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Altered expression of angiogenesis and lymphangiogenesis markers in the uninvolved skin of plaque-type psoriasis

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Abstract

Background Vascular alterations are significant events in the pathomechanism of psoriasis. A disorder in the mechanisms regulating skin angiogenesis and lymphangiogenesis could participate in the pathogenesis of the disease.
Objectives To quantify differences in the expression of angiogenesis and lymphangiogenesis growth factors, receptors, coreceptors as well as their antagonists in the uninvolved skin of patients with psoriasis compared with the skin of non-psoriatic volunteers.
Methods Skin biopsies were collected from the involved skin of 13 patients with untreated plaque-type psoriasis, from their nonlesional skin at distance from the lesions and from the skin of 16 healthy volunteers. The mRNA steady-state level of keratins 10, 14 and 16, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), vimentin, collagen I and IV, proliferating cell nuclear antigen, the various splice variants of vascular endothelial growth factor, VEGF-A, VEGF-C and VEGF-D, their receptors VEGFR1, VEGFR2 and VEGFR3, neuropilin (NRP)-1 and its soluble forms, NRP-2, semaphorin 3A and prox-1, was measured by reverse transcription—polymerase chain reaction. Immunohistochemistry was performed for Ki-67, von Willebrand factor and D2-40. Blood and lymphatic vessel density, area and distance from epidermis were estimated by morphological analysis coupled to an original computer-assisted method of quantification.
Results Skin from healthy volunteers and nonlesional skin from patients with psoriasis displayed similar histological, morphometric and proliferative features. However, a significant overexpression of VEGFR3, the VEGF-A isoform VEGF121, soluble 12 NRP-1 and GAPDH was observed in the nonlesional psoriatic skin as compared with that of normal volunteers.
Conclusions These data point to significant differences in the blood and lymphatic vascular transcriptome between the clinically normal-appearing skin of patients with psoriasis and the skin of volunteers without psoriasis.

Keywords: angiogenesis, lymphangiogenesis, psoriasis

The development of psoriasis lesions depends on multiple interactions between susceptibility genes and environmental factors. The pathogenesis of psoriasis includes epidermal hyperplasia, impaired epidermal differentiation, accumulation of inflammatory cells and excessive angiogenesis with enlarged, tortuous and hyperpermeable dermal blood vessels.[2] Vascular expansion is an early event that precedes epidermal changes[3,4] and persists after recession of the epithelial and inflammatory alterations[5]. This has led to the suggestion that psoriasis is an angiogenesis-dependent disease.

Both angiogenesis and lymphangiogenesis are complex multistep processes and the delicate balance between the numerous positively and negatively acting mediators leads to expansion or recession of the vascular network[6]. The vascular endothelial growth factor (VEGF) family plays a significant role in these processes. VEGF-A, that induces vascular endothelial cell proliferation and increased permeability, binds to two related tyrosine kinase receptors, VEGFR1 and VEGFR2, the latter being the major signalling mediator of the angiogenic effects. VEGF-A is overexpressed in the psoriatic epidermis and increases the expression of VEGFR2 in endothelial cells[7]. Alternative splicing of its gene generates at least six molecular variants. VEGF165 and the freely diffusible VEGF121 are the major isoforms. VEGF189, VEGF206 and, to a lesser extent, VEGF165 are associated to the extracellular matrix and are released upon proteolysis[8]. We recently described a new isoform, VEGF111, induced by genotoxic agents, biologically active and resistant to proteolysis.[9] Placental growth factor (PlGF), another key mediator of skin angiogenesis that is upregulated during hair follicle cycle and wound healing[10], may directly stimulate VEGFR1 and potentiate VEGF activity. Alternative exon splicing generates four different isoforms, PlGF-1-PlGF4,[11,12] with different binding affinities to heparin and neuropilins, PlGF-2

being membrane associated and PlGF-1 diffusible. PlGF-3 and PlGF-4 are expressed in placenta and umbilical vein endothelial cells[12].

During the last decade, the lymphatic system has been the subject of increasing interest with the discovery of specific factors among which are the lymphatic growth factors VEGF-C and VEGF-D[13]. Their tyrosine kinase receptor VEGFR3 found in venous and lymphatic endothelial cells during embryogenesis becomes restricted to lymphatic cells early in the postnatal period except for the fenestrated blood vessels of some organs such as endocrine glands.[14] The activation of VEGFR3 by VEGF-C or VEGF-D leads to proliferation of the lymphatic endothelial cells while targeted deletion of VEGF-C in mice impairs the development of small lymphatic vessels.[13] Lymphatics are expanded in psoriasis but little is known about the mechanism of their development in the lesions and potential alterations in the noninvolved skin of the patients.

