrophodermia idiopathica progressiva, followed by linear and plaque forms of morphea (40, 29 and 28% respectively being seropositive). Furthermore, in two seropositive cases morphea of the plaque type was combined with lichen sclerosus et atrophicans. In three patients the antibody titers decreased significantly after treatment of plaque-like morphea with penicillin. Antinuclear antibodies were present in 26% of the seropositive and in 39% of the seronegative sera.

In conclusion, serum antibodies to B.burgdorferi were significantly more frequent in patients with morphea than in controls. However, these findings should be interpreted with caution. An etiologic role of infection caused by B.burgdorferi seems possible at least in those patients with a history of tick bites, in whom the clinical exacerbation of morphea lesions was followed by in increase of antibody titers to B.burgdorferi or in whom a decline of antibody titers corresponded to the clinical improvement after penicillin therapy. It remains to be seen if such cases can be differentiated from classical morphea as a separate entity.

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MYCOBACTERIAL IMMUNE RESPONSE IN DERMATOSES, Pranab K. Das[1,2], Departments of [1]Dermatology and [2]Pathology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, NL-1105 AZ Amsterdam, The Netherlands.

Recent studies indicated that mycobacterial immunity (myc-imm) might be implied in autoimmune disease like rheumatoid arthritis (RA) [Lancet ii, 305-306, 1986]. In the present study, we carried out an extensive immuno-blotting analysis of antigen preparations (from M. tuberculosis, M. bovis, BCG and M. avium) using sera from controls and patients with DLE/SLE, RA, other dermatoses, leprosy and tuberculosis. We observed that 100% lepromatous lepra (L-lep), 100% tuberculoid lepra (T-lep), 75% tuberculosis (tb) patients showed antibody (Ab) activities to 29/33 kD, 64 kD and 58/40/18 kD cross species antigenic components of mycobacteria (CSAG-myc) respectively. Whereas > 80 kD CSAC-myc were reactive to 30% tb, 40% DLE/SLE and 30% of RA sera; in addition, 30% of both RA and DLE/SLE sera also reacted to 58/40/18 kD CSAC-myc. Further studies using ELISA serology with isolated antigens showed that DLE/SLE patients had significantly raised IgG/IgM Ab titre to > 80 kD in comparison to other patient groups whereas the IgG, IgM, IgA Ab titres to 64 kD, 29/33 kD, 58/40/18 kD and > 80 kD CSAC-myc could clearly differentiate between L-lep, T-lep, tb and patients with symptomatically non mycobacterial dermatoses. Additional studies on circulating immunocomplexes, rheumatoid factor IgM, autoantibodies and anti-DNA-idiotype in the sera of these patients strongly suggested, certain dermatoses might be related to myc-imm.

Acknowledgement: Thanks are due to late Prof. Dr. R.H. Cormane.

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LEISHMANIA MAJOR INFECTS AND REPLICATES WITHIN MURINE EPIDERMAL LANGERHANS CELLS, Anne Dompmartin, Alison T. Healy, Carol A. Nacy, Conrad Hauser, Monte S. Meltzer, Walter Reed Army Inst. Washington, DC 20307.

Leishmania major is a protozoan parasite of mononuclear phagocytes that causes cutaneous leishmaniasis. The parasite is transmitted through the bite of Phlebotomus sandflies. Sandfly bites are extremely superficial and it is likely that the first cells Leishmania encounter are those of the epidermis. Analysis of interactions between amastigotes of L. major and unfractionated murine epidermal cells (obtained from trypsinized epidermal sheets from normal C3H/HeN mouse ears) showed less than 1% of total cells were infected through 7 days of culture. All infected cells were phenotypically Langerhans cells defined by dendritic processes and presence of plasma membrane Ia antigen (immunofluorescence and immunoperoxidase cytochemistry). Infected Langerhans cells were detected in epidermal cell suspension (initial inoculum of 3 amastigotes/epidermal cell) at 24 hours and reached maximal levels of infection (about 25 to 40% of total Langerhans cells) at 48 hours. Keratinocytes remained uninfected through 7 days of culture. The number of parasites/infected Langerhans cell increased with time in culture: 1 parasite/cell at 24 hours increased to 2 to 4 parasites/infected cell at 96 hours. This increase with time suggests intracellular replication of the Leishmania parasite. The consequence to the host of initiating an infection intradermally is not yet known. Perhaps some of the more unusual aspects of the host immune response to Leishmania can be attributed to antigen-presentation by these infected cells. The recent observations that human immunodeficiency virus also infects Langerhans cells suggests that interactions between infectious pathogens and Langerhans cells may be more frequent that previously thought.

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SPECIFIC IGE-BINDING TO SCYBALA OF SARCOPTES SCABIEI VAR. HOMINIS, M. Böckers and K. Bork, Department of Dermatology, University of Mainz, Mainz, FRG.

IgE seems to be involved in the pathogenesis of scabies. Investigations using RAST or prick test revealed in a few patients the presence of anti-mite IgE. The site and nature of the scabies-mite antigen, however, is not exactly known. In order to identify the antigen location in the mite where the specific IgE-antibodies are bound, we performed an indirect immunofluorescence assay with mites and specific IgE of a patient.

The scales of a female patient with scabies norvegica contained an excessive number of mites, which were used as antigen as well as scales of four patients with "normal" scabies. The material was fixed on a transparent adhesive strip. In order to avoid non-specific binding, the scales were at first incubated with horse serum 1:10 in PBS. Subsequently, the adhesive strip was incubated with fresh serum of the patient with scabies norvegica in dilution 1:1 and 1:10 in PBS. The binding of specific IgE was demonstrated with a polyclonal FITC-labelled rabbit anti-IgE (Behringwerke, Marburg, FRG) in dilutions from 1:10 to 1:50. All samples showed a strong staining of the scybala of the mites, while body proteins of adult mites and the eggs did not show any binding at all. In the same way, the fecal material adherent to mite-nymphs fixed the IgE-antibody. Controls using anti-lysozyme and FITC-labelled IgE alone were negative. In none of the patients IgE-antibodies against D.pteronyssinus were found.

The results demonstrate that only the scybala of adult scabies mites and the fecal material of nymphs exhibit a specific IgE-binding and not any part of the body of the mite herself.

Wednesday, 22 June, 1988

10.45-11.45

Cuncurrent session – Cuvilliés

K. THESTRUP-PETERSEN, J. KNOP, Chairpersons

Contact Allergy (Abstracts 166-170)

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LOW DOSE EXPOSURE TO ANTIGEN INDUCES SUBCLINICAL SENSITISATION, P.S. Friedmann and J. Rees, Dermatology Department, Royal Victoria Infirmary, Newcastle upon Tyne, U.K.

We have previously shown that the immune responses to dinitrochlorobenzene (DNCB) follows predictable log-dose response relationships[1]. After exposure to small doses of antigen ($<ED_{100}$) only a proportion of subjects manifest clinically detectable sensitivity upon subsequent challenge. We wished to see whether these subjects have in fact been 'primed' to a state of sub-clinical sensitivity - in which case they should give boosted responses to a second sensitising stimulus. In a preliminary study the 50% effective sensitising dose (11 µg) of DNCB was applied to a 3 cm diameter circle on the forearm of 12 subjects. Four weeks later they were challenged with doses of 25, 12.5, 6.25 & 3.125 µg DNCB applied on standard 1 cm diameter patch test felts (Al test). The responses were measured as increase in skinfold thickness at 48 hours with Harpenden callipers. Six subjects (50%) were unresponsive; they were challenged again 4 weeks later and all responded: the mean response at the 12.5 µg challenge was 2.88 mm (SE 0.51). Since the challenge regimen (total 46.75 µg) has moderate sensitising potential, 12 controls were sensitised with the challenge regimen and rechallenged 4 weeks later. Compared with the experimental group they were significantly less responsive; at 12.5 µg challenge the mean was 1.28 mm (SE 0.37) P = 0.027, Anova. To see if such subclinical sensitisation would occur at even lower priming doses the experiment was repeated using the 20% sensitising dose. Fourteen subjects received 70 µg of DNCB. 11/14 (78%) showed no response to challenge at 4 weeks so were given a second challenge 4 weeks later. All responded: mean at 12.5 µg challenge was 2.05 mm (SE 0.34). 18 control subjects sensitised with the challenge regimen gave significantly lower responses at all challenge doses: e.g. at 12.5 µg challenge the mean response was 1.12 mm (SE 0.19), P < 0.02, Anova. Thus low sensitising doses of antigen induce a state of sub-clinical sensitisation which causes a boosted response to a second sensitising stimulus. Moreover, this effect is related to priming dose.

1. P.S. Friedmann, C. Moss, J.M. Simpson, S. Shuster (1983) Clin Exp Immunol, 53: 709-715.

167

AREA OF APPLICATION DOES AFFECT STRENGTH OF SENSITISATION BY DNCB, J. Rees and P.S. Friedmann, Department of Dermatology, University of Newcastle upon Tyne, U.K.

Sensitisation of humans by dinitrochlorobenzene (DNCB) has been shown to follow predictable log-dose response relationships[1]. We previously showed that when the area of application was kept constant changes in concentration of DNCB produce large differences in the degree of sensitisation[2]. By contrast, when the concentration per unit area of applied DNCB was kept constant changes in area of application over a defined range produced no significant change in sensitisation. This suggested that the density of antigen per Langerhans' cell was a more important determinant of sensitisation than the absolute number of Langerhans' cells with antigen. We have extended our study of this problem to see whether this applies at much smaller numbers of Langerhans' cells. Two groups of subjects were studied: in one 30 µg of DNCB was applied to an area of 0.8 sq.cm, in the other 3 µg was applied to 0.08 sc.cm. In both groups the dose per unit area of DNCB is the same (38 µg/sc.cm). One month later subjects were challenged with three doses of DNCB (8.8, 12.5, 17.7 µg) and increase in skinfold thickness at 48 hours measured with modified Harpenden callipers. There was a highly significant difference between the groups at all challenge doses ($p < 0.001$): the mean response for the 0.08 µg sc.cm group at the challenge dose of 12.5 µg was 0.3 mm (SE 0.1) compared with 1.6 mm (SE 0.3) for the 0.8 sc.cm group. This indicates that within the range of small areas tested here the total amount of antigen is of crucial importance in determining the magnitude of sensitivity. However our previous work using larger areas of application showed that when the concentration of DNCB was constant, increasing the amount of DNCB by increasing the area of application produced little effect on degree of sensitivity. This suggests that above a certain level simply increasing the number of Langerhans' cells presenting antigen does not significantly augment the afferent signal - although the strength of sensitising stimulus is augmented by increasing the number of antigen molecules per Langerhans' cell.

1. Friedmann PS, Moss C, Simpson JM, Shuster S (1983), Clin Exp Immunol, 83: 709-715.
2. White SI, Friedmann PS, Moss C, Simpson J (1986), 115: 663-668.

Wednesday, 22 June, 1988

10.45-11.45

Concurrent session – Effner

G. LANGE WANTZIN, H. GROSS, Chairpersons
Virology (Abstracts 171-175)

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SUCCESSFULL NICKEL PRESENTATION TO SENSITISED T LYMPHOCYTES REQUIRES PROCESSING OF THE ALLERGEN, E.P. Prens, K. Benne, H. Bremer, Th. van Joost and R. Brenner, Department of Dermatology and Immunology, Academic Hospital Rotterdam-Dijkzigt and Erasmus University, P.O. Box 1738, NL-3000 DR Rotterdam, The Netherlands.

In vitro nickel stimulated sensitised inducer T lymphocytes do not proliferate in the absence of antigen presenting cells (APC). Antigen presentation is thus necessary in this MHC class II restricted system and is performed optimally by epidermal Langerhans cells. On a per cell basis peripheral blood dendritic cells are less efficient. We investigated whether processing of nickel by peripheral blood APC is required for successful presentation to and activation of inducer T cells. Therefore peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-metrizoate density gradient centrifugation. T cells were purified by rosetting with AET treated sheep red blood cells. *Enrichment* of peripheral blood dendritic cells was performed by panning or rosetting using human IgG coated dishes or ox red blood cells. Antigen processing by APC was inhibited by incubation with the lysosomotropic agent chloroquine (10^{-4} M) for 1 hour. APC were fixed by incubation of these cells in 0.05% glutaraldehyde. Nickel was added to the culture in a final concentration of 5×10^{-5} M. Pulsing of APC was done by incubation for 2 hours with 6.6×10^{-4} M nickel. Immediately thereafter the cells were washed three times and added to the culture (*in vitro* antigen pulsing). Processing of Purified Protein Derivative (PPD) was included in this study and served as a positive control. Proliferation was measured by adding 0.5 µCi ^{3}H-thymidine to the culture, on days 6, 7 and 8 of culture, 8 hours before harvesting. The results showed that when nickel was added to purified T cells no proliferation occurred; this occurred only in the presence of APC. Fixation of APC prior to exposure to nickel resulted in a dramatic, almost complete inhibition of nickel specific T lymphocyte proliferation. Treatment of APC with chloroquine followed by nickel pulsing also resulted in a marked inhibition of T lymphocyte proliferation. Normal proliferation occurred if APC were fixed with glutaraldehyde or treated with chloroquine after *in vitro* nickel or PPD pulsing. *In conclusion:* Intracellular processing of nickel by APC is essential for induction of nickel specific T lymphocyte proliferation. We postulate a "reverse processing pathway" in which nickel ions are coupled to peptides, associated with MHC class II molecules, and presented to T lymphocytes in an immunogenic form.

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EPIDERMAL DERIVED LYMPHOKINES AND THEIR PRESENCE IN ALLERGIC AND IRRITANT SKIN REACTIONS, Christian Grønhøj Larsen, Thomas Ternowitz, Frederic Grønhøj Larsen and Kristian Thestrup-Pedersen, The University of Aarhus, Department of Dermatology, Marselisborg Hospital, DK-8000 Aarhus C, Denmark.

We have studied epidermal-derived lymphokines in 32 patients with allergic or toxic eczema of persons without skin diseases. We have performed patch testing in patients with known type IV cutaneous allergy or we tested the skin using 3% sodium lauryl sulphate in petrolatum in order to induce an irritant reaction. After 48 hours we used the suction blister technique to secure epidermal tissue and studied its content of interleukin 1 (Il-1) using the C3H thymocyte assay, and the presence of a newly described epidermal lymphocyte chemotactic factor (ELCF).

Il-1 was found in normal skin. During the evolution of an allergic patch test, Il-1 increased 2.8 fold in the test area and 1.9 fold in a non-test area compared with pre-test values. However, Il-1 was not increased in epidermis overlying an irritant skin patch test.

ELCF was not present in normal skin. However, ELCF activity could be measured in epidermis overlying a positive allergic patch test. Similar studies of a toxic-irritant patch test using sodium lauryl sulphate showed that ELCF was increased contrary to Il-1.

Il-1 and ELCF may thus be important lymphokines in immune-mediated skin inflammation. Preliminary studies of the time course in patch tests have shown that both Il-1 and ELCF exists in epidermis before the clinical appearance of a positive allergic patch test.

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IRRITANT SKIN REACTIONS DEMONSTRATE, IN CONTRAST TO ALLERGIC REACTIONS, DECREASED NUMBER AND FUNCTION OF EPIDERMAL $T6^{+}DR^{+}$ LANGERHANS CELLS. S. Lisby, O. Baadsgard, C. Avnstorp, O. Clemmensen, K.D. Cooper, G. Lange Wantzin, Laboratory of Immunology, Department of Dermatology, Bispebjerg Hospital, Department of Dermatology, Gentofte Hospital, Copenhagen, Denmark; Department of Dermatology, University of Michigan, Ann Arbor, MI, USA.

Antigen specific activation to T cells is dependent on antigen presenting cells (APC) that express major histocompatibility complex class II molecules such as HLA-DR (DR). Since the clinical picture and histopathology of irritant and allergic reactions often are indistinguishable we found it important to investigate immunological features during such reactions. Suction blister derived epidermal cells (EC) in suspension were derived from sites of irritant and allergic skin reactions at several time-points following challenge. Using monoclonal antibodies the number of antigen presenting $OKT6(T6)^{+}DR^{+}$ LC and $OKM5(M5)^{+}DR^{+}$ EC was determined. The functional antigen presenting capacity of the EC was determined by adding various numbers of EC to allogeneic mononuclear cells and the resultant T-cell proliferation was monitored using 3H-TdR uptake. Despite the appearance of an $M5^{+}DR^{+}$ EC population following irritant challenge, the total number of DR^{+} EC decreased from (mean +/- SEM) 3.1 +/- 0.2% to 1.2 +/- 0.1% (N = 9). This occurred in a time-dependent fashion. Concomitantly, the APC function decreased to 48 +/- 12% of control level (N = 4). In contrast, allergic reactions resulted in an increased number of DR^{+} EC. This was due to an increase in the number of $T6^{+}DR^{+}$ LC from 2.2 +/- 0.3% to 3.7 +/- 0.7% (N = 4), and to the appearance of 1.3 +/- 0.4% $M5^{+}DR^{+}$ non-LC EC. This increased number of DR^{+} EC was related to a 25 +/- 4% increase in antigen presenting capacity of EC in all patients.

In conclusion, allergic reactions resulted in an increased number of epidermal antigen presenting cells and an enhanced immunreactivity. This was in contrast to irritant reactions which resulted in decreased immunreactivity of the skin. Thus, irritant and allergic reactions have different immune patterns.

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CD4-EXPRESSION ON MONOCYTES OF PATIENTS WITH HIV-INFECTION, P. Thomas, P. Rieber, G. Riethmüller, J. Ring, G. Rank, Department of Immunology and Dermatology, Univ. School of Medicine, Munich, W.-Germany.

The tropism of HIV-1 for CD4+ cells can explain the reduction of T-helper/inducer lymphocytes in patients infected with HIV-1. Since monocytes/macrophages also express the CD4 antigen we asked whether CD4+ peripheral monocytes are also reduced in HIV-1+ patients. By using a sensitive erythrocyte rosetting technique, we first removed T-lymphocytes by density gradient centrifugation and subsequently enriched monocytes by ox erythrocytes coated with the monocyte specific monoclonal antibody M-M42. In healthy controls we found about 50-70% CD4+ monocytes (n = 12). HIV+ individuals showed a distinct decrease in CD4+ monocytes (20-30%, n = 10), whereas there was no difference in the number of circulating monocytes between the two groups. The role of monocytes as HIV carriers deserves further investigation.

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IMMUNOHISTOCHEMICAL STUDY OF THE SKIN OF HIV1-INFECTED PATIENTS. J. Kanitakis, D. Schmitt, J. Thivolet, C. Marchand, INSERM U 209, Clinique Dermatologique, Hôp. Ed. Herriot, F-69437 Lyon, France.

The Human Immunodeficiency Virus (HIV) presents a selective tropism for CD4+ cells, mostly T-helper lymphocytes and macrophages. In the skin, Langerhans' cells (LC) have been claimed to represent a potential target of HIV, since they express the CD4 antigen. In this work, we studied clinically normal skin of 16 HIV1-infected individuals (13M, 3F; CDC group II:8, group III:1, group IV:9), in order to investigate a) the potential expression of HIV antigens by skin cells, b) the number of epidermal LC during HIV-infection. Skin biopsies were obtained from the buttock and examined with monoclonal antibodies (mca) to the viral proteins p18, p24 and gp160. A HIV-infected promonocyte lineage (U937) was used as positive control. LC were revealed by an anti-CD1a mca (BL6); their number was evaluated by using a semi-automatic analyzer and expressed per mm2 of 4µ-thick epidermal section. Some cases were also studied by electron microscopy (EM). Mca to viral proteins were strongly positive on U937 cells. On skin sections, the mca to p18 revealed in all cases a cytoplasmic labelling of epidermal basal cells. However, the same reactivity was observed on *HIV-negative control* skin specimens, and was therefore interpreted as a cross-reactivity with an irrelevant antigen. Aside from this reactivity, no labelling could be observed with any of the three mca used. By EM, retro-viral particles were seen in U937 cells, but not in the skin specimens studied. On the other hand, the number of CD1a+ LC did not vary significantly among the different CDC groups (group II: 161 +/- 41, III: 189, IV: 155 +/- 12 per mm2), nor according to the number of circulating CD4+ lymphocytes (< 200/mm3: 149 +/- 48, > 200/mm3: 166 +/- 38 per mm2). These results do not support the possibility of detecting HIV-infected epidermal cells using mca to viral antigens, at least at the light-microscopic level. They also suggest that the number of CD1a+ LC in clinically normal skin is not directly correlated to the course or the severity of the HIV-infection. It seems reasonable to envisage that functional alterations (such as antigen-presenting capacity), rather than a numerical decrease, may reflect the impact of HIV infection on LC.