Differences between the uninvolved skin of patients with psoriasis and the skin of healthy individuals have been documented, such as increased expression of keratin 16[15] and phosphorylated NF-κB/RelA,[16] reduced keratin 15 and β1 integrin expression[17], or papillary oedema and endothelial cells swelling during psoriasis flares[18]. Vascular volume[19] and adhesion receptors such as intercellular adhesion molecule-1 and E-selectin[20] have been reported to be increased in the nonlesional psoriatic skin. These observations suggest the existence of a prepsoriatic phenotype in the distant nonlesional skin, but little is known about their potential significance in the pathomechanisms of the disease.

The aim of our study was to evaluate by reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry the expression of angiogenesis and lymphangiogenesis markers to search for potential differences between the normal-appearing skin in psoriasis and the skin of healthy volunteers. These markers were also evaluated in the psoriatic lesions of the same patients. Our study provides evidence that the distant uninvolved skin in psoriasis displays vascular and lymphatic transcriptomic differences when compared with healthy skin without major modification in the expression of epidermal or dermal genes or morphological alterations.

MATERIALS AND METHODS

Patients

Thirteen adult patients (eight women and five men) with moderate to severe plaque-type psoriasis (Psoriasis Area and Severity Index median score 8·5) who had not received any systemic treatment for 1 month or any topical therapy for 2 weeks underwent skin punch biopsy (4 mm) after local anaesthesia (1% xylocaine with adrenaline) in a lesion and in the distant uninvolved skin (at least 2 cm from the nearest plaque). Sixteen volunteers (six women and 10 men) who had no personal or familial history of psoriasis were also biopsied. The mean ± SD age was 37 ± 14 years (range 21-67) and 45 ± 19 years (range 21-76) for the psoriasis and volunteers group, respectively. Biopsies were snap frozen in liquid nitrogen and stored at -80°C until use. Biopsies from 15 additional untreated patients and 11 other volunteers were embedded in paraffin after fixation in 3% formalin or in OCT. This study was approved by the Ethics Committee of the University Hospital of Liège. The patients gave their written informed consent to participate in the study and the Declaration of Helsinki protocols were followed.

RNA isolation and reverse transcription-polymerase chain reaction

Total RNA was purified (High Pure RNA Tissue kit; Roche Molecular Biochemicals, Mannheim, Germany) from the frozen biopsies after crushing in liquid nitrogen (Dismembrator Braun Technology International, Melsungen, Germany), and quantified (RiboGreen, R-11491; Molecular Probes, Invitrogen, Merelbeke, Belgium). The RT-PCR amplifications were performed in an automated thermal cycler (GeneAmp PCR System 9700 using a GeneAmp thermostable rTth Reverse Transcriptase RNA PCR kit; Applied Biosystems, Foster City, CA, U.S.A.) with pairs of primers described in previous publications[21-25]. For RT-PCR amplification of PlGF, forward and reverse primers were chosen in regions surrounding the alternatively skipped sequences, allowing the discrimination the four isoforms on an electrophoretic mobility basis. A comparable strategy was used for VEGF-A.[9,26] For quantitative RT-PCR measurements, 3 ng of total RNA was used per 20 µL reaction mixture. The efficiency of the RT and the PCR reactions for 28S, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), collagen I, VEGF-A and semaphorin 3A was controlled by a synthetic RNA.[23-25] The amplification products were separated by polyacrylamide gel electrophoresis, stained (Gelstar, Cambrex, Rockland, ME, U.S.A.), and quantified (Fluor-S Multilmager; Biorad, Hercules, CA, U.S.A.). The results of quantification expressed in arbitrary units [per unit of 28S ribosomal RNA (28S rRNA)] are given as the mean ± SD percentage

of the values measured in skin from healthy volunteers taken as 100%.

RNA was extracted from several 50-µm sections from 10 OCT-embedded biopsies of each lesion category and the surface of each biopsy was measured. RT-PCR was performed to measure 28S rRNA and VEGF-A, VEGFR2 and VEGFR3 mRNA, using a Superscript II Reverse Transcriptase (Invitrogen) and a Takara Taq polymerase (Biomedical Group, Shiga, Japan). The mRNA level was calculated by unit volume of tissue.

Histology

Five-micrometre serial paraffin sections were stained with antihuman Ki-67 (Clone MIB-1; DakoCytomation, Glostrup, Denmark), antihuman von Willebrand factor (DakoCytomation) and anti D2-40 (Proteogenix, Illkirch, France). Secondary antibodies coupled to peroxidase were used for Ki-67 and von Willebrand factor (goat antimouse Envision and swine antirabbit, respectively; DakoCytomation). For D2-40, a goat antimouse antibody coupled to biotin was used, followed by incubation with streptavidin coupled to peroxidase (both from DakoCytomation). Peroxidase activity was revealed with diaminobenzidine for Ki-67 and with 3-amino-9-ethylcarbazole for D2-40 and von Willebrand immunohistochemistry.

Image analysis

Visual image quantification

Epidermal and dermal cellularity was evaluated by counting the number of cells per high power field in 5-µm sections of seven samples of uninvolved psoriatic skin and seven samples of healthy skin. The acanthosis score, evaluated by the ratio between epidermal area and horny layer length, and papillomatosis score, evaluated by the ratio between basement membrane and horny layer lengths, were calculated for each group of biopsies on 5-µm haematoxylin and eosin-stained sections (Axiovert 25 Zeiss equipped with a CCD camera and KS 400 software; Carl Zeiss Vision GmbH, Aalen, Germany). The Ki-67 labelling index was expressed as the number of positive cells as a percentage of the total basal and suprabasal cells. A minimum of 300 cells was counted on × 40 magnification photographs for each specimen.

Semiautomatic image quantification

Images of sections were observed and digitized with the Axiovert 25 Zeiss microscope coupled to a CCD camera (Carl Zeiss Vision GmbH). An original algorithm for image processing and measurements was implemented with the Aphelion 3.2i software (Adcis, Hérouville Saint-Clair, France) as detailed in Figure 1. The following parameters were measured: (i) the mean size of vessels, (ii) the mean distance of vessels to the basal membrane and (iii) the mean density of vessels per area of superficial dermis (dermis between the superficial vascular plexus and the dermoepidermal basement membrane).

Statistical evaluation

Results are expressed as mean ± SD. Statistical analysis was performed using ANOVA and Scheffé's test to compare the three groups and the paired Student's t-test to compare the non-lesional and lesional skin in the psoriasis group. Student's t-tests were applied to compare the histological parameters between the groups. To normalize the distribution, a log transformation was applied for some variables. Discriminant analysis was performed to identify variables enabling discrimination between the three groups. Results were considered to be significant at the 5% confidence level ($P < 0.05$). Within each group, Pearson correlation coefficients were calculated between each pair of variables. Calculations were done using the SAS software (version 9.1 for Windows) (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Morphological parameters

No difference was observed between nonlesional psoriatic and healthy volunteer skin in terms of cellularity, proliferating rate, papillomatosis or acanthosis indexes (Fig. 2). As expected, these parameters were significantly increased in the psoriatic lesions. The density of blood and lymphatic vessels in the superficial dermis as well as their surface area were similar in the uninvolved skin from patients with psoriasis and skin from healthy volunteers but were largely increased in the lesions of psoriasis. The mean distance of lymphatic vessels to the basement membrane was comparable in the normal skin from both psoriasis and volunteers, but lymphatic

capillaries were significantly closer to epidermis in the psoriatic lesions. Such a pattern was not observed for blood vessels, which were at similar distance from the basement membrane in the three groups.

Fig 1: *Illustration of the image analysis algorithm used to quantify the density, the size and the distance to the basal membrane of lymphatic and blood vessels.* (a) A typical D2-40-stained section in which lymphatic vessels (as well as part of the basal keratinocyte layer) can be distinguished by a brownish colour. After splitting the colour image to its grey level components, vessels were better contrasted in the green component (c) than in the red (b) or blue one (d). Vessels were segmented automatically using the entropy of histogram of grey level intensities and geodesic transformations were used to eliminate the vessels placed in the border of the image and to fill their lumen. Finally, an erosion filter was used to eliminate small artefacts, and the resulting image is used as marker to reconstruct the final binary image (e). The manually drawn contour of the region located between the basement membrane and the superficial vascular plexus (f) was binarized and the area filled automatically (g). The application of this mask on the segmented image of vessels (e) allowed us to obtain an image containing only those vessels placed between the superficial vascular plexus and the basement membrane (h). Then, each vessel was individualized by a colour (labelled image, i) in order to measure its corresponding area. The mean distance between vessels and the basement membrane was calculated as follows. The distance transformation[43] was applied to the binary image of the mask. In the resulting grey level image (k), the intensity associated to each pixel is the Euclidean distance to the nearest 'black' point, i.e. the nearest external border of the basement membrane. Then, the grey level intensities of the image obtained intersecting the image containing the centre of gravity of each vessel and the image distance of the mask (k) are the nearest distances from each vessel to the basal membrane. For the sake of clarity, (1) shows the intersection of the image distance (k) with the image of vessels (i), rather than with the image of their centres of gravity.