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ORAL HAIRY LEUKOPLAKIA, VIRAL CHARACTERIZATION EMPLOYING IN SITU HYBRIDISATION METHOD, Dario Tomasini, Amilcare Cerri, Stefano Cavicchini, Elvio Alessi, Emilio Berti, 1st Department of Dermatology, University of Milan, Milan, Italy.

Hairy leukoplakia (OHL), a newly described disease of the oral mucosa, had a remarkable importance in the infective, odonthoiatric and dermatological field because it is strongly indicative of HIV infection and evolution to AIDS. The aim of our study is identification of viral type involved in this disease. Six biopsies obtained from HIV positive patients suffering from OHL have been partly fixed in Bouin and embedded in paraffin, partly snap frozen in liquid nitrogen and partly fixed in glutaraldehyde 3% and OsO4 and prepared for electron microscopy (EM) studies. For viral characterization we have employed monoclonal antibodies (Mabs) specific for Herpes Simplex virus type I and II (HSV-I, HSV-II), Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), monoclonal and polyclonal sera against Papilloma Virus group (HPV), and DNA biotinylated probes specific for EBV (Ortho Diagnostic), HPV-1 (Ortho Diagnostic), HPV-16, HPV-18 (kind gift from Prof. zur Hausen). Immunohistochemical reactions have been performed using an Alkalyne Phosphatase or an Immunofluorescence method; in situ hybridisation (ISH) has been employed according to the methods of Brigati[1] and Brahic and Haase[2] to characterize specific viral involvement. Immunohistochemical characterization showed a strong nuclear positivity for EA-D, VCA, MA, EBNA-EBV antigens in all the tested cases; polyclonal and monoclonal antibodies against HPV group demonstrated nuclear positivity in four of six cases; HSV-I HSV-II and CMV were negative in all the tested cases. EM analysis of tissue samples always showed nuclear and intercellular presence of Herpesviridiae family viral particles; in two of six cases nuclear Papovaviridae family viral particles were also detected. ISH, employing EBV biotinylated probe, in high stringent conditions (50% deionized Formamide), showed Koilocytes nuclear positivity: HPV-1, HPV-16 DNA biotinylated probes were negative both in low (20% deionized Formamide) and high stringent condition. HPV-18 DNA biotinylated probe was positive in low stringent condition only in one case. These data suggest that EBV plays a primary role in the pathogenesis of this disease. The discordances among EM, immunohistochemical and ISH analysis regarding the significance of HPV reflect those already reported in the literature from other authors[3] and nowadays do not permit to clarify the pathogenetic role played by HPV.

1) Brigati D.J. et al., J of Virology, 1983, 126: 32-50.
2) Brahic M. et al., PNAS USA, 1978; 75: 6125-32.
3) Greenspan J.S. et al., NEJM, 1985, 313: 1562-71.

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HUMAN PAPILLOMAVIRUS (HPV) INFECTIONS IN RENAL TRANSPLANT PATIENTS. Y. Chardonnet, I. Guérin-Reverchon, J. Vlac, M.C. Chignol, S. Euvrard, J. Thivolet, INSERM U 209, CNRS UA601. Clinique Dermatologique, Pav. R, Hôpital E. Herriot, F-69374 Lyon Cédéx 03, France.

Cell mediated immunity plays an important role in HPV infections. Recurrent and persistent lesions are frequently observed in patients with compromised cell immunity. We examined 6 patients who had a renal transplantation 8 to 15 years ago. They developed profuse verrucosis within 4 to 7 days post transplantation. Multiple exophytic or plane warts were mainly first located on the back of hands, on the face or on the legs. Further lesions were seen later on uncovered zones of trunk or on the back of hands which were histologically recognized as squamous cell carcinoma (SCC) or keratoacanthoma (KA). We studied 18 different paraffinized or frozen biopsies taken from the 6 patients at various times after appearance of their lesions, for the presence of: 1. group specific viral antigen by indirect immunofluorescence, using a rabbit antiserum raised against SDS dissociated purified virus; 2. viral DNA by in situ hybridization with biotinylated probes (HPV types 1, 2, 6, 11, 16 and 18, under stringent conditions) and visualization of DNA-DNA hybrids with alkaline phosphatase.

In 8/18 biopsies, no viral DNA was detectable. Among 10 positive samples, several types were recognized: HPV type 1 (8), 2 (7), 16 (4) and 18 (7). Four of these lesions (2 SCC and 2 KA) from patient A were positive with 3 HPV probes, types 1.2 and 18. Both HPV 1 and 2 were found in 1 SCC of patient A and 1 KA of patient B: both HPV 2 and 18 were detected in 1 SCC of patient A: HPV 16 and 18 were also found in 2 SCC of patient E. One lesion from subject A showing typical histological features of vulgaris hand warts was positive only with HPV type 2. Only this specimen displayed group specific HPV antigen. The results obtained with in situ hybridization technique are compared with Southern blot on frozen tissues.

Our data show the presence of HPV DNA from common types (1 and 2) and oncogenic types (16 and 18) in SCC and KA developed on uncovered areas of renal transplant patients, several years after their surgery. In such patients, both their immunodeficient treatment and UV irradiation might play a crucial role in the recurrence of benign warts, their further evolution towards malignancy and coexistence of several HPV types in single lesions.

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CONDYLOMA ACUMINATUM-LIKE WARTS ON THE MAMILLA: DETECTION OF A NEW PAPILLOMAVIRUS TYPE AND SUCCESSFUL TREATMENT WITH INTERFERON ALPHA 2c, Gerd E. Gross and Herbert Pfister, Department of Dermatology, Univ. School of Medicine, Hamburg and Institute for Clinicial and Molecular Virology, University Erlangen-Nürnberg, W,-Germany.

Condylomata acuminata are papillomavirus-induced benign fibroepithelial tumors which are preferentially associated with the virus types HPV 6 and HPV 11. Condylomata acuminata are venereally transmitted and almost exclusively located at the lower genital tract.

We report about a 19-year-old male patient suffering from atopic dermatitis and recalcitrant condyloma-like warts located on the left mamilla. Prior to our therapy the warts were present over a period of 11 months increasing continuously in size despite podophyllin treatment. Histologically features of condyloma acuminatum were present and immunocytochemically papilloma-virus capsid antigens were identified moderate in nuclei of superficial epidermal cells using an antiserum against papillomavirus common antigen. The HPV DNA extracted from a punch biopsy was digested by the endonucleases BamH I, Hind III, EcoR I and Hpa I. The fragments received after digestion with Hpa I had a total molecular weight of 8.5 kb (5.0 kb and 2.6 kb each) which were significantly larger as compared to other HPV types (7.9 kb). Southern blot analysis revealed a comparable homology to HPV 6 and HPV 13. The latter virus type is regularly found in the orally located Heck's disease. Interestingly the viral DNA from the skin lesion of our patient hybridised only to a lesser intensity with HPV 11 which is in contrast to the classical HPV 6 DNA showing a strong hybridisation with HPV 11 but a minor hybridisation with HPV 13. These data suggest, that the isolate represents a new, as yet unclassified HPV type.

The warty lesions were treated at intervals with the systemic application of recombinant interferon (rIFN) alpha 2c (Thomae/-Boehringer Ingelheim, W.-Germany). RIFN alpha 2c was given at low doses (5 x 10^6 I.U. per day) over 7 consecutive days subcutaneously (lateral abdominal skin) followed by a therapy-free period of 4 weeks. After 3 therapeutic cycles the warts disappeared completely. Healing was accompanied by itching and moderate inflammation restricted to the warty lesions. The remission has been maintained for 14 months so far.

Wednesday, 22 June, 1988

10.45-11.45

Concurrent session - Holl

I. LEIGH, G. SCHULER, Chairpersons

Keratinocyte Immunology (Abstracts 176-180)

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ANTIGEN PRESENTATION IN THE AUTOLOGOUS MIXED EPIDERMAL CELL - T LYMPHOCYTE REACTION IN PSORIASIS, Th van Joost, E.P. Prens, K. Benne and R. Benner, Departments of Dermatology and Immunology, Academic Hospital Rotterdam-Dijkzigt and Erasmus University, P.O. Box 1738, NL-3000 DR Rotterdam, The Netherlands.

Recently activation and proliferation of T lymphocytes upon stimulation with autologous epidermal cells from psoriatic skin has been described (Schopf et al. 1986). We reproduced these studies and investigated the cells involved in antigen presentation in the autologous mixed epidermal cell - T lymphocyte reaction (MECLR). Therefore single epidermal cell suspensions (ECS), of involved and uninvolved psoriatic skin, were prepared by enzymatic digestion. T lymphocytes were isolated by rosetting with AET treated sheep red blood cells. The ECS was depleted of Langerhans cells by complement lysis using cytotoxic anti T6 and anti F_cR antibodies and by immunomagnetic separation. Monocytes were obtained from peripheral blood by plastic adherence or from density gradient interphase after AET rosetting. Enrichment for dendritic cells was performed by panning or rosetting using human IgG coated dishes or ox red blood cells. APC were fixed by incubation for 1 min in 0.05% glutaraldehyde. Results: Peripheral blood mononuclear cells as well as purified T lymphocytes from psoriasis patients showed a clear proliferative response to autologous epidermal cells from involved as well as uninvolved skin. In a control group of healthy persons and patients with contact allergy no significant T cell proliferation was observed in the autologous MECLR. In patients with contact allergy proliferation only occurred when antigen was added to the culture. In another control group consisting of patients with atopic dermatitis we also observed T cell reactivity to autologous epidermal cells. This response, however, has been shown to be due to persistence of antigen (e.g. house dust mite antigen) in the epidermis of these patients. The autologous MECLR involves HLA-DR since this response could be blocked by anti HLA-DR monoclonal antibody. Peripheral blood dendritic cells appeared to be more effective APC than peripheral blood monocytes, but epidermal APC were most efficient. Remarkably, depletion of (T6+) Langerhans cells from the ECS only resulted in a slightly decreased (mean: 15%) proliferation. In conclusion: These data show that epidermal cell specific autoreactive T cells occur in psoriasis patients. HLA-DR+/T6- APC present an "epidermal antigen" to these T cells. Based on these data psoriasis can be classified as a T cell mediated autoimmune disease.

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DETECTION OF CLASS II MHC GENE TRANSCRIPTION IN KERATINOCYTES CO-INCUBATED WITH INTERFERON-GAMMA, J.N. Barker, *L.A.P. Kerr, +H. Navsaria, +I. Leigh, *K.I. Welsh, D.M. MacDonald, Laboratory of Applied Dermatopathology and *Molecular Immunogenetics Laboratory, Guy's Hospital, London and +Experimental Dermatology Laboratory, The London Hospital, London, G.B.

Immunocytochemical studies have demonstrated that HLA-DR can be detected on the surface of human keratinocytes co-cultured with interferon-gamma (IFN-), while other studies have shown specific cell surface receptors for IFN- on human keratinocytes. IFN- may, therefore, induce HLA-DR surface expression either by modulating gene transcription or by increasing protein translation. To determine the effect of IFN- on human keratinocytes, class II MHC mRNA production was assessed both in resting and in stimulated keratinocytes.

Virally transformed human keratinocytes (SVK 14 cells) were used as an experimental model having established that they possessed identical characteristics to normal human keratinocytes when incubated with IFN-. Keratinocytes were cultured under standard conditions with recombinant human IFN- (Alpha-Therapeutic, W.-Germany), concentration 10 units/ml, for 48 hours. Surface expression of HLA-DR was confirmed by a standard immunoperoxidase technique. Total cellular RNA was extracted from the keratinocytes (10 cells approximately) and run on denaturing agarose gels with positive (B-lymphocytes) and negative (T-lymphocytes) controls for class II MHC mRNA. After Northern blotting class II mRNA was detected by hybridisation with radio-labelled cDNA probes for DR and DR chain mRNA.

Detectable levels of HLA-DR mRNA were obtained from keratinocytes incubated with IFN- but not from control keratinocytes. These results suggest that IFN- acts at the level of gene transcription to induce keratinocyte HLA-DR protein biosynthesis.

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EFFECTS ON RAT T-CELL PROLIFERATION BY SYNGENEIC EPIDERMAL CELLS EXPOSED TO τ-INTERFERON IN VIVO, Curt Skoglund, Annika Scheynius and Per Larsson*, Department of Clinical Immunology, Karolinska Institute, Stockholm and *Department of Medical and Physiological Chemistry, University of Uppsala, Uppsala, Sweden.

Normal epidermis contains one type of class II transplantation antigen expressing dendritic cell, the Langerhans cell, which is known to be antigen presenting. In many conditions in the skin, however, keratinocytes are induced to synthesize and express class II antigens. The biologic properties of this induced expression of class II antigens is still not fully understood.

In this study, expression of class II antigens on keratinocytes was induced in rats by daily intradermal injections of 10.000 U rat recombinant τ-interferon (IFN-τ) into the ears of two months old Lewis rats for three consecutive days. Epidermal cell suspensions were prepared from these ears as well as from ears of untreated rats. IFN-τ exposed epidermis expressed class II antigens on more than 50% of the cells as judged from immunocytochemical staining. In normal epidermal cell suspensions the numbers of positive cells were less than 5%. Titrated numbers of irradiated (1500 rad) epidermal cells, IFN-τ exposed or not, were co-cultivated on 0.2 ml medium with a syngeneic T-cell line specific for PPD in triplicate cultures, with or without PPD_1 (10 µg/ml). The proliferation of the T-cell line was measured by ^3H thymidine incorporation on day 3.

In three experiments, IFN-τ exposed epidermal cells induced lower T-cell proliferation than did normal epidermal cells. During one experiment, three mixtures of the two epidermal cell suspensions, containing 75, 50 and 25% IFN-τ exposed cells, were co-cultivated as above. The 75% mixture suppressed T-cell proliferation more than the other two suspensions (50 and 25%), but less than the original suspension containing 100% IFN-τ exposed cells.

Our data indicate that epidermal cells exposed to IFN-τ in vivo, thereby induced to express class II antigens, can suppress an antigen specific T cell proliferation.

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AN ALTERED RESPONSE BY PSORIATIC KERATINOCYTES TO GAMMA-INTERFERON, Barbara S. Baker[1], A.V. Powles[2], H. Valdimarsson[3] and Lionel Fry[2], Departments of Immunology[1] and Dermatology[2], St. Mary's Hospital and Medical School, Paddington, London, and Department of Immunology[3], Univ. Hospital, Reykjavik, Iceland.

To determine whether psoriatic keratinocytes differ from normal keratinocytes in their response to gamma-interferon, epidermal cell suspensions from normal, and lesional and uninvolved psoriatic skin were cultured in the presence of gamma-interferon and both induction of HLA-DR expression and inhibition of cell growth were measured.

The addition of 10^2 units of gamma-interferon/ml during a 7 day culture period significantly increased mean HLA-DR+ cell numbers in 21 epidermal suspensions of normal (from 3.9 to 24.1%, $p < 0.0001$), uninvolved psoriatic (from 8.4 to 33.1%, $p < 0.0001$), and to a lesser extent lesional psoriatic biopsies (from 12.6 to 18.3%, $p < 0.01$). However, the increase in HLA-DR+ cell numbers in these latter cultures was significantly less than that observed in normal epidermal cell cultures ($p < 0.001$).

Furthermore, ^3H thymidine incorporation was substantially decreased by gamma-interferon in 16/22 (73%) cultures of normal epidermal cells; this decrease was statistically significant, $p < 0.01$. In contrast, only 4/11 (36%) lesional and 9/21 (43%) uninvolved psoriatic epidermal cultures showed comparable inhibition of proliferation.

These findings suggest that psoriatic keratinocytes have an altered response to gamma-interferon; this could explain the infrequency of keratinocyte HLA-DR expression in psoriatic plaques in vivo and, also, may contribute to the increased epidermal proliferation which characterises this disease.

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ACCESSORY CELL FUNCTION OF DR+ AND DR- KERATINOCYTES, A. Murdoch, J.F. Morris and A.C. Chu, Unit of Dermatology, Hammersmith Hospital, London W12, G.B.

Keratinocytes have been shown to be phagocytic and can be induced to express HLADr by γ interferon. For these reasons, we studied the accessory cell function of DR+ and Dr- keratinocytes in presenting soluble recall antigen to autologous T cells.

4 volunteers gave blood and suction blisters of normal skin and skin overlying a positive Mantoux test. T cells were separated by ficol hypaque and E rosetting. Epidermal cell suspensions were separated by overnight trypsinisation and Langerhans cells depleted using OKT6 and dynal beads.

Cytospin preparations of all cell fractions were prepared and stained with monoclonal antibodies - OKT3, OKT6 and RFDR2 (anti DR). The following cell combinations were set up in a Terasaki hanging droplet technique - unseparated blood mononuclear cells (PBM), T cells, T cells + normal epidermal cells, T cells + CD1 depleted normal epidermal cells, T cells + Mantoux epidermal cells, T cells + CD1 depleted Mantoux epidermal cells - with PPD as the recall antigen.

Examination of the cytospin preparations showed that the T cells were > 98% pure and the unseparated epidermal cells contained 1.5 ± 0.6% Langerhans cells. In normal epidermal cells depleted of Langerhans cells, no CD1 or DR+ cells were present. In the Langerhans cells depleted Mantoux epidermal cells, no CD1 cells were present but 10.3 ± 2.1% cells were DR+.

PBM and T cells + unseparated epidermal cells gave similar proliferation responses to PPD. T cells, T cells + CD1 depleted normal epidermal cells (Dr-) and T cells + CD1 depleted Mantoux epidermal cells (DR+) gave no responses.

Keratinocytes, both DR- and DR+ are incapable of presenting soluble recall antigens to T cells.

Wednesday, 22 June, 1988

11.45-12.45

Plenary session – Cuvilliés

B. J. VERMEER, Chairperson (Abstracts 181-184)

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RAS ONCOGENE MUTATIONS IN BASAL CELL CARCINOMAS AND SQUAMOUS CELL CARCINOMAS, J.G. van der Schroeff, L.M. Evers, A.J.M. Boot and J.L. Bos, Department of Dermatology, University Hospital Leiden, and Department of Medical Biochemistry, University of Leiden, The Netherlands.

In the process of carcinogenesis, transforming oncogenes are thought to play a significant role. Activation of ras oncogenes may occur by point mutations in specific codons of genomic DNA, as has been shown for several epithelial tumours. At present there is hardly any information available regarding ras activation in human skin carcinomas. For this study DNA was extracted from 12 freshly obtained basal cell carcinomas and from 4 squamous cell carcinomas. Using synthetic oligonucleotide hybridization in combination with the polymerase chain reaction (an in vitro amplification procedure of specific segments of chromosomal DNA) we have analyzed tumour DNA for presence of mutations in the ras oncogenes. We have screened for mutations in codons 12, 13 and 61 of the N-ras and K-ras genes and in codons 12 and 61 of the H-ras gene. In DNA from 3 out of 12 basal cell carcinomas point mutations were found within the K-ras (2x) and H-ras genes. In DNA from 1 out of 4 squamous cell carcinomas a point mutation was found within the K-ras gene. These mutations result in aminoacid changes at position 12 of the K-ras gene, where the normal glycin is replaced by cysteine, and at position 61 of the H-ras gene, where the normal glutamin is replaced by histidine. These results indicate that activation of ras genes might be involved in the developent of carcinomas of human skin.

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G TO T MUTATION IN CODON 12 OF THE HUMAN HARVEY RAS ONCOGENE DERIVED FROM A BASAL CELL CARCINOMA, S.I. White and *A. Balmain, Department of Dermatology, University of Glasgow, and *Beatson Institute for Cancer Research, Glasgow, U.K.