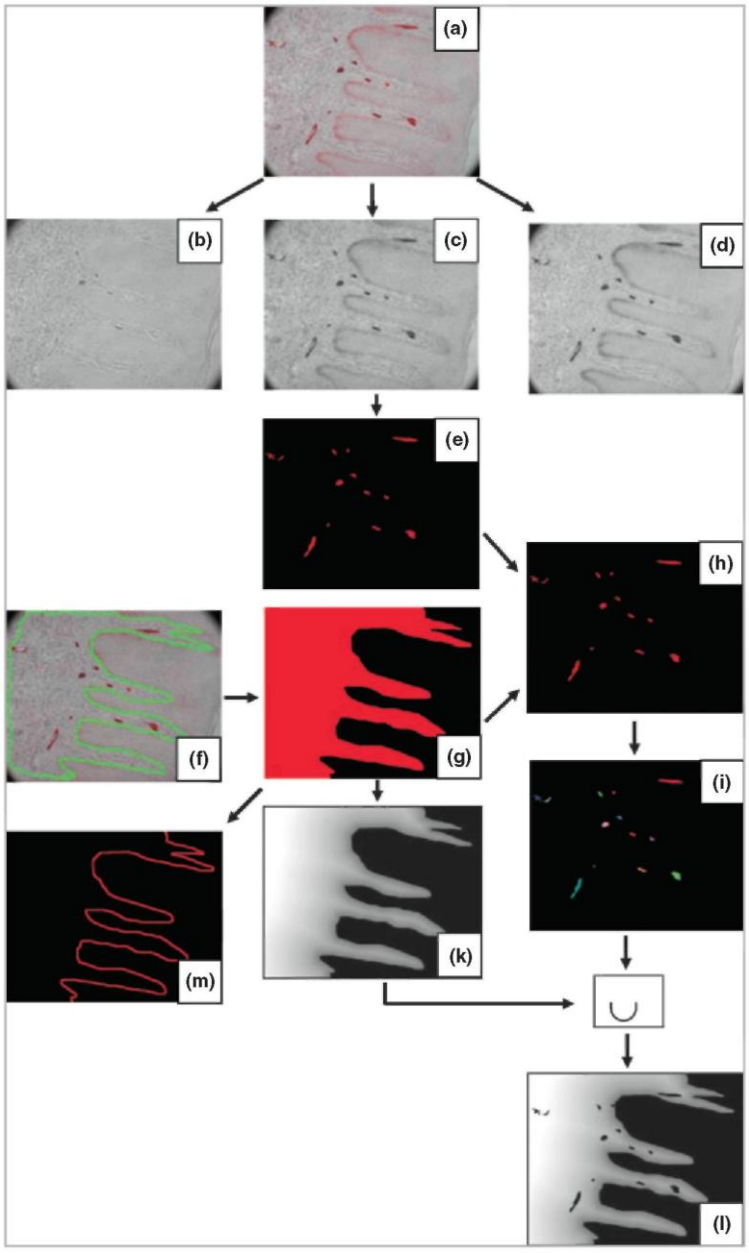
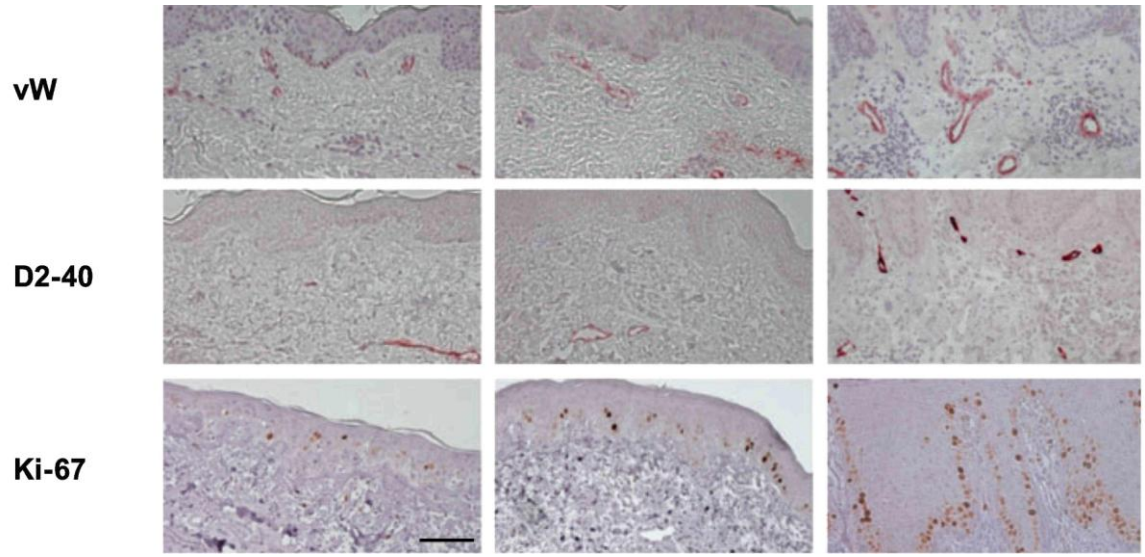


Fig 2: *Immunostaining of cycling cells, blood and lymphatic vessels in nonpsoriatic skin (NV), uninvolved psoriatic skin (NP) and lesional psoriasis (LP).* Paraffin-embedded sections were stained with antihuman von Willebrand factor (vW) (blood vessels), D2-40 (lymphatic vessels) and Ki-67 (cycling cells). Serial sections of LP were stained with D2-40 and vW. Cellularity was counted in epidermis and superficial dermis of the skin of seven healthy volunteers and seven patients (uninvolved skin) on haematoxylin and eosin-stained sections. Papillomatosis and acanthosis scores were evaluated for all the patients. The Ki-67 labelling index was expressed as the number of positive cells as a percentage of the total basal and suprabasal cells. Blood and lymphatic vessel sections were measured using computerized image analysis. A quantification of the morphological observations is given below the figure. Scale bar = 100 μm.



	NV	**NP**	LP
Cellularity			
Epidermis	387 ± 89[1]	324 ± 69	nd[2]
Dermis	182 ± 37	182 ± 60	
Papillomatosis[3]	1·4 ± 0·3	1·3 ± 0·1	3·1 ± 0·5[8]
Acanthosis[4]	80 ±26	86 ±32	257 ± 39[8]
Blood vessels			
Distance (μm)[5]	85 ±12	90 ±10	81 ± 5
Area (μm^2)[6]	253 ± 25	204 ±13	619 ± 78[8]
Density (%)[7]	1·6 ±0·3	1·3 ± 0·2	5·0 ± 0·6[8]
Lymphatic vessels			
Distance (μm)[5]	92 ±13	96 ±10	70 ± 4[9]
Area (μm^2)[6]	263 ± 54	254 ± 42	426 ± 25[8]
Density (%)[7]	10 ± 0·4	0·3 ± 005	2 ± 002[8]
Ki-67	22 ± 6%	25 ± 5%	50 ± 9%[8]

[1]Mean ± SD; [2]not determined; [3]ratio between basement membrane and horny layer length; [4]ratio between epidermal area and horny layer length; [5]distance to basement membrane; [6]mean area of vessels; [7]area of vessels in % of area of superficial dermis; [8]statistically significant (P< 0·05) difference when compared to NP and NV; [9]Statistically significant (P< 0·05) difference when compared to NP.

Critical analysis of the reverse transcription-polymerase chain reaction procedure to measure the mRNA of selected genes

The type of information used for quantifying the expression of representative gene products in the present study is illustrated in Figure 3. The results were expressed as arbitrary units of a specific mRNA per unit of 28S rRNA. This calculation is adequate for the comparison between nonlesional skin in psoriasis and skin from healthy volunteers, as cellularity is similar in the epidermal and dermal compartments as shown in Figure 2. As the psoriatic lesions contain a large excess of epithelial and inflammatory cells, a significant proportion of RNA collected in the lesional skin is of epithelial and inflammatory origin and dilutes the RNA originating from the less abundant dermal cells. The 28S rRNA collected from cryostat sections, calculated per unit volume of tissue, was indeed 3·5 times higher in the lesions than in the distant unaltered skin (data not shown). We therefore did not evaluate the statistical significance of the differences between lesional and nonlesional skin in Table 1. The

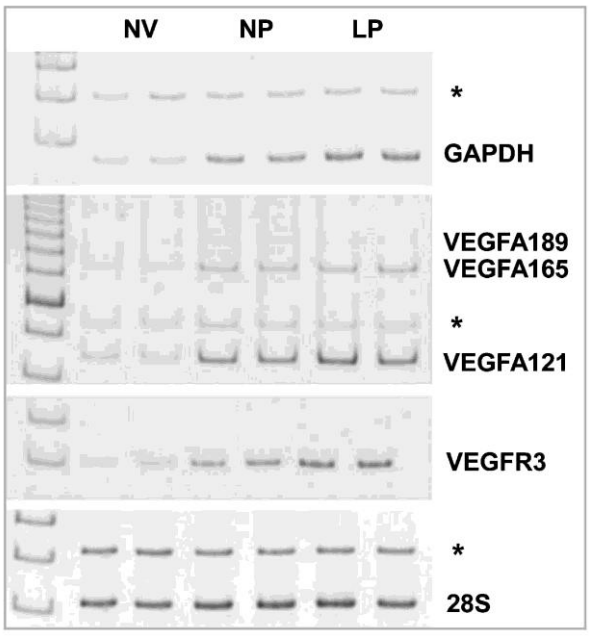
mRNA level values reported for the lesional skin were used only to identify angiogenesis and lymphangiogenesis markers that might be of interest in the comparison between the unaltered psoriatic and healthy volunteer skin.

Expression of the selected mRNAs in psoriasis lesions and in nonlesional skin

The amount of total RNA extracted from the punch biopsies of psoriasis lesions was on average 3·2 higher than the quantity recovered from nonlesional psoriatic and healthy skin biopsies of similar diameter, in agreement with the hypercellularity and the rRNA amounts measured in cryosections as described above. The results expressed in arbitrary units per unit of 28S rRNA represent a measure of the specific mRNA on a per cell basis.