In most cases of activation of the Harvey ras oncogene a point mutation at either codon 12 or 61 has been found. We have analysed the specific mutation at codon 12 of an activated Harvey ras oncogene derived from a basal cell carcinoma.

By the calcium phosphate co-precipitation method of DNA transfection we have detected an activated oncogene in a basal cell carcinoma. This oncogene proved active through three rounds of transfection. DNA from these transfections has been used in subsequent experiments. Polyacrylamide gel electrophoresis and immunoprecipitation of the p21 protein produced by a cell line containing the activated oncogene showed a similar pattern to EJ DNA (the human Harvey ras oncogene known to contain a codon 12 mutation). MSP1 endonuclease digestion of the transfection DNA and Southern blotting reveals the loss of an MSP1 site which confirms a codon 12 mutation.

To discover the precise mutation at codon 12, transfection DNA, normal WBC DNA and EJ DNA were amplified. Amplification was performed using synthetic deoxynucleotide amplimers for human Harvey ras codon 12 and Taq polymerase enzyme. Amplified DNA was spotted onto nylon filters and hybridised with a ^{32}-P-labelled oligomer probe. Separate filters were hybridised to the various mutation specific oligomers for codon 12 of human Harvey ras oncogene.

DNA amplification was confirmed by hybridisation to the normal human codon 12 oligomer (GGC), with reduced hybridisation to the EJ DNA. All possible mutations of the 1st and 2nd nucleotide of codon 12 were tested. First, 2nd and 3rd round transfection DNA and EJ DNA showed hybridisation to the oligomer containing a G to T mutation at the 2nd nucleotide (GTC).

We conclude that the mutation in codon 12 of the Harvey ras oncogene derived from a basal cell carcinoma is a G to T mutation. This mutation has been reported in other human malignancies.

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AUTOSOMAL DOMINANT LAMELLAR ICHTHYOSIS CAN BE IDENTIFIED BY A CHARACTERISTIC SCALE LIPID PATTERN, Bodo Melnik, Wolfgang Küster, Gerd Plewig, Henning Hamm, Heiko Traupe, Departments of Dermatology of Düsseldorf and Münster, RFG.

In the last years, lipid biochemical analyses have become a powerful tool in the classification of several disorders of keratinization. They proved to be particularly helpful for the differentiation of two types of autosomal recessive lamellar ichthyosis, an erythrodermic type (ELI), and a non-erythrodermic type (NELI).

We studied the lipid pattern of plantar scales of two patients, mother and daughter, suffering from autosomal dominant lamellar ichthyosis (ADLI). For comparison samples obtained from corresponding sites of patients with ELI (n = 4) and NELI (n = 3) were analyzed. A recently developed sequential one-dimensional high-performance thin-layer chromatographic micromethod permitting separation of all major human stratum corneum lipid classes was used. When compared to normal controls (n = 20) the two patients with ADLI revealed a pathological scale lipid pattern with pronounced elevations of free fatty acids, triglycerides, sterol esters and moderately increased n-alkanes, whereas total ceramides and free sterols were markedly decreased. The ratio of free sterols to cholesterol sulfate was reduced to a mean value of 2.5 (normal ratio for plantar stratum corneum: 16.1). Surprisingly, both ELI and NELI patients exhibited elevated n-alkanes in their scales and NELI patients did not show increased free sterols.

Our results differ from previous studies as far as NELI is concerned. The scale lipid analysis allows the differentiation of ADLI from NELI as well as from ELI in which marked elevations of n-alkanes and normal levels of free fatty acids have been reported. We conclude that ADLI has a characteristic scale lipid pattern which is useful for lipid biochemical differentiation from other types of autosomal lamellar ichthyosis.

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PIGMENT CELLS ARE ABLE TO SYNTHESISE MSH PEPTIDES, J. Lunec, *C. Fisher, C. Parker, G.V. Sherbet, *A.J. Thody, Cancer Research Unit and *Department of Dermatology, University of Newcastle upon Tyne, U.K.

Pigment cells including melanoma are regulated by melanocyte stimulating hormone (MSH). This peptide is known to be produced in the pituitary but in this study we show that pigment cells themselves are able to produce MSH.

Immunoreactive α-MSH was present in several murine melanoma cell lines with levels ranging from 339 ± 81 pg/10^6 cells (n = 9) in B16 BL6 cells to 445 ± 69 pg/10^6 cells (n = 7) in B16 F10 cells. HPLC confirmed the identity of this immunoreactivity and showed that it was composed almost entirely of des-acetyl and mono-acetyl MSH. Similar concentrations of α-MSH were found in the F1 variant, an amelanotic line (F10DD), the S91 murine cell line and in two human melanoma cell lines (RPMI5966 & MEL57). Normal murine melanocytes and cultured human melanocytes also contained MSH although the levels were lower than those in the melanoma cells. No immunoreactive MSH was detected in normal keratinocytes and other non-pigment cell lines (e.g. 3T3, A431).

Additional evidence for the expression of α-MSH by the B16 melanomas was obtained at the transcriptional level. Using a full length cDNA probe for the murine pro-opiomelanocortin (POMC) gene transcript, which includes the α-MSH coding sequence the presence of cDNA clones containing sequences homologous to the POMC cDNA probe were detected in a λ gt10 bacteriophage cDNA library made from mRNA isolated from B16 melanoma. Strong positive signals were obtained under high stringency hybridisation conditions at a frequency of 0.02% of the clones.

These results demonstrate that pigment cells, both normal and malignant are able to produce MSH. The significance of these findings is not yet clear but they could suggest that MSH peptides may act via autocrine mechanisms to regulate pigment cells. This could be particularly important in melanoma where MSH has been shown to stimulate growth and metastatic activity.

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LOW FREQUENCY OF MELANOMA-ASSOCIATED-PROGRESSION-ANTIGENS IN HALO NEVI, J.M. van der Wiel-Miezenbeek, P.M. Steijlen, W. Bergman, E-B. Bröcker, J. Johnson and D.J. Ruiter, Dept. Pathol., University Hospital Nijmegen, Nijmegen, Dept. Dermatol, University Hospital Nijmegen, Nijmegen, Dept. Dermatol., Leiden, University Leiden, The Netherlands, Dept. Dermatol. Wilhelms University, Münster, FRG, Dept. Immunol., Munich University, Munich, FRG.

In recent years monoclonal antibodies have been developed that recognize antigens with preference for different stages in the process of neoplastic transformation and progression in melanocytic lesions, the so-called melanoma-associated-progression-antigens (MAPA's). Sofar, the distribution of MAPA's has been investigated on a large series of melanocytic lesions, e.g. common nevi, dysplastic nevi, primary cutaneous melanomas, melanoma metastases and recently also congenital nevi. Based on these data a classification could be made in early, intermediate and late markers of tumor progression. The corresponding monoclonal antibodies are, in sequence of appearance during tumor progression: K-1-2, A1.43, Muc 54, PAL-M1, 15.75, P3.58, Muc 18. To test the hypothesis that a halo nevus represents a successful rejection of melanoma, we characterized the MAPA's in 7 nevocellular halo nevi, 5 dysplastic halo nevi and 2 halo nevi in terminal regression. We found that none of the MAPA's normally present in intermediate and late stage of progression were demonstrable in halo nevi, except for MoAb P.358 that showed sporadic positive cells in one nevocellular as well as in one dysplastic halo nevus. The early progression marker detectable by Mo-Ab K-1-2 could be demonstrated in 4 of the nevocellular halo nevi and in 3 of the dysplastic halo nevi. All lesions showed expression of HLA Class I antigens by nevus cells, whereas only one lesion, an end stage halo nevus, showed expression of HLA Class II antigens. Many OKT-6-positive dendritic cells in both epidermis and dermis were found in all lesions. It appears that regression of halo nevi is not associated with phenotype changes on the nevomelanocytes as one would expect in case of it being a "frustrated melanoma". Even the dysplastic halo nevi show a remarkable low frequency of MAPA's, suggesting that these antigens are not involved in the local cellular immune response observed in halo nevi.

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SOME EFFECTS OF UVA LIGHT ON CULTURED HUMAN MELANOCYTES, J. Zeegelaar, A.H. Siddiqui, W. Westerhof, Drs. N. Smit, Drs. A. Kammeyer, Department of Dermatology Academic Medical Center, University of Amsterdam, The Netherlands.

Ultraviolet (UV) radiation is one of the main activators of cutaneous melanin pigmentation. It has recently been shown that melanocytes respond directly to ultraviolet light. However, the photobiological effects of UV radiation are mostly ascribable to middle-wave UV light (280-320 nm, UVB). The long-wave UV light (UVA, 320-400 nm) is believed to play only an inferior role.

Using in vitro cultured human melanocytes we studied the responses of these cells to single and repeated doses of UVA light. The melanocytes were cultured in medium supplemented with 16 nM 12-0-tetradecanoyl-phorbol-13-acetate, 6 nM cholera toxin, 0.1 nM isobutylmethylxanthine. The melanocytes were irradiated by UVA light generated from a Waldmann PUVA 200 apparatus equipped with Sylvania F8T5/PUVA lamps. Melanocytes were equally distributed over 40 Petri dishes which were subsequently divided in 8 groups. Four of them (each consisting of 5 dishes) were irradiated with 7 J/cm2 per day. The remaining four groups served as controls. Each day only one group of irradiated cells and one control group were harvested and the melanocyte growth was quantified by counting randomly chosen microscopic fields.

Melanin concentration was determined by measurement of absorbance at 475 nm and comparison with a standard curve obtained using a synthetic melanin.

The irradiation of cultured melanocytes resulted in the inhibition of cell growth dependent on the number of exposures. A linear increase in melanin content per mg protein was found in relation to the number of irradiation doses.

Our results indicate that cultured human melanocytes respond to repeated doses of UVA light by diminished growth and increased melanin synthesis.

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EFFECT OF UVA AND UVB ON SKIN TOLERANCE TOWARDS ALKALINE SKIN IRRITATION, Renate von Kries, Eleonore Ritt, Gerd Plewig, Department of Dermatology and Institute of Electronmicroscopy and Biophysics, Univ. Düsseldorf, FRG.

Aim of this study was to evaluate the protective effect of repeated UVA- and UVB-irradiation towards an alkaline skin irritant. The protective effect was correlated with the concomitant thickening of the stratum corneum.

9 volunteers, skin types II and III, were repeatedly irradiated with 100 J/cm2 UVA from a high intensity UVA source, equipped with a high pressure metal halogenide lamp (330-460 nm), and with 1.5-fold MED-UVB using fluorescent bulbs (310-365 nm). Test sites on the back were irradiated three times a week up to three weeks. Before and after irradiation skin tolerance against a 0.5M NaOH solution was determined by the time interval necessary to induce ten erosions on the skin. Skin biopsies were taken and processed for electron microscopy using a special technique (teflon rings and dialysis tubes) in order to avoid loss of horny layers. The thickness of the stratum corneum was determined by counting the corneocyte layers on electron micrographs.

Time intervals necessary of skin damage by NaOH were increased in all subjects after UV-irradiation. Time intervals were longer after UVB than after UVA (mean values: before irradiation 11s, range 8-15 s; after UVB 19 s, range 15-25 s; after UVA 16 s, range 10-20 s). Increase of horny cell layers was seen in 8/9 subjects after UVB (mean values: before irradiation 15, range 12-20; after UVB 23, range 14-29). No hyperkeratosis was seen after UVA irradiation.

The assumption that hyperkeratosis is induced by repeated UVB- but not by UVA-irradiation is confirmed in this study using a pure UVA-light source. An increase in skin tolerance towards an alkaline irritant such as NaOH was seen both after UVB and UVA. Therefore tolerance is mediated by other means than a thickening of the stratum corneum.

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EVIDENCE FOR AN EPIDERMAL PHOTOALLERGEN IN SOLAR URTICARIA, V. Leenutaphong, E. Hölzle, G. Plewig, Department of Dermatology, University of Düsseldorf, Düsseldorf, FRG.

A patient with solar urticaria induced by wavelengths between 250 - 580 nm and maximal sensitivity at 300 nm is reported. Intradermal injection of in vitro irradiated serum or plasma of the patient failed to elicit urticaria. Injections of irradiated eluates of the epidermis or eluates of irradiated epidermis of the patient, however, induced an erythematous flare and sustained the injection wheal as compared to controls.

Irradiation of skin sites devoid of epidermis, after removal of suction blister roofs, caused no urticarial reaction. A single irradiation with 2 MEDs UVB suppressed urticaria for about 6 days on the test site. This refractory state induced by sunburn radiation in the present patient is different from tolerance induced by repeated exposures to radiation of the action spectrum, which requires irradiations for at least two or three times and lasts only two or three days.

Injection of codeine induced urticaria at the sunburned site as well as on normal skin. Damage or degranulation of mast cells by UVB, thus, is unlikely to be the cause of the refractory state. Removal of the stratum corneum at the sunburned sites by stripping with adhesive tape did not abolish suppression of urticaria suggesting that UVB absorption by UVB-induced parakeratosis is not responsible for the observed tolerance. It is conceived that epidermal damage due to sunburn prevented urticaria in this patient.

The present findings suggest an epidermal photoactivated factor as the cause of solar urticaria in this patient. This is as to own knowledge a unique observation.

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A MODEL OF MEASURED PERCUSSIVE MECHANICAL TRAUMA AND ITS EFFECTS ON SKIN, C.J. Graves, C. Edwards and R. Marks, Dept. of Medicine, University of Wales College of Medicine, Cardiff, U.K.

Repetitive mechanical injury to the skin is likely to play a contributory role in the development and possibly the cause of some industrial dermatoses. Percussive mechanical trauma is one type of repetitive injury received by workers in some heavy and service industries.

In the studies described, this type of mechanical trauma has been modelled using an oscillating device which can supply a range of standardised percussive and vibrational trauma doses. The instrument is electronically controlled and contains sensors that monitor precisely the stress and energy involved in each impact. Thus the dose "seen" by the skin is known. The rate or "quality" of dose is also controlled and monitored. Typical doses delivered were at a frequency of 10 Hz with impact stresses in the region of 1 N/sc.cm and energies delivered per stroke of the order of 1.5 mJ/sq.cm.

Sites on the forearm and hand were used. The concept of a minimal trauma dose was employed. The effects of this trauma on the skin were quantified using non-invasive measurements of erythema, skin thickness change and trans-epidermal water loss. Results confirm that erythema, skin thickness and water loss are all increased after mechanical challenge by amounts dependent upon the trauma dose applied.

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DRUG ALLERGIC REACTIONS: ANAPHYLACTIC REACTIONS TO SUPEROXID DISMUTASE: CHARACTERISATION OF THE ANTIBODIES BY IMMUNOBLOT, F. Jugert, H. Merk, Dep. of Dermatology; Cologne; W.-Germany.

Orgotein is a purified superoxid-dismutase (SOD) which has been extracted from bovine liver. It has become a widely used anti-inflammatory drug due to its ability to inactivate one of the activating oxygen species superoxid. The prescription rate increased about 700% during 1986/87 in West-Germany. We observed 5 patients with the history of an anaphylactic shock after treatment with orgotein. 1 mg orgotein was dissolved in 1 ml and used for Prick- and intracutaneous skin test. We found allergic reactions (+++) up to a dilution of 1:1.000.000.

Orgotein was electrophoresed on a SDS-PAGE-gel and on DISK-PAGE without SDS. 3 bands were found by Coomassie blue staining corresponding to a molecular weight of 16.000, 32.000 and 64.000 D, which again corresponds with the subunit, the native and the dimer of the orgotein molecule. The proteins were transferred by immunoblot technique onto nitrocellulose and the sheets were incubated with serum of the patients. The presence of antibodies (IgG/IgE) were checked and bands were found with all 3 moieties of orgotein. These results indicate that the antibodies of the sensitized patient recognizes even the 16.000 D subunit of orgotein.

This technique promises to be useful not only in the in vitro diagnosis of this allergic reaction but also to estimate whether there are cross-reactions to human SOD produced by cloned SOD-genes.

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IN VIVO EVIDENCE OF INVOLVEMENT OF UROKINASE IN PEMPHIGUS ACANTHOLYSIS, T. Lotti, P. Fabbri, P. Bonan, G. Cannarozzo, E. Panconesi, Dept. of Dermatology, University of Florence, Italy.

Plasminogen activators (PAs) are serine proteinases which activate the serum zymogen plasminogen to plasmin. Urokinase (UK, Mr 55.000) and tissue type PA (tPA, Mr 74.000) are the two major human PAs. It has been suggested that plasmin induces extracellular proteolysis, leading to acantholysis in pemphigus (P). We report here: 1) The immunohistochemical localization of UK in perilesional acantholytic P skin (3 cases) using an immunoperoxidase procedure based on the Biotin/Avidin system (Vectastain ABC kit, Vector Laboratories, Burlingame, USA). UK is not present in the epidermis of 3 control subjects matched for sex and age. 2) Cutaneous fibrinolytic activity (CFA, evaluated with autohistographic technique), dependent on the activity of PAs in exact correspondence with the P perilesional skin showing histopathologic evidence of acantholysis. When the epidermis appears histologically normal, but with typical direct immunofluorescence findings, CFA is not evident (20 cases). In control tests, with plasminogen free fibrin film (Sigma, St. Louis, MO, USA) and apposition of 2 ml of E-aminocaproic acid (5 x 10^{-5} Mol/L) there is inhibition of CFA in all cases. 3) Epidermal fibrinolytic activity (evaluated after apposition of monoclonal antibodies directed against the catalytic syte of UK and tPA, Monozyme-Lyngby-Denmark) is dependent on the activity of UK while the perivascular fibrinolytic activity is dependent on tPA (3 cases). 4) Pemphigus perilesional skin is provided with higher UK levels (321.6 mUl/µg, 7 cases) than normal skin (139.84 mUl/µg, 4 cases, spectrophotometric assay using S-2444, Kabi Diagnostica-Stockholm-Sweden). These data suggest that the release of UK from immunologically injured pemphigus keratinocytes in vivo represents one of the most significant events in extracellular proteolysis leading to acantholysis.

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MONOCLONAL ANTIBODY GB3 DEFINES A WIDESPREAD DEFECT OF SEVERAL EPITHELIAL BASEMENT MEMBRANES IN LETHAL JUNCTIONAL EPIDERMOLYSIS BULLOSA, Jean-Paul Ortonne[1], Patrick Verrando[1], Claudine Blanchet-Bardon[2], O. Schofield[3], Anne Pisani[1], R.A.J. Eady[3], 1 Lab. de Recherches Dermatologiques, Facultés de Médecine, F-Nice, 2 Service de Dermatologie, Hô. St-Louis, F-Paris, 3 Department of Cell Pathology, St-Thomas Hospital London, UK.

We have previously reported that the monoclonal antibody GB3, raised against an extract of human amniotic epithelium recognizes an antigenic structure expressed at the dermoepidermal junction (DEJ). Radioimmunoprecipitation performed on cultured human keratinocyte demonstrates 5 polypeptides of 93.5 kD, 125 kD, 130 kD, 146 kD, and 150 kD. It was found by immunoelectronmicroscopy in the lamina densa and lamina lucida, localized on the extracellular face of hemidesmosomes, and appeared different from other known components of basement membranes. In addition, the antigenic structure recognized by GB3 was lacking in the skin in lethal junctional epidermolysis bullosa (LJEB) whereas it is expressed normally in other types of epidermolysis bullosa. The aim of this study was to establish if the defect defined by GB3 at the DEJ in LJEB is found in other basement membranes.

Frozen sections of various tissues obtained from 21 week-old fetus with LJEB and its extra-embryonic membranes were studied by indirect immunofluorescence (IIF). For the characterization of BM components, the following monoclonal (MoAb) or polyclonal (PoAb) antibodies were used: anticollagen IV (PoAb), anticollagen VII (PoAb), antilaminin (PoAb), anti-EBA Antigen (MoAb). LH 7.2 (MoAb). In addition, sera from patients with herpes gestationis and bullous pemphigoid were also studied. The diagnosis of LJEB in the fetus was confirmed by light and electronmicroscopy. Tissues from two fetuses of the same age but without LJEB served als controls. No immunoreactivity with the MoAb GB3 was observed in the BM of skin, oral mucosa, oesophagus, stomach, small intestine, colon, rectum, bronchi, thymus and amnion from the fetus with LJEB: However, the staining was expressed as in normal adults in the two fetuses without LJEB. Furthermore, using the other antibodies, it was established that the other normal components of the various BM were normally expressed by IIF. Unfortunately, samples from all other epithelial BM could not be included in the study. We have demonstrated that the BM defect in LJEB, defined by the MoAb GB3, is widespread, it involves many organs and may be detected at 21 weeks gestational age.