Our data are consistent with previous studies[27] as keratin 16, synthesized by proliferating epidermal cells, keratin 14, expressed by dividing basal cells, collagen IV, a basement membrane collagen, and the proliferation marker proliferating cell nuclear antigen (PCNA) were overexpressed in the lesions, while the reduced expression of vimentin per unit of 28S also observed in the lesions is likely to be related to the dilution of RNA from dermal cells (Table 1). No significant difference existed in the expression of keratin 10, collagen I, VEGF121, VEGF165 and VEGF189, when expressed per cell unit. Only the PlGF-1 and PlGF-2 isoforms were observed in our samples. These factors were both overexpressed in the lesions as compared with the distant uninvolved skin, while the expression of the antiangiogenic factor semaphorin 3A, a VEGF competitor and antagonist that binds to neuropilin-1 (NRP-1)[25], was reduced. Neuropilin-2a (NRP-2a), that acts as a coreceptor for VEGF-A and VEGF-C by interacting with VEGFR2 and VEGFR3,[28-31] was also increased in lesions, while VEGF-D mRNA expression was reduced. The mRNAs of other lymph-angiogenic markers were not modified.

Fig 3: *Electrophoretic pattern of the amplified products of selected genes.* RNA was extracted from punch biopsies of uninvolved (NP) and involved (LP) skin of patients with psoriasis and from healthy volunteer skin (NV). Reverse transcription—polymerase chain reaction (RT-PCR) was performed in duplicate for 28S, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), vascular endothelial growth factor (VEGF)-A and VEGFR3. The efficiency of the RT and PCR reactions for 28S, GAPDH and VEGF-A was controlled by an internal standard (asterisks). The amplified products were separated by polyacrylamide gel electrophoresis, stained with ethidium bromide, and quantified.



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Epidermal and dermal markers expression in nonlesional psoriatic and healthy volunteer skin

As shown in Table 2, the expression of keratin 10, keratin 14 and keratin 16, vimentin, collagen I, collagen IV and PCNA was similar in both groups, in agreement with morphological and proliferative features. By contrast, GAPDH mRNA was significantly increased in the nonlesional skin in psoriasis as compared with the healthy skin (Fig. 4).

Table 1: Expression of general, angiogenesis and lymphangiogenesis markers in the uninvolved and lesional skin of patients with psoriasis

RNA	Uninvolved skin	Lesional skin
General markers		
Epidermal		
Keratin 10	817 ± 219	732 ± 202
Keratin 16	264 ± 350	4042 ± 4999
Keratin 14	228 ± 112	541 ± 798
Dermal		
Collagen I	245 ± 170	218 ± 149
Vimentin	367 ± 100	285 ± 130
Basement membrane		
Collagen IV	194 ± 98	452 ± 325
Proliferation		
PCNA	153 ± 181	418 ± 390
Metabolism		
GAPDH	216 ± 55	250 ± 117
Angiogenesis		
Ligands		
VEGF121	238 ± 104	246 ± 133
VEGF165	59 ± 40	60 ± 30
VEGF189	15 ± 9	18 ± 12
PlGF-1	193 ± 93	518 ± 606
PlGF-2	561 ± 319	1609 ± 1997
Semaphorin 3A	283 ± 190	227 ± 170
Receptors		
VEGFR1	226 ± 130	190 ± 50
VEGFR2	113 ± 44	104 ± 43
sVEGFR1	273 ± 104	311 ± 83
Coreceptors		
NRP-1	477 ± 141	407 ± 115
s11NRP-1	109 ± 56	113 ± 74
s12NRP-1	160 ± 59	201 ± 90
Lymphangiogenesis		
Ligands		
VEGF-C	141 ± 84	118 ± 59
VEGF-D	30 ± 26	15 ± 10
Receptor		
VEGFR3	33 ± 19	24 ± 19
Coreceptor		
NRP-2a	35 ± 29	107 ± 150
Transcription factor		
Prox-1	183 ± 81	185 ± 72

Results are shown as mean ± SD. The values are expressed in arbitrary units of mRNA per unit of 28S rRNA. PCNA, proliferating cell nuclear antigen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; NRP, neuropilin.

Expression of angiogenesis-related markers in nonlesional psoriatic and healthy volunteer skin

Ligands

As shown in Table 3, the VEGF121, VEGF165 and VEGF189 isoforms were expressed in all samples while VEGF111 was not detected. The VEGF121 mRNA level was significantly increased in nonlesional psoriatic skin as compared with healthy volunteer skin (Fig. 4) while the two other VEGF isoforms, PlGF-1, PlGF-2 and semaphorin 3A were expressed at a similar level.

Receptors and coreceptors

No difference was observed in the expression levels of VEGFR1, of its soluble antagonist form sVEGFR1 and of VEGFR2. NRP-1 is a coreceptor that binds and presents VEGF165 to VEGFR2, enhancing its biological activity[8]. Two soluble NRP-1 (sNRP-1) isoforms generated by pre-mRNA processing of intron 12 (s12NRP-l) and intron 11 (s11NRP-1) have been described as VEGF165 antagonists.[32,33] The soluble neuropilin s12NRP-1 was significantly increased in uninvolved psoriatic skin as compared with healthy individuals (Fig. 4) while no significant difference was observed for NRP-1 and s11NRP-1.