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CLONAL CHROMOSOME ABNORMALITIES IN FIBROBLASTS FROM A XERODERMA PIGMENTOSUM PATIENT BELONGING TO THE COMPLEMENTATION GROUP C, M. Stefanini, A. Casati, P. Lagomarsini, *R. Giorgi, **E. Berardesca and F. Nuzzo, Istituto di Genetica Biochimica ed Evoluzionistica CNR Pavia, *Dipartimento di Genetica e Microbiologia Università di Pavia, **Clinica Dermatologica, Policlinico S. Matteo Pavia, Italy.

Xeroderma pigmentosum (XP) is an autosomal recessive disease characterized by hypersensitivity to sunlight and predisposition to skin cancer. XP has been found to consist of nine complementation groups (c.g.) all defective in the excision repair of pyrimidine dimers and an excision proficient variant group.

Reduced levels of survival and DNA repair synthesis were observed in UV-irradiated lymphocytes and fibroblasts from a patient diagnosed as having XP. Complementation analysis in heterokaryons obtained after fusion of patient's fibroblasts with XP cells belonging to c.g. A, C, D allowed to assign the patient to c.g. C. Fibroblasts were harvested for cytogenetic analysis between the 44th and 18th passages and pseudodiploid metaphases with structural chromosome rearrangements (dicentric chromosomes, deletions and translocations) were observed. The frequency of abnormal mitoses was about 10% at the first passages and decreased thereafter. Identical structural changes involving chromosomes 2, 13, 14, 15 were present in different mitoses indicating a clonal expansion of chromosomally abnormal cells. A similar cytogenetic finding has been reported in other hereditary disorders showing a high incidence of neoplasia whereas in XP an increased frequency of chromosome aberrations has been observed only after UV irradiation.

Our data may represent an example of the correlation among DNA repair defects, chromosome instability and tumour predisposition and suggest a possible mechanism underlying the activation of oncogenes.

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ULTRASTRUCTURAL QUANTITATION OF HLA CLASS I ANTIGENS (HEAVY AND LIGHT CHAIN) EXPRESSED BY LANGERHANS CELLS AND KERATINOCYTES OF THE NORMAL HUMAN EPIDERMIS, *Véronique Gielen and **Daniel Schmitt, *Laboratory of experimental Dermatology, University of B-Liège and **Laboratory of dermatological Research, INSERM U 209, F-Lyon.

A knowledge of the precise quantitative distribution of HLA class I antigens among various cell subpopulations of the normal human epidermis would be very useful for the study and follow-up of cutaneous malignancies that are known to lose these molecules as well as for the understanding of immune responses, especially allospecific, that involve the skin. Using an immunogold labelling procedure, we quantified the density of major histocompatibility class I antigen on the surface of Langerhans cells (LC) and keratinocytes of the normal human epidermis. According to ultrastructural feature, keratinocytes were divided into 3 subpopulations: stratum basalis (SBK), stratum spinosum (SSK) and stratum granulosum keratinocytes (SGK), and analyzed separately. For this purpose, three monoclonal antibodies were employed: an anti-HLA A, B, C an anti-B2-microglobulin and a polymorphic anti-HLA A2 Aw69 monoclonal antibody. Quantitative analysis by electron microscopy demonstrated: 1) the presence of a high amount of HLA monomorphic determinants on SBK and SSK and a reduced but significant labelling of SGK; 2) the very low density of the major histocompatibility class I antigens on the surface of epidermal LC; 3) the expression, at an identical level, of the HLA heavy chain common determinant (HLA A, B, C), B2-microglobulin and the alloantigen HLA A2 by all epidermal cells apart from SGK and LC that presented far fewer HLA A2 sites than monomorphic determinants (B2-microglobulin and HLA A, B, C); 4) the absence of HLA class I on corneocytes and the moderate labelling of melanocytes. In epidermis HLA class I antigens are more expressed on keratinocytes than on LC or melanocytes. They undergo a maturational process as their density decreases from the basal to the superficial keratinocytes.

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EVALUATION OF HLA-DR EXPRESSION OF LANGERHANS CELLS AND INDETERMINATE CELLS WITH REGARD TO ULTRASTRUCTURAL FEATURES, G.C. Manara, *C. Ferrari, L. Pagani, C. Torresani, G. Bologna, S. Donelli and G. de Panfilis, Departments of Dermatology and *Histology, Parma University, Parma, Italy.

Among epidermal cells (EC), both Langerhans cells (LC) and the so-called indeterminate cells (IC) express the HLA-DR surface antigen. LC can be distinguished at the ultrastructural level from IC by the presence, in LC, of Birbeck granules (BG). We herein investigated the HLA-DR expression on LC/IC by using an immunogold approach with 5 nm colloidal gold particles. Moreover, we correlated the labelling degree to the cell ultastructural features.

Normal human skin keratotomed slices were incubated in 0.25% trypsin at 37°C for 60 minutes. Individual EC were enriched for LC by Ficoll-Hypaque. Cells at the interface were prefixed in 0.1% glutaraldehyde. Subsequently, they were firstly incubated with 20% heat-inactivated AB human serum, then with an anti-HLA-DR monoclonal antibody, finally with a goat anti-mouse IgG antibody coupled to 5 nm colloidal gold particles. Cells were than fixed in 1% glutaraldehyde.

The number of colloidal gold particles bound to the membrane of cells with the nuclear profile in the section plane were counted to determine the extent of surface labelling. Particle counts were obtained from at least fifty LC and fifty labelled cells lacking BG in their cytoplasm.

LC had a mean particle-binding of 396.70 ± 493.87. The number of gold granules linked to the cell surface of IC was 955.23 ± 685.22. Thus, IC displayed a significantly higher labelling ($p < 0.01$) in comparison to LC. Interestingly, almost all IC showed a high labelling degree, whilst among LC we observed a wide range of labelling degrees (from 88 to 1927). Moreover, intensely labelled cells displayed an ultrastructural pattern characterized by a low nuclear/cytoplasmic ratio, folded nuclei and an irregular surfacé with many microvilli. Some of these cells contained few BG, while the majority lacked them. Conversely, weakly labelled cells displayed a more even surface, numerous vesicles, lysosomes and a high number of GB. The existence of different density of membrane HLA-DR antigen, corresponding to a different ultrastructural pattern, might suggest a difference in the capacity of antigen presentation. The weak labelling of most cells provided with GB could be due to internalization of class II antigen with corresponding enrichment in vacuoles, lysosomes, receptosomes and GB, at the ultrastructural level.

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DIFFERENTIAL DISTRIBUTION OF SEVERAL MONOCYTE/MACROPHAGE SUBPOPULATIONS IN NORMAL HUMAN SKIN, K. Weber, V. Mielke and W. Sterry, Department of Dermatology, University of Kiel, FRG.

Out of the members of the monocyte/macrophage system of human skin, the epidermal Langerhans cell has been investigated in greatest detail, but less is known about other members of this system. Recently, a new series of antibodies (KiM series) has been raised detecting interdigitating reticulum cells (KiM1), dendritic reticulum cells (KiM4) and phagocytic macrophages (KiM6 and M8). In this study, we examined the densitiy and distribution of cells with dendritic or macrophage-like morphology within the epidermis and dermis of 10 volunteers, using the following antibodies: anti-CD1a, anti-HLA-DR, -DP-, -DQ, KiM1, KiM4, KiM6, KiM8. As expected, CD1a positive Langerhans cells are found most frequently wihtin the epidermis, but regularly occur in low numbers within the papillary body. In contrast, KiM1 positive cells with their highly dendritic morphology are confined almost exclusively to the papillary body, where they are more frequent than CD1a positive cells. Dendritic reticulum cells as recognized by KiM4 do not occur in normal human skin. Cells labelled by KiM6 and M8 are evenly distributed throughout the papillary and reticular dermis; the expression of the KiM8 antigen on macrophages is higher than KiM6 antigen. The expression of different class II antigens varies between these subpopulations. In conclusion, by use of these antibodies several distinct subpopulations within the skin monocyte/macrophage system with differential localisation may be defined.

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IMMUNO-SCANNING ELECTRON MICROSCOPY STUDY OF LANGERHANS CELLS AND HISTIOCYTOSIS X CELLS, Amilcare Cerri, Gianluca Tadini, Davide Soligo, Emilio Berti, Ruggero Caputo, 1st Department of Dermatology, University of Milan, Milan, Italy.

A very sensitive immunoscanning electron microscopy method (ISEM) was employed in order to study the expression on Langerhans cells (LC) and on histiocytosis x cells (HXC) of the antigens recognized from monoclonal antibodies (Mabs) OKT6 (CD1a), NuT2 (CD1b), M241 (CD1c), LeuM5 (CD11c) and 4B4 (CD29). An epidermal cell suspension was obtained by standard trypsinization procedures from skin biopsies taken from involved skin of 2 patients with Letterer-Siwe disease and from normal skin of healthy subjects. Cells were resuspended in RPMI 1640 and dendritic cells, enriched by centrifugation on a Ficoll-Hypque gradient, attached to poly-l-lysine pretreated glass coverslips, prefixed with 0.2% glutaraldehyde and incubated for 60 min with the above cited Mabs. Cells were subsequently incubated with goat anti-mouse immunoglobulins coupled with 40 nm colloidal gold particles, postfixed with 2% glutaraldehyde for 30 min and finally dehydrated and dried with routine techniques. Samples were then observed with a scanning electron microscope (SEM) equipped with back scattered electron detectors to allow the specific identification of colloidal gold particles. HXC when compared to LC are slightly bigger and show a more richly endowed surface with pleimorphic projections. Cell surface immunolabelling both in LC and HXC was negative with NuT2 (CD1b), weak with 4B4 (CD29) and intense with all other Mabs tested. These data indicate that the expression of CD1a on LC is at least double than that of CD1a; CD1c is also heavily expressed on the cell surface of malignant HXC and with labelling density similar to CD1c. In conclusion the ISEM employed is very sensitive and allowed a quantitative evaluation of the labelling densities with different Mabs.

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LANGERHANS CELLS HISTIOCYTOSIS: A NEW IMMUNOPHENOTYPICAL EVALUATION, Emilio Berti, Raffaele Gianotti, Lucia Crosti, Emilio Clementi, Cristina Pellegrini, Ruggero Caputo, 1st Department of Dermatology, University of Milan, Milan, Italy.

In the last years several studies showed that the proliferating cells of histiocytosis x (HX) express the same immunological and electron microscopial markers (CD1a+, S100+, DR+, Birbeck granules) of Langerhans cells (LC). Recently however same authors reported the existence of new entities in the field of histiocytic tumors strongly positive for CD1a and S100 antibodies. These findings led us to investigate three cases of HX and two cases of non HX CD1a+ cutaneous infiltrates (one case of indetermined tumor cells and a case of myelo-monocytic leukemia) with a large panel of monoclonal antibodies (Mabs) of the Third International Workshop of Leucocyte Differentiation Antigens. One or several Mabs of all the defined cluster (CD1 to CD45) and the 13 anti macrophages Mabs of the unclustered group 12 (Leucocyte Typing III Ed by A.J. McMichael, Oxford University Press 1987) were studied employing a very sensitive immunoalkaline technique (APAAP method) or an immunofluorescence double labelling procedure on frozen or paraffin embedded tissues. HC cells showed CD1a+, CD1c+, CD4+, CD9+, CD11b+, CD11c+, CD14+, CD18+, CD29+, CD39+, CD40+, CD45+ (epitopes 1 and 2 only) and CD45R+ (weak staining) immunophenotype. HX cells were also stained by some antimacrophage Mabs of the group 12 (Y-2/160, EBM11, HL-21, 10.1 and KI-M7). Interestingly the infiltrating cells of the two CD1a+ non HX tumors showed a strong positivity for KI-M6 and KI-M8 anti-macrophage also in paraffin embedded tissue. The same Mabs never stained HX cells in the three cases studied. Our data confirm that HX cells express some immunological markers of tissue macrophages and suggest that two new anti-macrophage Mabs (Ki-M6 and Ki-M8) could be useful to differentiate CD1a+ non X proliferations from the typical LC cell histiocytosis.

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PAGETOID RETICULOSIS: A PHENOTYPICAL COMPARISON OF THE LOCALIZED AND DISSEMINATED TYPE, V. Mielke, *H.H. Wolff and W. Sterry, Depts. of Dermatology, Universities of Kiel and *Lübeck, FRG.

Pagetoid reticulosis (PR) is a very rare but fascinating skin disease defined by a nearly exclusively intraepidermal infiltrate of clonal large atypical lymphoid cells. Clinically, a localized benign variant (Woringer Kolopp type) is distinguished from a disseminated form (Ketron Goodman type), which may show extracutaneous involvement. We asked whether detailed immunophenotyping might contribute to the differentiation between both types and therefore analyzed three cases of PG (two localized, one disseminated) with a panel of more than 40 monoclonal antibodies against different leukocyte antigens. The following results were obtained: 1) The atypical intraepidermal lymphoid cells belong to the T cell lineage, but exhibit a surprisingly variable phenotype (CD4+CD8-, CD4-CD8+ or CD4-CD8- in our cases). 2) The atypical cells are highly activated, expressing the transferrin receptor (T9), CD25 (IL2 receptor), CD30 (Ki1 antigen), class II antigens (HLA-DR, -DP, -DQ), and show a high proliferative activity (Ki67 above 30%). 3) There is a reactive subepidermal infiltrate of T cells, made up of T cells both of the helper and suppressor phenotype. 4) Within the dermis and epidermis there is a relatively high percentage (up to 40%) of cells belonging to the monocyte/macrophage series (KiM1, 6, 8); this finding may explain earlier ultrastructural results that PG might be of monocytic origin. In conclusion, PR - like lymphomatoid papulosis represents a disease of highly activated atypical T cells with benign or sometimes low grade malignant course; detailed immunophenotyping is at present of no value in discriminating the localized and disseminated types.

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CYTOGENIC EFFECTS OF THE TREATMENT OF CUTANEOUS T-CELL LYMPHOMA (CTCL) BY EXTRACORPOREAL PHOTOPHERESIS. Wolfgang Küster[1], Ursula M. Peterseim[1], Hans-Jürgen Gebauer[2], Rolf Meschig[2] and Gerd Plewig[1]. Department of Dermatology[1] and Institute of Human Genetics and Anthropology[2], University of Düsseldorf, Federal Republic of Germany.

It is supposed that isolated lymphocytes can be damaged or functionally impaired by a distinct phototoxic reaction as by photochemotherapy (extracorporeal photopheresis). The retransfusion of the altered cells still expressing their own antigenic pattern may possibly reduce the activity of a malignant population of T-cells responsible for a T-cell-lymphoma. To test this hypothesis, mitotic index, chromosomal aberrations and SCE-rate after extracorporeal photopheresis (EP) were studied in two patients suffering from CTCL.

Extracorporeal photopheresis therapy has been established for the treatment of extracorporeal isolated white blood cells by a photosensitizer and UV-A irradiation. Two hours after oral administration of 0,6 mg/kg body weight 8-methoxypsoralen leukocyte-enriched plasma was collected using a leukopheresis centrifuge. Thereafter, this buffycoat passed as a 1 mm film through a UV-A irradiated cassette (1-2 J/cm2) for 4.5 hours. The entire amount was returned to the patient.

After 48 hours of incubation of the lymphocytes the mitotic index after EP was decreased. This effect may be interpreted as cytotoxicity of the treatment. After an incubation of 72 hours assessment of the mitotic index showed an increase. The cytotoxic effect of the therapy was covered using this standard time of incubation.

Chromosomal aberrations as breaks, fragments, deletions, and interchanges were markedly increased after EP. The SCE-rate was significantly enhanced after the treatment.

The results are signs of DNA lesions caused by EP, but point to an enhanced repair activity. Thus at the beginning of the next cycle of treatment (1-5 weeks later) all these parameters returned to basic levels. Therefore the mutagenic risk of EP can rather be estimated as low.

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NICKEL PENETRATION THROUGH SKIN. EDX ANALYSIS OF SKIN PENETRATION. Magnus Lindberg[1,2], Samuel Sjögren[1,3], Godfried M. Roomans[1,3], Bo Forslind[1], [1]Expetimental Dermatology Research Group. Department of [2]Medical Biophysics, [3]Department of Dermatology, Södersjukhuset, and [3]Department of Ultrastructure Research, University of Stockholm, Sweden.

Plastic vessels were glued to the shaved skin of female albino guinea pigs. Two groups of animals comprising five animals per group were exposed to $NiSO_4$ in distilled water and to $NiSO_4$ in 5% SLS (sodium lauryl sulfate) respectively. A third group of animals served as unexposed controls. Skin specimens were taken after sacrifice at 24 hours exposure time. The biopsies were processed, one piece for conventional light microscopy, the other frozen and freeze-sectioned on a cryostat at -30°C. The sections were subsequently freeze dried. Elemental analysis was performed using a Kevex energy X-ray analysis system attached to Jeol 100C. Na, Mg, P, S, Cl and K were analysed quantitatively using a standard. Ni was determined on a qualitative basis at four levels in the skin.

The quantitative changes in the physiological elements indicated a mild stimulation of growth (Na↑, Mg↑, P↑) at exposure to a plain $NiSO_4$ solution, whereas $NiSO_4$ + 5% SLS indicated cellular damage (Na↓, Mg↓, P↓, K↓). The light microscopic changes were in harmony with these results.

The semiquantitative analysis shows that Ni is only detectable in the stratum corneum after exposure to a plain $NiSO_4$ solution. Addition of 5% SLS dramatically changes the picture allowing conspicuous amounts of Ni to be recorded in all layers of the skin.

Conclusions: Work environmental factors such as SLS produces conspicuous changes in the skin barrier function. The resulting increased penetration of elements such as Ni may readily be detected by EDX analysis in addition to changes occuring in the concentrations of physiologically important elements.

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CROSS REACTIVITY OF ANTI IL2 ANTIBODIES WITH EPIDERMIS. Brigitte Dréno*, Yannick Jacques**, Richard J. Robb***, Pierre Litoux* and Jean Paul Soulillou**, *Department of Dermatology, CHU Hôtel Dieu, Nantes 44035, France - **Laboratory of Immunology, INSERM, U 211, Nantes 44035, France - ***Glenolden Laboratory E.I du pont de Nemours al Co, Glenolden PA.

The 15-2 monoclonal antibody.(MAb) directed against unglycosylated recombinant interleukin 2 (IL2) has been shown to react with a human skin epitope located on the granular layer (1). To examine possible structural relationships between the epidermal antigen recognised and the IL2 molecule, we have extended our study to 7 other anti-IL2 antibodies recognising different epitopes on the IL2 polypeptide chain: MAb 1H 11-1AS, 9B11-1ES (mouse MAb) R135, R225 (Rabbit IgG) were raised against IL2 peptides comprising residues 1-8, 33-54, 8-27 and 38-58 respectively (2). MAb F1-441-SD2, F1-307-4BS and F1-307-2F 11 (murine MAb) are of unknown specificities.

The anti-IL2 antibodies were used on cutaneous frozen section of normal epidermis, as well as on smears of cells prepared from a SV 40 transformed keratinocyte cell line (SVK 14). Their reactivity was revealed with an indirect strept-avidin-biotin immunofluorescence technique. This study showed that among the eight MAb tested, four of them (9B11, R221, F1-441-SD2 and 15-2) reacted with the granular layer of epidermis and with SVK14 keratinocytes. Each of these four MAb gave similar labelling patterns, although with different intensities and cross competed each other. The other four MAb gave background staining. The 33-54 amino acid region has recently been suggested to comprise the site for IL2 binding to the 55 Kd α chain (Tac antigen) of the IL2-receptor, whereas the NH2 terminal region (residues 1-27) likely comprise the binding site for the newly described 75 Kd βchain (3). Our results therefore raise the possibility that the human skin antigen detected on the granular layer would be able to bind the human IL2 receptor chain (Tac antigen). Since Langerhans cells have recently been shown to express the Tac antigen (4) in culture and since important infiltrates of Tac positive T lymphocytes are often encountered in cutaneous diseases, a potential role for this IL2-like antigen in skin physiopathology can be suggested.

1) J. Invest Dermatol 1986, 86, 359-362, 2) J. Immunol 1986, 137, 1538-1543
3) Proc. nat. Acad. Sci. 1987, 84, 2002-2006, 4) J. Immunol 1986, 137, 157-159

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INTERLEUKIN 1 (IL1) AND NEUTROPHIL CHEMOKINETIC ACTIVITY IN NORMAL AND PSORIATIC STRATUM CORNEUM. N. Fincham, R. Camp, *A. Gearing and *C. Bird, Institute of Dermatology, London, England and *National Institute for Biological Standards and Control, South Mimms, England.

In view of past reports that ETAF/IL1 derived from epidermal cells in vitro possesses neutrophil chemotactic and chemokinetic properties in in vitro assays (Luger et al. J Immunol 131: 816-820, 1983; Sauder et al. J Immunol 132: 828-832, 1984), we have measured the IL1-like activity in aqueous extracts of normal and psoriatic lesional stratum corneum (s.c.), and compared it with the neutrophil chemokinetic activity in the same samples.

Samples of s.c. (15 mg) obtained by abrasion of psoriatic lesions (n = 3) or the heels of normal volunteers (n = 3) were homogenised in aqueous medium (0.8 ml) and supernatants ultrafiltered through YM30 and YM10 membranes to yield 10-30 kD fractions. These fractions were assayed in serial dilution for IL1-like activity in a two-stage bioassay incorporating EL-4 NOB-1 and CTLL cells, and for neutrophil chemokinetic activity in an agarose microdroplet assay.

Concentration-related IL1 activity was found in all normal heel s.c. fractions, optimal dilutions causing maximal assay responses. The 10-30 kD samples from psoriatic s.c., assayed in parallel with the normal s.c. samples, also showed dilution-related IL1 activity, but this was consistently less than that in the normal s.c. extracts and did not provoke a maximal assay response. In contrast, the normal heel samples contained negligible neutrophil chemokinetic activity on serial dilution, whereas the psoriatic samples caused marked dilution-related chemokinetic activity associated with maximal responses.

The contrasting IL1 and neutrophil chemokinetic activity in the normal and psoriatic s.c. samples confirms recent evidence that the neutrophil chemoattractant activity in lesional psoriatic s.c. is unlikely to be due to IL1. The presence of substantial amounts of IL-1-like material in normal human s.c. is also confirmed, levels as high as 20,000 units per 15 mg s.c. being recovered. The lack of neutrophil chemokinetic activity in these normal s.c. extracts suggests that IL1 derived from epidermis in vivo does not possess inherent neutrophil chemoattractant properties.

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STUDIES ON MEDIATOR RELEASE FROM HUMAN SKIN IN RESPONSE TO SUBSTANCE P AND ANTIGEN. R.M. Barr, A. Kobza Black, C. Benyon*, M. Church*, and M.W. Greaves. Institute of Dermatology, St. Thomas's Hospital, London, UK, and *Clinical Pharmacology Group, University of Southampton, Southampton, UK.

Substance P (SP) causes a weal and flare reaction when injected into human skin. The mechanisms involved are not entirely clear but SP is reported to release histamine in skin in vivo and from isolated human mast cells in vitro. Mast cells can generate a number of pharmacologically active products. We have investigated prostaglandin D_2 (PGD_2) and histamine release in human skin challenged by application of SP to superficial abrasions.

Seven atopic subjects, skin test positive to D. pteronyssinus (Dp) antigen, took part. Three 1 cm2 abrasions were prepared on the thigh. Plastic chambers were attached and filled with 0.8 ml Tyrode solution which was changed at 15 min intervals. At 30 min the abrasions were challenged with 10 µM SP or 800 units Dp antigen. Chamber contents were analysed for PGD_2 by RIA and histamine by a radioenzyme technique.

Six of the 7 subjects responded to antigen with wealing and erythema (mean area 26.2 ± 6.1 cm2). In all cases SP caused wealing of the abrasion but no erythema. PGD_2 was markedly raised in response to antigen in three subjects and minimally raised in the other 4 subjects. Substance P did not cause PGD_2 release, (means ± sem, pg/site, n = 7: antigen, 291 ± 120; SP, 100 ± 15; control, 118 ± 8). Histamine was released in response to antigen from 6 of the 7 subjects but in only one subject challenged with SP, (mean ± sem, ng/site, n = 7: antigen, 16.5 ± 5.6; SP, 5.0 ± 4.2; control 1.0 ± 0.7).

At a concentration which induced weal but not flare at the abraded sites, SP failed to stimulate detectable mediator release from mast cells. Its ability to cause wealing may be due to direct action on the blood vessels.

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EPIDERMAL PROLIFERATION AND ACCUMULATION OF POLYMORPHONUCLEAR LEUKOCYTES IN THE PSORIATIC LESION. T. Beurskens, A. Chang, P.E.J. van Erp, R. Happle, and P.C.M. van de Kerkhof, Department of Dermatology, University of Nijmegen, The Netherlands.

The aim of the present study was to find out whether epidermal proliferation and exocytosis of polymorphonuclear leukocytes (PMN) are related or independent processes in the pathogenesis of psoriasis.

In 11 patients punch biopsies were taken from the utmost periphery of the lesions. Following cryostat sectioning, alternate sections were stained with H & E, in order to assess the intraepidermal accumulation of PMN. On the remaining sections immunohistochemical staining with the monoclonal antibody Ki67 was carried out, to estimate the number of cycling epidermal cells. Fixation of the sections was carried out in acetone/ether. Air-dried sections were then incubated in PBS containing Tween 80 and subsequently Ki67, diluted 1:5 in PBS, was added. After washing in PBS a peroxidase-conjugated antimouse antibody was added at a dilution of 1:25 in PBS. The bound peroxidase was developed with the 3-amino-9-ethylcarbazole/H_2O_2 reaction. Finally these sections were counterstained with Mayer's hematoxylin and mounted in glycerine gelatine. From each biopsy 40 fields of 1 mm diameter were scanned for Ki67 positive nuclei. In the corresponding sections, the number of microabscesses and spongiform pustules were counted in 40 fields of 1 mm diameter and further an estimate was given as to the prevalence of "small sized", "medium sized" or "large sized" accumulation of PMN. The correlation between the density of PMN accumulations and Ki67 positive nuclei was assessed. In addition the topographic relation between PMN accumulation and density of Ki67 positive nuclei was studied.

A positive correlation ($r = 0.798$; $P < 0.005$) was shown between the density of microabscesses and the number of cycling epidermal cells. On areas where PMN invaded the basal cell layers and formed micropustules, a marked decrease of cycling epidermal cells was evident compared to areas with microabscess formation. These data indicate a pathogenetic link between the intraepidermal accumulation of PMN and epidermal proliferation and suggest that PMN exocytosis precedes epidermal proliferation.

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INTRAEPIDERMAL ACCUMULATION OF POLYMORPHONUCLEAR LEUKOCYTES IN ANNULAR PUSTULAR PSORIASIS. T. Bouman, A.M. Lammers, R. Happle, and P.C.M. van de Kerkhof, Department of Dermatology, University of Nijmegen, The Netherlands.

The annular zone of the lesions of pustular psoriasis of the Lapière type is a model 'par excellence' for studying the dynamics of the discharge of polymorphonuclear leukocytes (PMN) in psoriasis. In the different zones of evolution and involution of a typical lesion in a patient with annular pustular psoriasis we investigated the histopathological picture. In the different zones of the lesion we studied the micropustule formation induced by epicutaneous application of a standard dose leukotriene B_4 (LTB_4).

A punch biopsy was taken from the clinically normal skin at 20 cm distance from the lesion (I), clinically normal skin adjacent to the lesion (II), peripheral erythema (III), the pustular zone (IV), the erythematous paracentral zone (V) and the healed center (VI). Aliquots of 100 ng of LTB_4 in 10 µl ethanol were applied topically on zones I, V, and VI. From the LTB_4 treated areas biopsies were taken 24 h after application. For comparison 100 ng LTB_4 was applied to the clinically uninvolved skin of 8 patients with chronic stable plaque psoriasis. Biopsies were processed for H and E staining.

The histopathological picture in zone I was essentially normal. In zone II we found a mixed perivascular infiltrate, predominantly mononuclear, with an essentially normal epidermis. In zone III epidermal acanthosis was seen with predominantly PMN, which penetrated focally into the epidermis. The picture in zone IV was characterised by massive intracorneal accumulation of PMN which had a degenerated appearance in zone I. Zone VI showed no histological abnormalities apart from a slight acanthosis. Following epicutaneous application of 100 ng LTB_4 a massive invasion of PMN was seen in patients with chronic plaque psoriasis. In contrast, only sporadic PMN accumulation occurred in zone VI and no PMN accumulation at all was observed in zone I and V.

In annular pustular psoriasis the appearance of mononuclear cells preceded the accumulation of PMN and epidermal acanthosis. A marked habituation for a standard dose of LTB_4, as to PMN accumulation in vivo, was evident in the symptomless skin although active pustular lesions were advancing into the symptomless skin.

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EFFECTS OF POLYMORPHONUCLEAR NEUTROPHILIC GRANULOCYTES (PMN) FOLLOWING STIMULATION WITH TUMOR NECROSIS FACTOR α ON ENDOTHELIAL CELLS - AN ULTRASTRUCTURAL STUDY. Gabriele Zeck-Kapp, *Alexander Kapp, **Rudi Busse, and Urs N. Riede, Depts. of Pathology, *Dermatology and **Applied Physiology, University of Freiburg, FRG.

As reported previously human Tumor Necrosis Factor α/Cachectin (TNF) represents a potent activator of the oxidative metabolism in human PMN. Release of reactive oxygen species (ROS) by activated PMN is an essential prerequisite for the destruction of microbes and parasites. ROS, however, are also capable of inducing tissue damage in the host. Therefore, in the present study we investigated the effect of TNF-stimulated isolated human PMN on cultured bovine aortic endothelial cells (EC) grown on microporous membranes. For this purpose ultrastructural techniques were used: Scanning and transmission electron microscopy (SEM and TEM resp.) and ultrastructural detection of H_2O_2 production. When isolated PMN were added to EC in the presence of recombinant human TNF (10^3 U/ml) the EC-monolayer was disrupted within 4 hours and EC changed their shape by exhibiting a spindle-like structure. PMN were seen in the intercellular spaces. Similar, but even more pronounced effects could be detected when TNF was substituted by phorbol-myristate-acetate (PMA, 10 ng/ml) as a control stimulus. Release of H_2O_2 was observed at the surface of the PMN plasma membrane, the luminal part of the small intracytoplasmic vacuoles in the PMN as well as in the contact zone between PMN and EC. Within the EC no reaction product could be demonstrated. On the other hand, stimuli when added in absence of PMN or PMN alone did not show any effect on EC. The effect of TNF-stimulated PMN could be blocked almost completely by addition of NaN_3 (0.1 mM), an inhibitor of myeloperoxidase. However, scavengers of ROS, such as superoxide dismutase and catalase or D-mannitol were ineffective. Furthermore, combinations of NaN_3 and ROS scavengers did not enhance the effect induced by NaN_3 alone. The results obtained suggest that TNF-stimulated PMN effectively cause the disruption of EC monolayers by a mechanism which is probably mediated by a myeloperoxidase-dependent mechanism. These findings could play an important role in the pathogenesis of inflammatory vascular diseases.

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ULTRASTRUCTURAL LOCALISATION OF S-100 PROTEIN AND KERATIN IN HUMAN ECCRINE SWEAT GLANDS. G. Metzler and G. Schaumburg-Lever, Department of Dermatology, University of Tübingen, West Germany.

S-100 protein is an acidic calcium-binding protein which is present in nerves, Langerhans cells, melanocytes, apocrine and eccrine gland cells. Its function is connected with the calcium-metabolism. Keratin is part of the cytoskeleton, present in cells of ectodermal origin. The purpose of this present investigation was to study be distribution of S-100 protein and keratin within human eccrine sweat glands and their possible association with a particular structure.

Human eccrine sweat glands were excised, fixed, dehydrated and embedded in Lowicryl K4M at -35°C. Thin sections were mounted on formvar coated nickel grids and incubated as follows: Initially the sections reacted with PBS-Glycin and PBS-BSA to block unspecific binding of the following primary antibody. 1. For the detection of S-100 protein, a polyclonal antibody against cow S-100 protein (Dakopatts) was adsorbed with formvar powder and diluted 1:20 with PBS-BSA. The sections were incubated with the antibody for 120 mins, rinsed and incubated with gold-labelled protein A (Janssen), diluted 1:80 with PBA-BSA for 60 mins and rinsed in dist. water. 2. For the detection of keratin the sections were incubated with a monoclonal antibody against human keratin (Dianova), diluted 1:80 in PBS-BSA for 120 mins, rinsed and incubated with gold-labelled goat anti-mouse IgG (Janssen), diluted 1:80 for 60 mins and rinsed in dist. water. The sections were stained with uranyl acetate and examined in a Zeiss EM 9. The positive reaction product consisted of distinct black 15 nm gold particles. Experiments to assess the specifity of the immunocytochemical reaction included omission of the primary antibody.

Gold particles indicating the presence of S-100 protein were found within the cytoplasm and nucleus of the myoepithelial cells, in the villous folds of adjacent clear cells, in and around the granules and in the nucleus of the clear and dark secretory cells. Within the intradermal duct the gold particles were associated with the nucleus and the mitochondria of the basal cells. Gold particles indicating the presence of keratin were always found in the luminal cells of the intradermal duct, being deposited on the tonofilaments of the apex of the cell.

In conclusion the post-embedding immunocytological localisation of intracellular antigens has proved to be a reliable method for the localisation of S-100 protein and keratin in eccrine sweat glands.

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INVESTIGATON OF DIFFERENTIATION MARKERS IN HUMAN SEBACEOUS GLANDS BY INDIRECT IMMUNOFLUORESCENCE. J. Latham, C. Redfern, A.J. Thody, Dermatology Department, University of Newcastle upon Tyne, U.K.

We have established conditions for growing human sebaceous gland cells in vitro but it has proved difficult to unequivocally identify cultured cells as sebocyte derivatives. A range of monoclonal antibodies known to stain sebaceous glands was used to identify combinations of monoclonals that could be used to discriminate between basal and differentiated sebocytes and other cell types present in the pilosebaceous unit. Cryostat sections of human skin were stained with monoclonal antibodies to involucrin, a range of cytokeratins, epithelial membrane antigen (EMA) and an ovarian cystadenocarcinoma antibody (OM1) reported to recognise sebaceous gland antigens (de Kretser et al, Eur. J. Cancer Clin. Oncol., 21: 1019, 1985).

Both the EMA and OM1 monoclonals specifically recognised differentiated sebocytes: no staining of basal sebocytes or other epidermal cell types was seen. OM1 recognised three proteins of apparent molecular weight 380, 210 and 180 kDa in western blotting experiments, presumably analogous to those identified in the original ovarian carcinoma cell line (de Kretser et al, Int. J. Cancer, 37: 705, 1986) which may represent different intermediates in the synthesis of the antigen. Differentiated (but not basal) sebocytes were also stained by a cytokeratin 10 monoclonal (LH2). Conversely, the basal sebocytes were recognised by an antibody to basal keratinocytes (LH6). Cells of the sebaceous duct stained with both LH2 and LH6 and also with the anti-involucrin monoclonal. Cytokeratin 4 is one of the few skin keratins reportedly specific for sebaceous glands. This keratin has been identified in sebaceous glands by protein analysis but has not been detectable by immunohistochemical methods (var. Muijen et al, Exp. Cell Res., 162: 97, 1986). We showed by immunofluorescence after limited proteolysis that cytokeratin 4 epitopes are distributed in all sebaceous gland cells including the duct cells.

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PRODUCTION AND CHARACTERISATION OF A MONOCLONAL ANTIBODY DIRECTED AGAINST THE LAMELLAR BODIES OF HUMAN EPIDERMIS. Jacques Bailly, Yves-Michel Darmon, and Michel Demarchez. Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, 06565 Valbonne, France.

Lamellar bodies, intracellular organelles also termed "membrane coating granules", "Odland bodies", "keratinosomes", or "cementsomes", are synthesised within the keratinocytes of the stratum spinosum of epidermis. In the stratum granulosum, they are then translocated to the apex and periphery of the keratinocytes. Eventually, at the interface between the stratum granulosum and the stratum corneum, they fuse with the cell plasma membrane and secrete their contents into the intercellular spaces. This secretion is thought to play an important role in the barrier function of epidermis and in desquamation.

In the present study, we describe a monoclonal antibody, named BC 12, recognising the epidermal lamellar bodies. It was raised by immunising BALB/c mice against epidermal cell suspensions enriched in spinous cells. By indirect immunofluorescence, it is found to decorate the upper layers of the stratum spinosum and the stratum granulosum of human epidermis. By immunoelectron microscopy it is found to react with vesicules localised at the lamellar granules emplacement, namely intracellular vesicules in the spinous cells and extracellular vesicules at the junction between the stratum granulosum and the stratum corneum. By 2D-immunoblotting of epidermal cell extracts, it is found to recognise a triplet of approximatively 32kD with a pI between pH 6 and pH 7.

In conclusion, BC 12 appears to be an interesting probe to study the lamellar granules and their role in barrier function and desquamation.

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A NEW MONOCLONAL ANTIBODY CAPABLE OF RECOGNISING ANTIGEN(S) ON BOTH SKIN KERATINOCYTES AND PERIPHERAL BLOOD T CELLS. Hilliard Festenstein and Ahmad M.E. Nouri. Departments of Immunology and Experimental Dermatology. The London Hospital, London, UK.

We have developed a new mouse monoclonal antibody, designated AN1 which recognises the basal layer cells of normal individuals and the whole of epidermis in skin sections of patients with psoriasis. The antibody is also capable of recognising human peripheral blood T cells. This recognition however, disappears when the cells are activated by either OKT3 antibody, indicating the co-modulatory nature of antigen(s) recognised by our antibody with T1/T3 complex present on the T cells. It is interesting to mention that established human T cell lines like Molt4, CEM and HSB2 were also found to be negative with AN1. Investigation of its (1/1000 dilution of ascites) biological activities on MNC by looking at the incorporation of tritiated thymidine (3H-TdR) into cellular DNA showed (each value represents the mean± SD from three replicates of a representative experiment, the antibody was tested on cells of in some cases on as many as 15 individuals) that: a) it inhibited 3H-TdR uptake by MNC in allogeneic (from 10,633 ± 304 to 799 ± 145 cpm), autologous (from 2,020 ± 372 to 570 ± 145 cpm) mixed lymphocyte reactions. MNC activated by PHA (from 14,629 ± 186 to 402 ± 34 cpm), Con-A (from 14,691 ± 2,617 to 2,367 ± 345 cpm) and LPS (from 8,065 ± 1,539 to 726 ± 246 cpm) were also inhibited by AN1, b) it enhanced the proliferative response of MNC activated by TPA (from 18,869 ± 241 to 28,361 ± 903 cpm) or PDA (from 18,869 ± 241 to 23,361 ± 903 cpm), c) it did not have any effect on MNC activated by OKT3 antibody (from 4,638 ± 59 to 4,159 ± 265 cpm).

These results showed that using AN1: a) there is a common antigen(s) on human T cells and basal layer of normal skin section and the whole of epidermis of skin sections in psoriatic patients, b) this antibody has modulatory properties on cellular proliferation depending on the nature of stimulatory signal used for cell activation, c) the antigen(s) recognised by our antibody is absent on both activated normal T cells and established human T cell lines. The implication of these findings is presently been investigated.

PHA Phytohaemagglutinin, Con-A Concanavalin-A, LPS Lipopolysaccharide, TPA Phorbol myristate acetate, PDA Phorbol 12,13-didecanoate.