Expression of lymphangiogenesis-related markers in nonlesional psoriatic and healthy volunteer skin

Ligands, receptor and coreceptor

As shown in Table 4, the mRNA level of the lymphangiogenesis receptor VEGFR3 was significantly increased in the nonlesional psoriatic skin as compared with healthy volunteer skin (Fig. 4) whereas the lymphatic endothelial cell growth factors VEGF-C and VEGF-D displayed the same level. The mRNA of NRP-2a tended to be higher in uninvolved psoriatic skin ($P = 0{\cdot}08$). The other isoform of NRP-2, NRP-2b, and its soluble form, s9NRP-2[33], were not detected in our skin samples.

Differentiation marker

Prox-1 is a homeotic protein that specifies the lymphatic cell fate by reprogramming the transcriptome of embryonic venous endothelial cells[30]. Prox-1 mRNA expression tended to be increased in uninvolved psoriatic skin ($P = 0{\cdot}052$) and correlated positively with that of NRP-2a ($P = 0{\cdot}02$, not illustrated).

Table 2: *Expression of epidermal, dermal and metabolic markers in skin from healthy volunteers and uninvolved skin from patients with psoriasis*

Markers	Healthy skin	Uninvolved skin
Epidermal		
Keratin 10	100 ± 37	121 ± 32
Keratin 16	100 ± 130	233 ± 309
Keratin 14	100 ± 48	181 ± 89
Dermal		
Collagen I	100 ± 89	155 ± 107
Vimentin	100 ± 56	129 ± 35
Basement membrane		
Collagen IV	100 ± 78	115 ± 58
Proliferation		
PCNA	100 ± 105	82 ± 97
Metabolism		
GAPDH	100 ± 43	162 ± 41[a]

Results are shown as mean ± SD percentage of the value measured in healthy volunteers taken as 100%. [a]Significant statistical difference between uninvolved psoriatic skin (n = 13) and healthy skin (n = 16) ($P < 0{\cdot}05$), ANOVA and Scheffé's test. PCNA, proliferating cell nuclear antigen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Fig 4: *GAPDH, VEGF121, s12NRP-1 and VEGFR3 mRNA expression values (mean ± SD percentage of the value measured in healthy volunteers taken as 100%) in healthy volunteers (NV; black) and uninvolved psoriasis skin (NP; grey) groups (*P < 0·05). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; VEGF, vascular endothelial growth factor; NRP, neuropilin.*

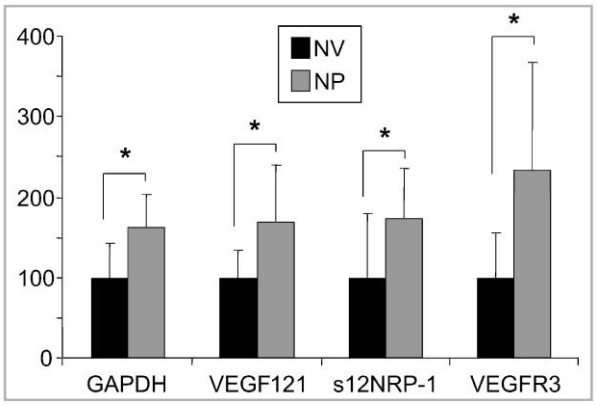

Table 3: *Expression of angiogenesis related markers in skin from healthy volunteers and uninvolved skin from patients with psoriasis*

Markers	Healthy skin	Uninvolved skin
Ligands		
VEGF121	100 ± 35	168 ± 73[a]
VEGF165	100 ± 45	133 ± 90
VEGF189	100 ± 161	123 ± 73
PlGF-1	100 ± 49	117 ± 56
PlGF-2	100 ± 46	111 ± 63
Semaphorin 3A	100 ± 62	135 ± 91
Receptors		
VEGFR1	100 ± 65	123 ± 70
VEGFR2	100 ± 51	149 ± 58
sVEGFR1	100 ± 39	104 ± 40
Coreceptors		
NRP-1	100 ± 39	103 ± 29
s1 1NRP-1	100 ± 64	195 ± 100
s1 2NRP-1	100 ± 80	173 ± 63[a]

Results are shown as mean ± SD percentage of the value measured in healthy volunteers taken as 100%. Significant statistical difference between uninvolved psoriatic skin (n = 13) and healthy skin (n = 16) (P < 0·05), ANOVA and Scheffé's test. VEGF, vascular endothelial growth factor; PlGF, placental growth factor; NRP, neuropilin.

Table 4: *Expression of lymphangiogenesis markers in skin from healthy volunteers and uninvolved skin from patients with psoriasis*

Markers	Healthy skin	Uninvolved skin
Ligands		
VEGF-C	100 ± 40	109 ± 65
VEGF-D	100 ± 66	169 ± 148
Receptor		
VEGFR3	100 ± 55	233 ± 134[a]
Coreceptor		
NRP-2a	100 ± 54	148 ± 127
Differentiation		
Prox-1	100 ± 50	136 ± 60

Results are shown as mean ± SD percentage of the value measured in healthy volunteers taken as 100%. Significant statistical difference between uninvolved psoriatic skin (n = 13) and healthy skin (n = 16) (P < 0·05), ANOVA and Scheffé's test. VEGF, vascular endothelial growth factor; NRP, neuropilin.