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ABERRANT EXPRESSION DURING TWO STAGE MOUSE SKIN CARCINOGENESIS OF A TYPE I 47KD KERATIN, K13, NORMALLY ASSOCIATED WITH TERMINAL DIFFERENTIATION OF INTERNAL STRATIFIED EPITHELIA. Roswitha Nischt, Dennis R. Roop, Thomas Mehrel, Stuart H. Yuspa, Martin Rentrop, Hermelita Winter, Jürgen Schweizer. Institute of Experimental Pathology, German Cancer Research Center, Heidelberg, FRG; National Cancer Institute, Bethesda, Maryland, USA.

Specific keratin cDNA probes and monospecific anti keratin antisera were used to analyze mouse epidermis and epidermal tumors for the expression of a type I 47kd keratin, K13, normally associated with the prpcess of terminal differentiation of internal stratified epithelia. We show that this keratin is virtually absent from the entire body epidermis at various stages of development. It can also not be detected in various forms of acute and chronic epidermal hyperproliferation and in epidermal cells cultured under conditions which either favour cell proliferation or in vitro differentiation. In contrast, K13 is consistently expressed in DMBA/TPA-induced squamous cell carcinomas of the skin, whereas papillomas obtained by the same two stage protocol are distinctly heterogeneous with regard to the expression of this keratin. These findings hold true for two different strains of mice, i.e. NMRI and SENCAR. Papillomas collected from SENCAR mice after 12 weeks or from NMRI mice after 15 weeks of TPA promotion are either negative for K13 or elicit variable amounts of this keratin. As in normal stratified internal epithelia, in all cases of tumoral K13 expression, both the keratin protein and its mRNA invariably occur in the differentiating cell compartments. Unlike in internal stratified epithelia, however, K13 is expressed without its commonly encountered type II partner, K4. Papillomas negative for the K13 protein are also devoid of the K13 transcript. This indicates that the aberrant tumoral K13 expression is regulated at the level of transcription. Our results suggest that K13 provide a marker for malignant conversion in the mouse two stage skin carcinogenesis model and may be expecially suited for studies on regulation of gene expression.

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CYTOKERATIN EXPRESSION IN SKIN EPITHELIAL CYSTS INVESTIGATED WITH A SET OF SELECTIVE MONOCLONAL ANTIBODIES. D. Broekaert, L. Goeman, H. Eto[1], E.B. Lane[2], I.M. Leigh[3], F.C.S. Ramaekers[4], G.N.P. Van Muijen[4], P. Coucke, J. De Bersaques and E. Gillis. Lab. Physiol. Chem. and Dept. Dermatol., State Univ. Ghent, Belgium; 1) Dept. Dermatol., Kitasato Univ., Kitasato, Japan; 2) Imp. Canc. Res. Fund, Clare Hall Labs., S. Mimms, UK; 3) Dept. Dermatol., The London Hospital, London, UK; 4) Dept. Pathol., Nijmegen Univ., Nijmegen, The Netherlands.

An immunohistochemical study was performed to evaluate the cytokeratin (CK) distribution in epidermal cysts, trichilemmal cysts and milia. Healthy epidermal tissues and outer root sheaths of hair were analyzed as reference tissues. Our range of antibodies included specific probes for CK no 4, 7, 8, 10, 13, 14, 18 and 19, two cross-reacting probes slightly more specific for CK5 and two vimentin probes. Indirect immunoperoxidase studies were performed on cryotome sections (5 µm) fixed in acetone (10 min, 20°C) using optimal dilutions of the primary antibodies. Rabbit anti-mouse Ig conjugated to peroxidase was used as a second antibody and 3-amino-9-ethyl carbazole as the reaction electron donor.

The data obtained sofar indicate that expression of CK5, 10 and 14 in cystic lesions corresponds to the epidermal CK pattern, although quantitative and topographic differences have been regularly noticed. Expression of CK10 was subject to serious limitations in 12 out of 13 trichilemmal cysts and 1 milium cyst (1/3). This was paralleled by extensive parakeratosis, the absence of a distinct stratum granulosum and an extended staining area of CK14. Generally though, non-epidermal CKs (no 4, 7, 8, 13, 18 and 19) were not expressed. However, exceptions to this rule were frequently noted. Epidermal cysts expressed CK4, either focally (6/13) or generally (1/13) during terminal keratinization, while trichilemmal cysts showed the presence of CK4 focally in only one case. CK13 was found focally expressed in just one epidermal cyst. Expression of CK8 and 19 was restricted to one trichilemmal cyst where it was preferentially located in the upper and lower parts of the cyst wall respectively. Merkel cells observed in epidermal and trichilemmal cysts displayed CK7, 8, 18 and 19. The vimentin expression was limited to Langerhans cells irregularly arranged amongst keratinocytes.

We conclude that skin-epithelium cysts manifest a disturbed CK expression, pointing to suppression of a phenotypic keratinization program, either focally or more generally.

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LOCALIZATION OF A NOVEL mRNA IN KERATINIZING EPITHELIA OF THE MOUSE: EVIDENCE FOR A SEQUENTIAL ACTIVATION OF DIFFERENTIATION-SPECIFIC GENES. Martin Rentrop, Roswitha Nischt, Jürgen Schweizer, Hermelita Winter, Institute of Experimental Pathology, German Cancer Research Center, 69 Heidelberg, FRG.

In the course of studies on the differentiation of stratified epithelia of the mouse, we have screened a variety of cDNA libraries with two known type II and type I keratin cDNAs under low stringency conditions. Besides several new keratin clones, the screening yielded a clone, subsequently referred to as p4e4, which did not contain sequence information for a keratin. Apparently the cross hybridization with the type II screening probe is due to the extremely high GC content of p4e4. Northern blot analysis with p4e4 reveals a single 1.7 ± 0.1 kb mRNA exclusively in orthokeratinizing epithelia. In situ hybridization of p4e4 to different normal and pathologically altered epithelia confirms the restriction of the p4e4-hybridizing mRNA to orthokeratinizing epithelia. This could best be shown in the papillary region of the tongue epithelium and in tail epidermis in which the p4e4-hybridizing mRNA is absent from the parakeratotic scale epidermis, however, induced after orthokeratotic conversion of the latter by repetitive vitamin A treatment. A comparative analysis of in situ hybridizations with cDNAs of basal and suprabasal keratins and with p4e4 reveals characteristic labeling patterns. In both the epidermis and in internal stratified epithelia, the expression of the mRNAs of the basal keratins K5 and K14 is virtually restricted to the basal cell layer. In contrast the mRNA coding for the epidermal suprabasal keratins K1 and K10 and the suprabasal keratins K4 and K13 of internal stratified epithelia are predominantly expressed in lower spinous cells and exhibit a gradually decreasing intensity towards the granular layer. The occurrence of the p4e4-hybridizing mRNA is also limited to the living suprabasal cell layers. However, the density gradient of its hybridization signals is clearly opposite to that observed for suprabasal keratins, i.e. low in early spinous cells and high in the granular cell layer. Thus in situ hybridization with specific cDNA clones is ideally suited to demonstrate the sequential activation of differentiation related genes in keratinizing epithelia.

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KERATIN BIOSYNTHESIS IN THE PILOSEBACEOUS DUCTS IN ACNE PATIENTS. Annette R. Kluznik and William J. Cunliffe. University of Leeds, Department of Biochemistry, Leeds, LS2 9JT and Leeds General Infirmary, Department of Dermatology, Leeds LS1 3EX, UK.

Acne vulgaris typically occurs in skin areas richly endowed with pilosebaceous units, but only a small percentage of these units become involved. Changes in the character and rate of production of lipids, keratins and other factors within the pilosebaceous duct may be important in the initiation and development of an acne lesion. Since the majority of ducts do not appear to participate in these processes, it is necessary to study individual pilosebaceous units. Single pilosebaceous units may be isolated by microdissection following enzyme digestion. We report here results on keratin biosynthesis in individual units obtained by such procedures. Normal chest skin was obtained at cardiothoracic surgery, and 4mm punch biopsies were taken from the upper backs of acne patients with their informed consent. The pieces of skin were soaked for 16 h at 4°C in 2 mg/ml dispase, and the epidermis with its associated pilosebaceous units was then peeled away from the dermis. Individual units were dissected under a X45 binocular microscope and then incubated with 10uCi of [^{35}S]-metnionine at 37°C. After 5 h the keratins were extracted in 50 ul 0.05 M Tris/HCl, ph 7.5, containing 2% sodium dodecyl sulphate, and 10 mM dithiothreitol. Aliquots of the supernatant containing 0.5 - 1.0 ug protein were electrophoresed on 8.5% mini-gels. The gels were silver stained, dried, and autoradiographed, or immunoblotted. Analysis of active and inactive acne areas revealed that keratin Nos. 1, 5, 10, 7, 14 and 16 were present and being synthesized in the pilosebaceous unit, which, apart from keratin 16, are those found in normal epidermis. No differences between keratins synthesized in units from acne-prone and non-acne-prone areas were observed in the present work. Studies using polyclonal and monoclonal antibodies have shown that keratin Nos. 1, 5, 10, 7 and 14 are present in the pilosebaceous unit. However, keratin No. 19 could not be detected: this is only reported to be present in terminal hair follicle.

Further studies using this micro-technique may produce valuable results relating to the aetiology of acne.

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THE EFFECT OF ISOTRETINOIN ON SEBUM EXCRETION IN ACNE: A 4 YEAR STUDY. Jayne L. Blake, B.R. Hughes and W.J. Cunliffe, Department of Dermatology, The General Infirmary at Leeds, Leeds LS1 3EX, England.

Sebum excretion rate was measured using a gravimetric technique in 100 patients with acne. This was done before starting isotretinoin and at intervals during and after treatment for up to 4 years. Two doses were given; 0.5 and 1.0 mg/kg bw/day.

Mean pre-treatment SER was 1.84 and 1.79 $\mu g\ cm^{-2}\ min^{-1}$ respectively. Both groups demonstrated a dramatic reduction of SER within the first 4 weeks ($p = 0.0005$). At the end of treatment SER was significantly suppressed to 0.42 and 0.21 $\mu g\ cm^{-2}\ min^{-1}$ in the lower and higher dose groups respectively ($p< 0.003$). After stopping treatment SER increased with time. This was most pronounced within the first 4 months, 1.05 (0.5 mg) and 0.80 (1.0 mg) $\mu g\ cm^{-2}\ min^{-1}$, still significantly lower than pre-treatment ($p= 0.0005$). Up to 18 months off treatment the 1.0 mg group showed a significantly greater sebum suppression at 59% compared to the 39% of the 0.5 mg group ($p= 0.03$). After 4 years, post treatment SER suppression was 32% (1 mg) and 17% (0.5 mg) but the difference was not significant.

Patients on the 1 mg dose had the best clinical success rate up to 4 years off therapy, 73% did not require further oral treatment, whereas 48% of the 0.5 mg group did. Two further groups were defined: a successful group (no further oral treatment) and an unsuccessful group (further oral treatment). The successful group maintained post treatment sebum suppression at 36% of pre-treatment level and the unsuccessful group at 19%.

This data demonstrates correlations between long term resolution of acne and sebum suppression with treatment with isotretinoin. These are both dose dependent. However, it is unlikely that sebum suppression alone explains the long term clinical benefit of isotretinoin, since sebum suppression at 4 years was at best only 36%.

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SEBUM EXCRETION RATE AND DENSITY OF ACTIVE SEBACEOUS FOLLICLES. Gérald E. Piérard and Claudine Piérard-Franchimont. Dermatopathology, University of Liège, Belgium.

In the literature, there is little information concerning the relationship existing between seborrhea and the number or rate of excretion of individual sebaceous follicles. We have used a recently introduced technique of computerized image analysis of sebum sensitive films (Sebutape^{R}) allowing the quantification of active sebaceous follicles and the determination of the mean follicular sebum excretion rate. Fifty young caucasian women and 50 untreated acneic women (grade 0.25 to 2 according to the Leeds method) participated in this study. A total of 254 measurements were made at weekly intervals.

In normal young women only 10 to 25% of the sebaceous follicles are active at a given moment. There is a positive relationship between the overall sebum excretion rate and the number of active sebaceous follicles. There is also a positive relationship between the number of active follicles and their mean individual follicular sebum excretion rate.

In acne the severity of the clinical grading is linked to the intensity of seborrhea and to the level of the mean individual follicular sebum excretion rate, but not to the number of active follicles.

The presently reported data stress the fact that the physiological activity of sebaceous glands is regulated both by the number of active sebaceous follicles and by their individual activity. In acne the mean follicular sebum excretion rate is increased without recruiting a larger amount of active glands.

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DEGRADATION OF CALLOUS BY THE RESIDENT CUTANEOUS MICROFLORA OF HUMAN FEET. J. Marshall, E.M. Gribbon, K.T. Holland and P.D. Wilkes*. Dept. of Microbiology, Leeds University, Leeds, and *Scholl Consumer Products Ltd., St. Johns St., London, UK.

Pitted keratolysis (PK) is characterised by the presence of pits in the stratum corneum of the soles of the feet. It is likely that pits are due to the presence of microorganisms with keratinolytic activity, and various microorganisms have been implicated as the causative agent of PK. These include species of Corynebacterium, Micrococcus, Actinomyces, Streptomyces and Dermatophilus. Recent work (Arch Dermatol. 123,1320.1987) has provided evidence for PK induced by Micrococcus sedentarius, a microorganism resident on normal feet (Marshall, unpublished). The purpose of this investigation was to determine whether other members of the resident cutaneous microflora possess the ability to degrade callous in vitro, which may be indicative of activity in vivo.

The Williamson and Kligman scrub wash technique was used to sample the bacterial populations on the sole of the foot of 19 male volunteers. Bacterial isolates were identified, and screened for callous-degrading ability using an in vitro assay.

Of the 224 bacterial isolates, 58 (26%) were able to degrade callous. These comprised; 17% of the staphylococci (12/72), 47% of the micrococci (32/68) and 24% of the aerobic coryneform bacteria (14/59). None of the propionibacteria isolated exhibited this ability. Callous-degrading organisms were recovered from 84% of the subjects. Eight of the subjects demonstrated mild superficial erosions in the stratum corneum, particularly around the base of the toes and these areas were avoided when sampling. There was, however, no association between the presence of these pits and the carriage of microorganisms with the ability to degrade callous. There were no significant differences in the bacterial types recovered from those feet with or without pits, however, those with pits supported higher total bacterial population densities ($p = 0.0207$) and had a higher skin surface pH ($p = 0.0166$). These results demonstrate that callous-degrading ability is a property exhibited by various species of bacteria resident on human feet, and support the reported associations of PK with several different microorganisms. Clearly, factors other than the presence of callous-degrading organisms predispose to pitting. Pitted keratolysis is often associated with hyperhidrosis, and skin pH may also be important.

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UROKINASE AND TISSUE TYPE PLASMINOGEN ACTIVATORS ARE PRESENT IN HUMAN DERMATOPHYTES AND RELATED TO THEIR DIFFUSION INTO THE SKIN. T. Lotti, A.M. Fedi, C. Senesi, E.M. Difonzo and E. Panconesi. Dept. of Dermatology; Univ. of Florence (Dir. Prof. E. Panconesi).

Urokinase (UK, M_r 55,000) and tissue type plasminogen activator (tPA, M_r 74,000) are the major human serine proteinases which activate the serum zymogen plasminogen to plasmin. Serine proteinases are involved in extracellular protein catabolism and in many other physiologic (tissue remodelling, epidermal shedding) and pathologic (cancer invasion) events. We report here that: 1) UK and tPA are present (Direct IF technique, MAb Monozyme, Lyngby, Denmark) in the cell wall and in the cytoplasm of the human dermatophytes Microsporum Canis (MC), Trichophyton Mentagrophytes (TM) and Trichophyton Rubrum (TR). 2) MC, TM and TR are provided "in vitro" with fibrinolytic activity (FA) when inoculated in a plasminogen-rich fibrin film. FA is not evident using a plasminogen-free fibrin film (Sigma Chemical Company, St Louis, USA). TM has more FA than TR which shows more FA than MC (respectively ranging from 4 to 8 mm, 2 to 5 mm and 1 to 3 mm, respectively, after 24 hrs and 12 to 15 mm, 8 to 10 mm and 4 to 7 mm after 48 hrs). 3) Cutaneous FA (CFA) dependent on the UK and tPA activity is not directly related to the in vitro FA. CFA is abnormally present in inflammatory dermatophytosis (7 cases), while it is absent in granulomatous dermatophytosis (3 cases). These data suggest that plasminogen activators which are released by dermatophytes play a major role in fungal invasion of the skin.

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IN VIVO RELATIONSHIP BETWEEN CORNEOCYTES SIZE AND SKIN PERMEABILITY ACCORDING TO ANATOMIC SITE, AGE AND SEX IN MAN. Claire Lotte+, André Rougier+, Pierre Corcuff+, and Howard I. Maibach++. +Centre de Recherche Fondamentale de l'Oréal, Aulnay sous Bois, France. ++University of California San Francisco, USA.

The relationship between the barrier properties of the stratum corneum in terms of percutaneous absorption of benzoic acid and transepidermal water loss (TEWL), and corneocyte surface area was studied in man according to anatomic site, age and sex.

In subjects aged from 20 to 30 cutaneous permeability to both water and benzoic acid is: forearm (ventral-elbow) < forearm (ventral-mid) < arm (upper-outer) ≤ abdomen < forearm (ventral-wrist) < postauricular < forehead.

No difference between sexes in cutaneous permeability was observed. As regards age, there did not appear to be any changes in permeability up to 55 years. From 65 to 80 years of age, percutaneous absorption of benzoic acid decreased by a factor of 4, but there was no change in TEWL.

As regards corneocyte surface area, in subjects from 20 to 30 years of age, the rank order is: forearm (ventral-elbow) = forearm (ventral-mid) = arm (upper-outer) > abdomen > forearm (ventral-wrist) > postauricular > forehead.

No changes were observed in corneocyte surface area between sexes. In the 65 - 80 group of age, corneocyte size increases substantially. Except in the elderly (65 - 80), benzoic acid penetration and TEWL bear the same relationship to corneocyte size. When the corneocytes have large surface area (around 1000 μm^2), they are no longer a factor limiting percutaneous absorption and water loss. Conversely, when corneocyte surface area decreases TEWL and percutaneous absorption increase, but only to a limit.

These results suggest that water transport and percutaneous absorption of topically applied material can be in part related to corneocyte surface area.

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THE EFFECT OF DIETARY SUPPLEMENTATION WITH EVENING PRIMROSE OIL ON SKIN SURFACE TEXTURE IN ATOPIC DERMATITIS. A QUANTITIVE METHOD OF ASSESSMENT. Gail Coskery, Noreen Cowley, Roger Allen. Department of Dermatology, Queen's Medical Centre, Nottingham, NG7 2UA. - U.K.

This study was undertaken to investigate the effect of Evening Primrose Oil on skin texture in atopic dermatitis using the technique of profilometry. In a trial with a double blind design and random assignment, 15 patients with mild to moderate atopic eczema were given 8 capsules of Evening Primrose Oil or placebo (olive oil) daily for 4 weeks.

At the beginning and end of treatment silicone rubber impressions of the skin surface pattern were taken and epoxy resin casts (replicas) made of each impression. Profiles of the skin surface were obtained by tracing the surface of a replica of the skin with a stylus instrument (profilometer) and using the roughness parameter Rtm to quantify the surface measurements. Clinical assessments including macrophotography were also made at the time of taking the skin impressions.

Patients on Evening Primrose Oil showed Rtm values before and after treatment of 56.9 and 56.4 respectively, and those on placebo 57.3 and 56.9 respectively. There were thus no measurable differences in either group. There was however a good correlation between the Rtm value and the clinical and macrophotographic assessments ($r = 0.45$ $p< 0.005$) thus confirming profilometry as a valid technique for assessing skin roughness.

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KINETICS OF THE CALCIUM INDUCED MIGRATION OF HUMAN KERATINOCYTES IN VITRO. Heenen M., De Graef Ch., Galand P.. Département de Dermatologie, Université Libre de Bruxelles, Hôpital Erasme, route de Lennik, 808 à 1070 Bruxelles, Belgique.

Control of the decision to proliferate or to differentiate is probably regulated at a distinct cell cycle state (late G_1). During calcium induced stratification of human keratinocytes in culture, a selective migration of terminally differentiating cells has been described (involucrin pos. cells). Human keratinocytes cultivated on collagen were used to determine if the sensitivity to calcium is related to a specific position in the cell cycle. Cell kinetics parameters were measured on keratinocytes forced to grow as a monolayer by reducing the calcium concentration in the culture medium: cell cycle time was 40 h., S phase duration, 12 h. and growth fraction = 1. Raising the level of calcium to induce keratinocyte monolayers to stratify was performed at various times after a pulse labelling by 3H-Tdr. Position of labelled cells in the cell cycle at the time of calcium addition can therefore be determined. A high proportion (over 50%) of labelled cells were found in a suprabasal position for each time tested (labelled cells in S-G_2, early G_1, mid G_1 or late G_1). This suggests:

1. That the majority of cells committed to leave the basal layer after calcium addition are not post mitotic maturing cells.
2. The existence of distinct subpopulations of epidermal cells: a small proportion of stem cells probably unsensitive to the calcium induced stratification and a majority of cells in a dividing transit compartment where they are already committed to differentiation and undergo a further round of cell division in a suprabasal position.

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EFFECT OF 1α-HYDROXYVITAMIN D_3 ON EPIDERMAL KERATINS IN PSORIASIS. D.B. Holland[1], E.J. Wood[2], W.J. Cunliffe[1], M.L. West[3], and D.M. Turner[3]. [1]Department of Dermatology, The General Infirmary at Leeds, Leeds, LS1 3ES, [2]Department of Biochemistry, University of Leeds, Leeds, LS2 9JT and [3] Department of Cell Biology, Glaxo Group Research Ltd., Greenford, UB6 OHE, UK.

In psoriasis, a modified set of epidermal keratins is expressed compared with epidermis from normal individuals. The levels of keratins 1 and 2 are reduced and keratins 6, 16 and 18 appear or increase in amount. Recently successful treatment of psoriasis with 1α-hydroxyvitamin D_3 /1α(OH)D_3) has been reported. We have investigated both the clinical efficacy of oral 1α(OH)D_3 and its effects on epidermal differentiation in psoriasis as measured by changes in epidermal lesional keratins in 14 psoriatic patients receiving 1μg 1α(OH)D_3 per day. Keratome shavings (0.1 mm) were taken before treatment and at monthly intervals during therapy. Keratins were extracted in pH 2.65 buffer, analysed by SDS/polyacrylamide gel electrophoresis and quantified by scanning densitometry. Clinical resolution occurred in 11 patients: of these 2 resolved rapidly within 6 weeks, whilst the remainder required 4 - 6 months of therapy. Serum calcium and phosphate levels remained within the normal range and no side effects were recorded. A significant increase in the level of keratin 2 occurred with lesion resolution, to levels greater than those in normal epidermis. In contrast, the level of keratin 1 started low and remained lower than normal. Changes in amounts of keratins 10, 5, 14, 7 and 17 were very small. Amounts of keratins 16 and 18 decreased significantly and normalised at clinical resolution. These changes were reminiscent of those observed with PUVA therapy. The decrease in level of keratin 16, a proliferation marker, suggests that 1α(OH)D_3 inhibits keratinocyte proliferation. Simultaneously 1α(OH)D_3 stimulated the overproduction of keratin 2, which is a major keratin of the granular cells. This might indicate an increase in cell numbers and/or a retention of these cells within the granular layers.

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VITAMIN A AND CALCIUM IONS HAVE SEPARATE EFFECTS ON KERATIN EXPRESSION IN SCCI2F CELLS. M.J. Raxworthy*, D.B. Holland, W.J. Cunliffe & E.J. Wood*. *Department of Biochemistry, University of Leeds, Leeds. LS2 9JT and Department of Dermatology, Leeds General Infirmary, Leeds LS1 3EX, UK.

Differentation of epidermal keratinocytes is controlled by vitamin A, Ca^{2+} and other factors and the pattern of keratin polypeptide expression provides information on the state of differentiation of these cells. We have examined the influence of treatments both favouring and repressing differentiation on keratin expression using the well differentiating cell line SCCI2F (derived from human epidermis).

SCCI2F cells were grown in submerged culture for 10 days in medium supplemented with 10% fetal calf serum depleted of (1) vitamin A by serum delipidisation, (2) Ca^{2+} by treatment with Chelex-100 resin or (3) grown in serum-free medium containing no vitamin A. Cells were extracted with 2% SDS and 20mM dithiothreitol and samples were analysed by one- and two-dimensional gel electrophoresis. Stained gels were scanned and the amounts of keratin polypeptides were quantified.

Depletion of vitamin A from serum was found to reduce the levels of keratins 4,5 and 18 (-19.0, -21.4 and -13.3% respectively compared with controls) and increase the levels of keratins 10, 6, 16 and 17 (+12.0, +11.3, +25.9 and +12.7% respectively). When cells were grown in serum-free medium without vitamin A the trend was similar except for keratins 10 and 6 (-17.5 and -28.5%). However, the levels of keratins 7 and 8, unaffected by serum delipidisation, were now markedly increased (+56.0 and +41.5%). Cells grown under conditions of reduced Ca^{2+} demonstrated altered expression of keratins 5 (-18.9%) and 14 and 16 (+17.4 and +9.0%). Each value was determined by reference to an internal standard and is the mean of at least five experiments.

Serum delipidisation appears to have more profound effects on SCCI2F cells than those reported for normal keratinocytes in submerged culture and these effects were even more pronounced when cells were grown in serum-free medium without vitamin A. Depletion of vitamin A influenced the expression of at least seven keratins whereas Ca^{2+}-depletion modulated the expression of only three. There was no clear reversal of effects of one treatment by the other, indicating that vitamin A and Ca^{2+} modulate keratin expression by separate mechanism.

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EFFECTS OF RETINOIC ACID AND THE ADDITIONAL COMPONENTS OF A DEFINED MEDIUM ON KERATIN EXPRESSION IN HUMAN EPIDERMAL KERATINOCYTES. J.M. Page, M.R. West and *E. Wood, Cell Biology, Glaxo Group Research, Greenford Road, Greenford, Middlesex, UB6 OHE. *Department of Biochemistry, Leeds University, Leeds, LS2 9JT.

We have studied the effects of retinoic acid and different medium components on the growth and differentiation of human epidermal keratinocytes. The effects of several concentrations of retinoic acid and EGF, hydrocortisone, insulin, selenium, transferrin, cholera toxin and calcium ions on the keratin profile were assessed using SDS-PAGE and quantitative scanning densitometry.

Cholera toxin, hydrocortisone, selenium and transferrin had little direct effect on the keratin profile and also did not influence the response to retinoic acid. High Ca^{2+} could promote, but not induce the expression of keratin 1 (67kD), whilst retinoic acid and EGF both inhibit it. Retinoic acid also decreases the amount of keratin 16 and increases the amount of keratin 14 and a 46kD protein.

The effect of retinoic acid on keratins 14 and 16 is dependent on the presence of EGF (> 1 ng/ml) and the sensitivity of this response is increased by insulin (> 1 μ/ml). However, without retinoic acid, neither EGF nor insulin have any effect on these proteins. In contrast, the effect of retinoic acid on the 46 kD protein is not EGF or insulin sensitive. Therefore it appears that retinoic acid acts on these cells by at least two mechanisms, one of which is dependent on EGF and enhanced by insulin whilst the other is independent of both factors.

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EFFECTS OF RECOMBINANT HUMAN TUMOR NECROSIS FACTOR-ALPHA ON THE IN VITRO PROLIFERATION OF TRANSFORMED HUMAN KERATINOCYTES. Michael Detmar, Dieter Littkemann, Rudolf Stadler, and Constantin E. Orfanos. Department of Dermatology, University Medical Center Steglitz, The Free University of Berlin, Berlin, West Germany.

The effect of recombinant human tumor necrosis factor-alpha (rTNF) on the proliferation of two transformed human keratinocyte cell lines was studied in vitro.

The spontaneously transformed cell line HaCaT, and the oncogen-transfected cell line II/3 (EJ ras), obtained from P. Boucamp, DKFZ Heidelberg, FRG, were cultured for 1 to 8 days in the presence of 10, 50, 100, 500, 1000, 5000, and 10000 U/ml rTNF. The cell proliferation was examined daily by determination of cell counts, cell viability, DNA content, incorporation of (3H)-thymidine into DNA, and autoradiographic labeling indices. The effect of rTNF on the cell attachment was studied by determination of plating efficiencies after 4 days of rTNF treatment.

Treatment with rTNF induced a dose-dependent reduction of cell numbers and DNA content in both cell lines. A 50% inhibitory dose of 500 U/ml was found in HaCaT cells. The II/3 cells were less sensitive to rTNF, and 10000 U/ml rTNF only induced a 30% reduction of cell proliferation. The antiproliferative effect of rTNF was observed within the first day of treatment, and was reversible within 2 days after discontinuation of the treatment. The incorporation of (3H)-thymidine into DNA was slightly elevated, while the autoradiographic labeling indices were unchanged compared to control cultures. Daily evaluation of attached and shed cells showed that under rTNF influence, 20 to 40% of the cells became non-viable. Treatment with rTNF did not influence the cell attachment. The plating efficiency of treated cultures was unchanged compared to untreated controls.

In conclusion, rTNF exerts a dose-dependent cytotoxic effect on transformed human keratinocytes in vitro. rTNF did not inhibit the DNA synthesis.

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HEXADECANE-INDUCED ORNITHINE DECARBOXYLASE ACTIVITY AND EPIDERMAL PROLIFERATION. S. Owusu and E.J.Wood, Department of Biochemistry, University of Leeds, Leeds LS2 9JT, U.K.

The polyamines, putrescine, spermine and spermidine, are intimately involved in the regulation of growth and proliferation of mammalian cells. Polyamine concentrations are higher in rapidly proliferating tissues and levels of ornithine decarboxylase (ODC), the key enzyme in polyamine biosynthesis, are significantly elevated in psoriasis compared with normal epidermis. Topical application of hexadecane to guinea pig skin induces epidermal hyperproliferation and hyperkeratosis similar to that observed in psoriasis (Cowan and Mann, Brit. J. Dermatol., 84, 353-360. 1971) and following the application there is a rapid increase in the activity of protein kinase C, a decrease in the levels of cAMP, and an elevation in DNA and glycogen synthetic activities. We have investigated the effect of topical hexadecane on ODC activity in pig skin with the aim of trying to throw light on the mechanisms of proliferation and differentiation and also of evaluating this system as a possible animal model for psoriasis.

Hexadecane was applied to keratotomed pig epidermis floating in nutrient medium (DMEM containing 5% foetal calf serum). At intervals over 2h, pieces of epidermis, as well as pieces of control, untreated epidermis, were removed, rinsed and homogenized and ODC assayed in the supernatant by means of $^{14}DO_2$ release from labelled ornithine. A rapid and biphasic increase in ODC activity was observed. In the first phase, ODC activity increased 3 - 4 fold in 5 - 10 min and this increase was insensitive to cycloheximide. This effect was therefore independent of protein synthesis and was due to an activation of pre-existing enzyme. After approx. 60 min the level of ODC increased by about 6-fold over untreated epidermis, this second phase of increase being sensitive to cycloheximide and was presumable due to de novo synthesis of enzyme. These rapid changes in ODC activity in response to hexadecane will lead to increases in polyamine concentrations and therefore offer insights into the early events leading to hyperproliferation of the epidermis in response to the application of hexadecane.

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THE IN VITRO DIFFERENTIATION OF KERATINOCYTE CULTURES DERIVED FROM THE INTERNAL AND EXTERNAL PART OF HUMAN FORESKIN IS NOT IDENTICAL. Long-Qing Xia, and Michael Detmar, Department of Dermatology, University Medical Center Steglitz, The Free University of Berlin, Berlin, West Germany.

The aim of this work was to investigate whether or not the differences of epithelial differentiation between the internal and external part of human foreskin are maintained in vitro.

Keratinocytes were isolated from both the internal and external part of the preputium of 1 year-old children, and cultured separately on collagen type I gels at the liquid-air interface, using Dulbecco's MEM with 10% FCS, 10^{-9}M cholera toxin, 10 ng/ml EGF, 0.4 µg/ml hydrocortisone, and antibiotics. Cryostat sections of 14 day-old cultures were processed for immunocytochemistry with monoclonal antibodies (McAbs) against cytokeratins (McAbs KL1, PKK-2, CK8.12, CK8.60), filaggrin, fibronectin, and collagen type IV, using the alkaline phosphatase-anti alkaline phosphatase technique.

14 day-old keratinocyte cultures from both locations consisted of a multilayered stratified epithelium with identical reactivity to McAbs KL1 and CK8.60 in the upper cell layers, and to McAbs PKK-2 and CK8.12 in all layers except the basal cell layer. Anti-filaggrin McAb labelled more cell layers in cultures derived from the internal part, in a similar pattern to what was observed in vivo. The deposition of fibronectin and collagen type IV in the area between the basal cells and the substratum was markedly increased in keratinocyte cultures from the inner part of human foreskin. Similar distribution was observed in vivo.

In conclusion, keratinocyte cultures from the internal and from the external part of human foreskin display distinct patterns of epidermal differentiation, mimicking the in vivo situation, and should be cultured and examined separately.

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MONOCLONAL ANTIBODIES TO KERATIN 14 AS A MARKER OF KERATINOCYTE LINEAGE. Leigh I.M., Lane E.B., Purkis P.E. Experimental Dermatology (ICRF) London Hospital E1 2BL.

Basal keratinocytes in normal skin synthesise the keratin pair 5/14 (Moll catalogue) and suprabasal keratinocytes synthesise the pair 1/10. Monoclonal antibodies can be obtained which are specific for a particular keratin and these may be used to examine the expression of keratins in vivo as markers of differentiation. Monoclonal antibodies to keratin 5/14 react with basal cells predominantly (AE 1, LH 6, LH 8). We have developed a new monoclonal antibody to keratin 14 which reacts with all cells of keratinocyte lineage, including tumours. A peptide was synthesised to the 20 amino acid terminal region of keratin 14 and used to immunise BAlb C mice to develop hybridomas using standard techniques. The resulting hybridomas were screened against tissue by immunocytochemistry in addition to dot blot assay against the peptide. Positive hybridomas were cloned and retested. Monoclonal antibody LL001, was found to react with the synthetic peptide by dot blotting, and with keratin 14 by western blotting of 1D and 2D SDS PAGE gels. Immunocytochemistry showed reaction of LL001 with the full thickness of epidermis and other squamous epithelia, including oral and genital mucosa. In glandular epithelia the antibody reacts with basal (myoepithelial cells) only. Basal cell and squamous cell epitheliomas and cell lines derived therefrom also react with LL001. Thus LL001 provides an excellent marker for cells of keratinocyte lineage.

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THE cDNA CLONING OF A DIFFERENTIATION-SPECIFIC KERATIN OF RAT EPIDERMIS AND ITS USE AS A PROBE TO STUDY THE ATTACHMENT AND PROLIFERATION OF KERATINOCYTES IN VITRO IN RELATION TO DIFFERENTIATION. C.P.F. Redfern and G. Allan, Department of Dermatology, University of Newcastle upon Tyne, Newcastle upon Tyne, U.K.

An epidermis-specific cDNA clone, pEL3.25 was isolated from an epidermal cDNA library in pAT153. Hybrid arrest and hybrid selection experiments show that the 700 bp insert codes for a 66 kDa type II keratin which accumulates during epidermal differentiation. The cDNA clone detects a 2.5 kbp mRNA in northern blotting experiments and in situ hybridisation to skin sections indicates that expression of this mRNA is largely confined to the suprabasal epidermal cells. These data suggest that expression of mRNA complementary to pEL3.25 is confined to differentiating cells. However, northern analysis showed that 'basal' epidermal cells prepared by percoll-density-gradient centrifugation contained pEL3.25 mRNA but within 20 hours after culture in vitro this mRNA was no longer detectable. Basal cell preparations may well be heterogeneous, containing true undifferentiated basal cells and cells at an early stage of terminal differentiation. The absence of pEL3.25 mRNA from cells 20 hours after plating was not due to selective attachment of true basal cells since both northern analysis and in situ hybridisation show that pEL3.25 mRNA was also present in cells attached to tissue-culture plastic only one hour after plating. If it is correct that expression of pEL3.25 mRNA is confined to cells that have ceased proliferation and started to differentiate then these data suggest that the early stages of terminal differentiation are reversible. It is possible that in vivo basal-like cells expressing pEL3.25 represent a transient amplifying population of differentiating cells which can attach and proliferate in vitro; maintenance of pEL3.25 mRNA synthesis may then be dependent on exogenous factors which are absent in vitro. Experiments to distinguish between these possibilities are in progress.

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REPRODUCTION OF THE CHARACTERISTIC MORPHOLOGIC CHANGES OF FAMILIAL BENIGN CHRONIC PEMPHIGUS (FBCP) IN CULTURES OF LESIONAL KERATINOCYTES ONTO HEALTHY HETEROLOGOUS DEAD DEEPIDERMIZED DERMIS (D.E.D.) G. De Dobbeleer, C. De Graeve, J.M. Gourdain and M. Heenen. Département de Dermatologie, Hôpital Erasme, Université Libre de Bruxelles.

The aim of this study is two fold: 1. We wonder whether the system of keratinocyte culture proposed in 1983 by Prunieras et al. on D.E.D. is a good model for the in vitro study of disorders of keratinocytes. 2. We want to determine if the pathological process involved in FBCP has an epithelial origin and is possibly reproducible in this model of culture.

The model of recombining epidermal and dermal elements we used has been described by Prunieras et al. (1983). Normal heterologous deepidermized dermis (D.E.D.) is killed by repeating freezing, sterilized by τ rays (3.500 rads) and placed on a grid in contact with the tissue culture medium. Epidermal cell suspensions coming from lesional skin of a patient with a long standing FBCP are seeded on the top of D.E.D. and are processed for routine photonic and electron microscopy after 14 days of culture.

Histologically, D.E.D. was covered with an epidermis showing suprabasal acantholytic clefts; occasional dyskeratotic cells were observed. Electron microscopy showed numerous microvilli in the suprabasal area, a dramatical decrease of the number of desmosomes and dyskeratotic keratinocytes suggestive of the so called "grain".

These data suggest that this model of culture could hold great promise for studying other disorders of keratinization or blistering diseases. In addition to that, the yet unknown defect involved in FBCP is well located in the epidermis, and is independent of the dermis.

References: Prunieras M., Régnier M. and Woodley D.: "Methods for cultivation of keratinocytes with an Air liquid interface". J. Invest. Dermatol., 81 : 28_s -33_s, (1983).

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ULTRASTRUCTURAL AND IMMUNOLOGICAL STUDIES ON THE ALOPECIC (DEBR) RAT. H.J. Michie, C.A.B. Jahoda, R.F. Oliver and T.A. Poulton*, Departments of Biological Sciences and *Pathology, University of Dundee, Scotland.

The hairless condition in the DEBR strain of rat, in common with alopecia in man, is associated with a marked peri- and intrafollicular lymphocytic infiltrate. The pathogenesis of this immuno-deficient lesion in the vibrissa follicle of the DEBR rat has been further investigated using electron microscopy (TEM), peroxidase anti-peroxidase staining and indirect immunofluorescence. Levels of circulating T-lymphocytes in lesional and control rats were studied using indirect immunofluorescence on peripheral blood lymphocytes separated by gradient centrifugation. The TEM study revealed that the apical area of the papilla-follicular epidermis interface appears to be the primary target of the invading inflammatory cells. Disruption and then breakdown of the basement membrane, which was confirmed by indirect immunofluorescence, occurred as the infiltrate passed into the epidermal component where, as the lesion progressed, it caused damage and disruption to the differentiating epidermal cells culminating in the non-production of hair. While collagen deposition occurred in the papilla in the presence of inflammatory cells, papilla cell morphology appeared unchanged. T-lymphocytes were the predominant cell type in the infiltrate. Examination of peripheral blood lymphocytes showed that while total T-cell numbers were similar in lesional and control rats the T helper/suppressor population was elevated in lesional rats.

The observations focus on lymphocytic disruption of the biologically important papilla-epidermal interface and/or cytotoxic effects on differentiating hair cortex cells as possible mechanisms underlying cessation of hair production. A causal link with changes in T-cell subsets has yet to be established.