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DISCUSSION

With nine susceptibility loci already described, the predisposition to psoriasis probably results from a combination of inherited genes that interact. This has led us to search for alterations in the expression of genes related to angiogenesis and lymphangiogenesis in the uninvolved skin of patients with psoriasis.

In agreement with recent data,[34,35] blood and lymphatic vessel density was similar in uninvolved psoriatic skin and in skin of normal volunteers. The same conclusions were reached for the other morphological parameters and the expression of epidermal, dermal and proliferative markers, indicating a comparable biosynthetic activity and supporting the absence of subclinical psoriasis lesions in the biopsies collected at distance. The increased expression of GAPDH in the psoriasis group, however, suggests the existence of an increased mitochondrial activity not only in the lesions, as we and others found,[36] but also in uninvolved skin. Our morphological investigations also demonstrate that the lymphatic capillaries are not only more abundant in the superficial dermis in the lesions when compared with the uninvolved tegument but are also significantly closer to the epidermis.

By using discriminant analysis on the angiogenesis markers, the best variable to discriminate the nonlesional and normal volunteers groups was VEGF121 which was overexpressed in the uninvolved psoriatic skin. Focal over-expression of VEGF-A mRNA was previously detected by in situ hybridization[7] in the nonlesional skin in psoriasis. However, the isoform was not identified and microscopic abnormalities were suggestive of early psoriasis activity. Our results indicate that the uninvolved psoriatic skin displays either an increased VEGF121 transcription, and/or stability of the transcript and/or regulated alternative splicing. This freely diffusible form could be responsible for the increased vascular permeability described in this group[18] and therefore could promote an abnormal inflammatory response for minor trauma (Köbner phenomenon). This is also in agreement with previous observations showing that the VEGF gene, located on chromosome 6p21 relatively close to the PSORS1 locus,[37] is polymorphic and that a single polymorphism in the untranslated region of the gene is associated with psoriasis type I[38]. The absence of any significant alteration of VEGF expression in the lesions when compared with the nonlesional skin in the same patients, as we observed in our study, is related to the normalization by 28S rRNA that gives an estimation of mRNA value per cell unit, suggesting a similar level of expression of VEGF per cell. The overall increase of VEGF mRNA in psoriatic lesions[7] could therefore be related to the increased cellularity. We indeed measured a higher expression of the different isoforms of VEGF-A, as well as of VEGFR2, VEGF-C, VEGFR3 and prox-1 in lesional skin as compared with nonlesional in a series of 10 patients with psoriasis when the mRNAs levels were expressed by unit volume (μm^3) of tissue instead of 28S rRNA (data not shown).

The increased expression of the soluble s12NRP-1 in the uninvolved skin of patients with psoriasis might interfere with the binding of VEGF-A to its receptor VEGFR2 and offers a counterbalance to its angiogenic effects. sNRP targeted to the skin is indeed able to inhibit vascular permeability[39]. In the lesions, the reduced expression of semaphorin 3A coupled to the overexpression of PlGF-1 and PlGF-2 could promote angiogenesis.

Among lymphangiogenesis markers, the best variable enabling discrimination between nonlesional psoriasis and healthy volunteer skin was VEGFR3. The similar lymphatic vessel density in those groups might indicate that the production of VEGFR3 mRNA per lymphatic endothelial cell is increased in uninvolved psoriatic skin. The increased number of lymphatic vessels seen in the psoriasis lesions might be related to the overexpression of NRP-2a, which has a crucial role in endothelial cell survival and migration, and could represent a mechanism leading to an increased number of lymphatic vessels by promotion of VEGFR3 activity. Recent work suggests that keratinocytes express VEGFR3 and NRP-2 in vitro and in vivo[40], and could be another potential source for these factors.

The present study provides additional evidence that the distant uninvolved skin in psoriasis presents vascular and lymphatic transcriptomic differences when compared with healthy skin without major modification in the expression of epidermal or dermal genes. This is consistent with the fact that the characteristic vascular features of psoriasis are among the first phenotypic variations observed in the genesis of a lesion. It adds evidence for a predisposition to inflammatory and vascular over-responsiveness to unspecified stimuli in the disease. Alterations in the expression of those mRNAs in clinically uninvolved skin distant from active plaques could result from genetically determined alterations in their transcription but could also be triggered by factors (cytokines) released by the active lesion. Whether the corresponding proteins are increased or not is under investigation. Whatever the issue, the post-transcriptional/ translational mechanisms regulating their expression are worthy of investigation and are at the centre of our future interests.

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