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THE PLEOMORPHISM OF LAMINA DENSA BLISTERING DISEASES INCLUDING TYPE VII COLLAGEN DISEASES. Whitehead P., Bogal B., Wojnarowska F.T., Leigh I.M.. Experimental Dermatology. The London Hospital. London E1 2BL.

Type VII collagen is a newly characterised component of the epidermal basement membrane which can be detected immunocytochemically using the monoclonal antibody LH7.2 which reacts with the carboxy-terminal region. This antigenic determinant is missing from the basement membrane zone of skin biopsies from recessive dystrophic epidermolysis bullosa (RDEB) which shows that this is a hereditary disease of type VII collagen synthesis or breakdown (Leigh et al. J. Invest Dermatol, in press). In addition LH7.2 reacts with the same polypeptide by western blotting as sera from patients with epidermolysis bullosa acquisita (EBA) confirming that this is an autoimmune disease of type VII collagen. However in linear IgA disease, which shows the same binding site of auto antibody to lamina densa by immunoelectron microscopy, the antigen on Western blotting is at a different molecular weight (285,180kD) compared to the LH7.2/EBA antigen (250kD). Thus linear IgA disease autoantibodies bind a different antigen to EBA, confirming the pleomorphism of lamina densa blistering disease. Immunoblotting techniques will further subdivide blistering diseases at a molecular level and may require a reclassification on biological grounds.

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INTERACTION OF HUMAN KERATINOCYTES AND FIBROBLASTS WITH COLLAGEN LATTICE. Jan E.M. Souren, Roeland van Wijk, Erna van Wilsem, and *Maria Ponec, Department of Molecular Cell Biology, State University, Utrecht, and *Department of Dermatology, University Hospital Leiden, The Netherlands.

The aim of the present investigation was to study the interaction of human fibroblasts and keratinocytes with hydrated collagen lattice. The cells were incorporated into and/or placed on top of the collagen gel. A 2 ml mixture of rat tail collagen, NaOH, Dulbecco's Modified Eagles Medium and foetal calf serum prepared on ice was subsequently placed in a petri dish (8 cm2 surface area) and gel formation was achieved by raising temperature to 37°C. Keratinocytes or fibroblasts derived from human foreskin (first to fourth passage) were either added to the mixture before or placed on top of the gel one hour after gel formation.

Keratinocytes incubated within or on top of a collagen gel induced gel contraction, the extent of which was dependent on both the cell number and the location of the cells. The gels contracted by 40% when 20,000 cells were placed on top of the gel, but only by 10% when 50,000 cells where incorporated into the gel. Marked differences were observed between arrangements of cells incorporated into or placed on top of the gel. While keratinocytes on top of the gel formed large clusters, no cell-cell contact was observed when they were incorporated into the gel matrix.

In the presence of fibroblasts the gel contraction occurred also in a cell-density dependent manner. Contrary to keratinocytes, the extent of contraction was higher when fibroblasts were incorporated within the gel as compared with those placed on top of the gel. Furthermore, fibroblasts placed on top of or incorporated within the gel formed uniformly distributed cellular network.

The simultaneous presence of keratinocytes on the top and of fibroblasts within the gel resulted in gel contraction, the extent of which was the sum of the individual reduction effects of both cell types.

The results clearly show that both the keratinocytes and the fibroblasts can affect the process of collagen arrangement. This process was dependent on both the cell localization and the cell number. Furthermore, the cellular distribution and shape on top of the gel was different with the two types of cell is used.

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INCREASE IN TENASCIN IN THE DERMIS OF INVOLVED SKIN IN SCLERODERMA. Jean-Paul Ortonne[1], Annie Vitetta[1], Ruth Chiquet-Ehrismann[2], Jean-Philippe Lacour[1], Anne Pisani[1], Françoise Bernerd[3]. 1 Lab. de Recherches Dermatologiques, Faculté de Médecine Nice-France, 2 Friedrich Miescher-Institut, Basel, 3 Centre International de Recherche Dermatologique, Valbonne-France.

Tenascin (T) is an extracellular matrix glycoprotein consisting of several disulfide-linked subunits (mw 150.000-240.000), synthesized by fibroblasts and muscle cells. In Rat Fetal Skin, T is selectively present in the mesenchyme surrounding organs such as vibrissae where the mesenchyme is absolutely required for normal development, suggesting its important role in mesenchyme-epithelial interactions. Very little is known on the distribution of T in human skin.

We report a study of the distribution of T in normal adult human skin. In addition, scleroderma (S) skin, in which an abnormal expression of fibronectin, another ECM protein, has been reported was also investigated. A recently described antiserum to T (Chiquet-Ehrismann R. et al., 1986) purified from conditioned medium of embryo fibroblasts culture has been used. This antiserum cross reacts with human T. Frozen sections of the normal skin of 14 adult volunteers (N) was studied by indirect immunofluorescence. The involved skin of 13 patients with systemic scleroderma (SS) and of 7 patients with localized scleroderma (LS) was also investigated. In the LS group, frozen sections of clinically uninvolved (U) skin served as controls. An immunoelectronmicroscopy (IEM) study was also performed in one sample of normal skin. (Immunoperoxidase and immunogold techniques).

In N skin, T was concentrated at the dermo-epidermal junction, in the dermal papillae, but was usually absent in the dermis below the epidermal rete ridges. T was also present in the dermis surrounding hair follicles, dermal blood vessels and sweat glands. By IEM, no reactivity was observed in the lamina lucida and in the lamina densa of the epidermal basement membrane. By IEM, gold particles were observed in the vicinity of collagen fibers of the papillary dermis. A marked increase of T was found in the superficial dermis of involved SS and LS skin compared to N skin of volunteers of U skin of LS patients, in all cases examined. The mechanism by which T accumulates in the upper dermis is unknown. It is likely that this abnormality is the consequence of an increase in synthesis of connective tissue elements by S dermal fibroblasts.

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THE EFFECT OF EGF ON AGING FIBROBLASTS. Alain Colige, Betty Nusgens and Charles M. Lapière, Laboratory of Experimental Dermatology, University of Liège, Belgium.

The expression and function of EGF receptors in human skin fibroblasts might be modified with aging. Fibroblasts from donors of increasing age were tested (4 males and 4 females in the age groups of 25, 35, 55 and 84 years). Two days after confluence, the cultures were incubated for 18 h with increasing concentrations of EGF (0,1 and 25 ng EGF per ml of medium), and then labeled for 24 h with 3H-proline in presence of EGF. Non-collagen protein (NCP) synthesis was determined as 3H-labeled non-dialyzable proteins and collagen synthesis from 3H-hydroxyproline measurements. In the control cultures in absence of EGF, the synthesis of NCP varied from 5 x 10^4 to 11 x 10^4 cpm/µg DNA but these variations were not related with the age or the sex of the donor. The collagen biosynthesis in the 2 strains of 84 year-old donors was slightly decreased as compared to other age groups. The percentage of 3H-protein synthesis devoted to collagen (% PC) was 6.7 ± 0.1% for old donors as compared to 8.6 ± 1.7% for the young and adult donors. Addition of EGF to cultures induced a slight stimulation of the NCP synthesis in the age group of 25, 35 and 55 years while no effect was detected for the 2 older strains. On the opposite, EGF induced a dose dependent inhibition of the collagen synthesis in all the strains (5 to 44% inhibition). The % PC was more markedly reduced by EGF in the age groups of 25, 35 and 55 (-30 ± 12% and -37 ± 9% for 1 and 25 ng EGF) than in the olders (-12 ± 5% and -21 ± 1%). The pretranscriptional effect of EGF on the collagen biosynthesis was proved by collagen nRNAs measurements. Total cellular RNAs were slot-blotted and hybridized with a cDNA collagen I probe. By densitometric scanning of the autoradiogramms a decrease in the α 1 (I) mRNA of about 50% and 80% was observed after, respectively, 24 and 48 h of incubation with 25 ng EGF per ml. The different effects of EGF on the older strains did not seem to be related to alterations of the EGF receptors since no significant difference was observed in the number of EGF receptors per cell or their Kd as a function of the age of the donor. These results suggest that the regulation of the biosynthetic activity of fibroblasts by EGF can be modified with aging.

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QUANTITATIVE AND QUALITATIVE EVALUATION OF MIGRATION AND INVASIVENESS OF FIBROBLASTS AND TUMOR CELLS IN VITRO. Carl Georg Schirren, Rüdiger Hein, Hans-Werner Ziegler-Heitbrock, Thomas Krieg and Otto Braun-Falco, Dermatologische Klinik der LMU München, FRG.

Attraction of cells by tissue mediators and other substances plays an important role during normal repair processes, but also in fibrosis and tumor metastasis and has therefore been extensively studied in in vitro systems. In the tissue, however, fibroblasts as well as tumor cells do not only show a chemotactic response, but have also the potential to cross interstitial connective tissue and basement membranes, which often serve as barriers separating different biological compartments. It was therefore the aim of the present study to use an in vitro system, which allows to investigate the ability of cells to migrate through connective tissue barriers and to analyze the influence of mediators and drugs. Chemotaxis of cells was measured in a Boyden chamber. In addition type I collagen as well as basement membrane extracts were prepared and allowed to form a threedimensional gel within the Boyden chamber. Normal human fibroblasts, squamous carcinoma cells and fibrosarcoma cells were seeded onto this barrier and fibroblast conditioned medium as well as fibronectin were used as chemoattractants. Whereas fibroblast migration was inhibited by the basement membrane barrier as well as by the interstitial collagen gel, squamous carcinoma cells migrated through the reconstituted basement membrane but were inhibited by type I collagen. Compared to normal skin fibroblasts, fibrosarcoma cells showed an enhanced ability to migrate through both barriers. Preincubation of skin fibroblasts with TNF resulted in a marked induction of the potential of the cells to invade the type I collagen gel without altering the low migration through the reconstituted basement membrane. These data demonstrate that the invasive migration of cells can be measured in vitro and that the ability to cross different connective tissue barriers can be selectively induced by mediators.

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MODULATION OF TUMOR CELL-FIBROBLASTS INTERACTIONS BY TWO EXTRACELLULAR MATRIX GLYCOPROTEINS: LAMININ AND FIBRONECTIN. Agnès Noël*, Betty Nusgens* , Jean-Michel Foidart**, and Charles M. Lapière*, *Laboratory of Experimental Dermatology and **Laboratory of foetoplacental physiopathology, University of Liège, Belgium.

During metastasis, neoplastic cells come into contact with basement membrane (BM) and interstitial stroma. These interactions play a significant role in determining proliferation synthesis and metastatic potentials. Laminin (LAM) and Fibronectin (FN) are known to be extracellular matrix glycoproteins involved in these processes. We have investigated these interactions in vitro in coculture of MCF 7 (human breast adenocarcinoma cell line) and human skin fibroblasts on various substrates. On plastic or on laminin coated dishes, the two cell types spread and grew in separate colonies. On matrigel (a gel reconstituted by polymerizing BM macromolecules and containing 85% of LAM) clusters of MCF 7 formed and attached to fibroblast aggregates. The influence of LAM on cell-cell interactions was determined on agar gel. In absence of additives, MCF 7 on 1% agar gel formed floating aggregates without interactions with the substrate. By immunofluorescence, the presence of endogenous LAM was demonstrated at the cell surface. Addition of anti-LAM antibodies or of the peptide YIGSR, the sequence homologous to the cell binding domain of LAM induced disruption of the clusters into isolated cells. On 1% agar gel containing LAM, MCF 7 cells formed clusters attached to the substrate. In coculture with fibroblasts on LAM coated dishes, the addition of exogenous LAM induced MCF 7 cells to exhibit a rounding morphology. In the same way, addition of anti FN antibodies or the synthetic peptide RGDS, containing the sequence of the cell binding domain of FN prevented MCF 7 cell spreading and promoted clustering on fibroblasts. The control peptide GRGD, the peptide YIGSR or anti LAM antibodies did not affect cell spreading. These results indicate that FN and LAM have opposite and antagonistic effects on tumor cell-fibroblast interactions. FN inhibits the MCF 7 clustering induced by LAM and promotes cell spreading.

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COLLAGENASE ACTIVITY DURING THE REMODELLING OF A DERMAL EQUIVALENT. P.J.E. Rowling*, M.J. Raxworthy*, E.J. Wood*, J.N. Kearney*, W.J. Cunliffe* . Department of Biochemistry, University of Leeds, Leeds LS2 9JT; *Yorkshire Regional Tissue Bank, Pinderfield General Hospital, Wakefield, WF1 4DG; Department of Dermatology, Leeds General Infirmary, Leeds LS1 3EX, UK.

Human dermal fibroblasts are able to contract a gel of repolymerised collagen Type I and remodel it into a dermis-like structure termed a dermal equivalent. Fibroblasts within a dermal equivalent have been postulated to display some of the characteristics of fibroblasts in healing wounds which are themselves associated with a 200-fold increase in collagenase activity compared to normal skin. We have therefore investigated the activity of collagenase during dermal equivalent contraction.

Dermal equivalents were formed by mixing 7.7 mg acetic acid-solubilized rat-tail tendon collagen, 1 x 10^5 human dermal fibroblasts, Dulbecco's modified Eagle's medium, 5% newborn calf serum, 0.1M NaOH in a total volume of 15 ml in a 85 mm bacteriological petri dish and were maintained in culture at 37°C. Collagenase was partially purified from the culture medium and from dermal equivalents at days 5, 10, 20 and 30 and was assayed by its ability to digest a dried film of fibrillar collagen.

All the collagenase activity associated with the dermal equivalent during the first 20 days of culture was found to be in an active form since trypsin pretreatment was unable to increase the activity. Collagenase activity was essentially constant during the first 20 days of culture (400.3, ± 32.0, 438.8, ± 29.9, 417.3, ± 34.0, units on days 5, 10 and 20 respectively, where one unit equals 1 g collagen digested in 24 h) but decreased to 208.0. ± 50.4 units by day 30. Latent collagenase activity of 128.1 ± 20.6 units was detected on day 30. Collagenase activity in the culture medium was greatest on day 5 (115.3 ± 10.4; no latent activity detected). Over the course of 30 days in culture total activity fell to 28% of that on day 5. However, the proportion of latent collagenase activity increased so that on day 30, 85% of the activity was in this form. Collagen within a dermal equivalent is remodelled over the first few days in culture and this process is complete by day 20. Our present findings indicate that collagenase activity is associated with the remodelling process.

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EFFECT OF AROMATIC RETINOIDS ON PROCOLLAGEN SYNTHESIS AND GROWTH OF SKIN FIBROBLASTS IN VITRO. Chresten Godt, Klaus P. Müller, Rudolf Stadler, Constantin E. Orfanos, Dermatologische Klinik, Klinikum Steglitz der Freien Universität Berlin.

The aim of this study was to evaluate the effect of some newer synthetic retinoids on the collagen metabolism and growth of skin fibroblasts in vitro.

Normal skin fibroblasts were grown in Dulbecco's MEM with supplements. Retinoids were dissolved in DMSO giving final concentrations of 10^{-12} to 10^{-5} and 0.1% DMSO respectively, where DMSO alone served as control. The cultures were kept under retinoids for up to 14 days. Procollagen synthesis was analysed after labelling with L-(2,3)-^{3}H-proline on SDS-PAGE. Growth characteristics were evaluated by automated cell counting and determination of labelling indices after ^{3}H-thymidine incorporation.

Arotinoid acid and arotinoid ethylester were found to be the most potent inhibitors of collagen synthesis. This effect was seen even in the micromolar range, whereas arotinoid was only effective at millimolar concentrations. Etretin, etretinate and demethyletretin had no or only little effect on total collagen synthesis. The relative amount of Pc-procollagen increased up to threefold after treatment with arotinoid acid and ester. Dose dependence was only seen in the small range of 10^{-10} to 10^{-8} M. Etretin was thousand fold less effective and the other tested coumpounds had virtually no effect. Arotinoid acid and ester were also found to reduce cell growth most strikingly, whereas etretin and etretinate were less effective by the factor 10^3. Arotinoid, arotinoid sulfon and demethyletretin showed no remarkable influence on the growth rate. Inhibition of cell growth was strictly dose dependent and cytotoxicity was not observed.

In conclusion, 3rd generation retinoids markedly influence the metabolic activity and proliferation rate of human fibroblasts, whereas reduction of procollagen synthesis is accompanied by enhanced aminopropeptidase activity.

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DERMATOSPARAXIS IN A CALF WITHOUT PROCOLLAGEN ACCUMULATION. Klaus P. Müller, Andreas Nerlich*, Peter K. Müller+, Dermatologische Klinik, Klinikum Steglitz der FU Berlin, Pathologisches Institut der Universität München*, Max-Planck-Institut für Biochemie, Martinsried+.

Dermatosparaxis is a recessively inherited general disorder of the connective tissue in several animals, e.g. calf and sheep, where the normal processing of procollagen is disturbed by a defect of the aminopropeptidase. In man, similar pathological conditions are found in Ehlers Danlos syndrome type VII. We saw a sporadic case of a calf exhibiting all clinical symptoms of dermatosparaxis, that virtually did not show an accumulation of procollagen in the skin. We tried to elucidate the underlying pathological mechanism by histological, ultrastructural and biochemical methods.

The skin of the calf appeared thin and fragile, showing thin, loosely woven collagen bundles. Electron microscopy revealed normally striated fibers and fragments of fibers, interspersed with amorphous material. In cross sections of the fibers, unequally reduced diameters were detectable but not the "hieroglyphic" pattern, typical for common dermatosparaxis. Rotary shadowing of the SLS-crystallites from neutral salt extracts showed the regular striation pattern but were always contaminated with coprecipitating globular material. The relative amount of collagen in the dermis was markedly reduced as shown by hydroxyproline assessment. Whereas examination of different skin extracts on 6% SDS-PAGE exhibited a normal banding pattern, an additional peptide of apparent MW of 25.5 kD was detectable in 5-20% gradient gels, when neutral salt or guanidinium extracts were analysed. CM-chromatography of the neutral salt extract showed broadening of the peaks. The relative amount of intact collagen chains in that extract was about half of the control. Additionally, the melting point of type I collagen triple helix showed a decrease of 2°C.

We speculate, that a defect in the collagen chains may lead to reduced stability of the triple helix, followed by enhanced degradation and accumulation of small peptides.

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IN SITU T CELL ACTIVATION IN ALOPECIA AREATA. Henning Hamm, Imke Kreuzer, Silke Klemmer, and Eva-B. Bröcker, Dept of Dermatology, University of Münster, Münster, F.R.G.

Alopecia areata (AA) is characterised by peri- and intrabulbar infiltrates mainly composed of T lymphocytes. We asked how far T cell activation, as determined by in situ expression of interleukin-2 receptor (IL-2 R), is correlated to clinical and histopathological features in AA.

Fifty-five patients (29 males, 26 females, mean age 32 ± 13 years) with AA of varying extent and duration were studied. Serial cryostat sections of scalp biopsies were immunohistologically investigated with monoclonal antibodies against CD 25 (IL-2 R), CD 1, CD 3, CD 4, and CD 8 antigens. Peri- and intrabulbar mononuclear cells were counted, and the percentages of antigen positive cells were determined.

IL-2 R^+ cells in peribulbar location were seen around 91/111 bulbs (82%). Intrabulbar IL-2 R^+ cells were present in only 27/102 bulbs (26.5%). The percentages of IL-2 R^+ cells were neither related to the extent of scalp involvement nor to the duration of AA. In peribulbar infiltrates, we found a clear-cut correlation between the percentage of IL-2 R^+ cells and the amount of overall inflammatory infiltrates, the amount of peribulbar T cells (CD 3), and the percentage of Langerhans/indeterminate cells (CD 1) in the infiltrates. IL-2 R expression was not correlated to the peribulbar CD4/CD8 ratio. In intrabulbar location, IL-2 R^+ cells occurred mainly in bulbs which contained intraepithelial CD 1^+ cells.

IL-2 R expression seems to reflect continuous disease activity even in long-standing AA, because no decline in proportion to the duration of disease was observed. Our data provide indirect evidence for in situ T cell activation by accessory cells of the Langerhans/indeterminate cell phenotype.

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