NEUROENDOCRINE-IMMUNOLOGY : FROM SYSTEMIC INTERACTIONS TO THE IMMUNE TOLERANCE OF SELF NEUROENDOCRINE FUNCTIONS

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INTRODUCTION

In recent years, it appeared more and more evident that the three major integrating and adaptive systems of intercellular communication, nervous, endocrine and immune systems, were closely interconnected. Through these interactions, psychological and neurological influences can modulate the immune response (neuroimmunomodulation), while immune cells may communicate to the neuroendocrine system by a regulatory feedback loop. On the basis of our own observations, it has been shown that the neuroendocrine-immune dialogue occurred in the thymus during the early steps of T-cell differentiation, and could be involved both in T-cell positive as well as negative selections.

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1° NEUROENDOCRINE INFLUENCES ON IMMUNE CELLS

According to several authors, the immune system may be regarded as a chemical sensory organ since its cellular components express specific receptors for various kinds of messengers (neurotransmitters, hormones, cytokines and tissue growth factors) (1, 2). The pathways by which the nervous system may influence the immune system are the following ones: the peripheral autonomic and sensory innervations of target organs, the hypothalamo-hypophysial axis, and the diffuse neuroendocrine system (to which paragraph 3 is specially devoted in this review).

Primary (thymus, bone marrow) and secondary (spleen, lymph nodes, gut-associated lymphoid tissue) lymphoid organs receive extended sympathetic and sensory innervations (3, 4). The reality of the communication has been established both at the level of the neurotransmitter signal and of the reception system expressed by lymphoid organs. Several neuropeptides like substance P (SP), neurotensin (NT), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), cholecystokinin (CCK) may be detected in nerve-like profiles of lymphoid organs but the localization of their cognate receptors has rarely been determined. This point is important since the target cells of nerve fibers in these organs may be immune cells, accessory or stromal cells, and the vascular bed. Innervation of lymphoid organs may therefore be implicated in the modulation of the activity of immunocompetent

cells, in the control of local tissue factor synthesis and secretion, as well as in the regulation of local blood flow, but a general conclusive view is still difficult to be drawn from the different experimental observations. Altogether, they have nevertheless enlightened the ability of peripheral nervous system (PNS) to regulate inflammatory processes and its potential etiopathogenic contribution to several disorders such as asthma, inflammatory gut diseases and arthritis (5).

Through the hypothalamo-hypophysial axis, the central nervous system (CNS) intervenes in the modulation of endocrine functions, either directly as for growth hormone (GH), prolactin (PRL), and neurohypophysial hormones, or indirectly via hypophysial trophic hormones which control the activity of peripheral endocrine parenchymas (thyroid, adrenals and gonads). The idea of an hypothalamo-hypophysial-thymic axis has also emerged from several observations (6) and could be implicated in the regulation of T-cell differentiation. GH and PRL exert well-established thymotrophic activities and contribute to a normal stage of immune functioning. Like its other peripheral actions, GH actions on the thymus are probably relayed by the induction of local insulin-like growth factor-I (IGF-I) since this latter has been shown to be expressed in the thymus, both at the peptide and messenger RNA (mRNA) levels (7). The importance of GH and PRL in immunophysiology is further enlightened by the structure of their respective receptors which make them belong to the superfamily of *hematopoietin* receptors (8), together with those of interleukin-2 (IL-2), IL-3, IL-4, IL-6, IL-7, erythropoietin, and granulocyte-macrophage colony stimulating factor (GM-CSF). Indirectly, the decrease of immune defenses observed in aging could be partially related to the GH deficiency of old patients. The reference immunosuppressive agent, cyclosporine, was also reported to act on lymphocytes through a PRL-type receptor (9) and not through a selective plasma-membrane drug-receptor, although the precise mechanism of the inhibition of cyclosporine is not yet fully understood. In the near future, some physiological significance may also emerge from the paracrine/autocrine systems evidenced for adreno-corticotrophic hormone (ACTH)-like, PRL-like and GH-like factors produced by several immunocompetent cells (10). Some pathological conditions, rather rare however, were found to be associated with excessive ACTH secretion by inflammatory cells (11) or in acute myeloblastic leukemia (12).

At the systemic level, steroid hormones are also implicated in a fine-tuned regulation of the immune reactivity. From a physiopathological point of view, this immune-endocrine connection intervenes to modulate immune responses in crucial periods of life such as pregnancy, along which a natural immunosuppression is usually guided by an increase of the *progestative/oestrogen* balance (13); at the opposite, during the post-partum and lactation, an enhancement of immune response and an increased risk of autoimmune processes seem to be induced or launched by the *gonadal steroid* environment (decrease of the progesterone/oestrogen balance) and by the *lactating peptide hormones*, prolactin and oxytocin (OT), known to raise unspecifically the level of immune reactivity (14).

The effects of *glucocorticoids* (GC) upon the immune system have been extensively investigated and have been presented in excellent recent reviews (15). At pharmacological doses, GC induce a lymphopenia resulting from cell death (apoptosis) in primary lymphoid organs and from a redistribution of circulating immunocompetent cells. *In vitro*, even physiological doses of GC inhibit the production of various cytokines like IL- 1, IL-2, IL-6 and interferons (IFNs), and this inhibition is not caused by immune cell death. The natural killer (NK) activity of some lymphocyte subpopulations is also depressed by GC *in vivo* and *in vitro*. These immunosuppressive effects are underlying the generalized use of GC in the pharmacological treatment of inflammatory disorders and autoimmune pathologies, while

their cytolytic action is exploited in the therapy of lymphoproliferative disorders. They also provide some explanation for the increased risk of infection of patients receiving chronic administration of GC. More importantly, these effects have contributed to the redefinition of the GC role in stress physiology. The concept of stress proposed by Selye (16) as the General Adaptation Syndrome (G.A.S.) was defined as all nonspecific, systemic reactions of the body subjected to various forms of aggression or challenges to homeostasis (hemorrhage and fluid loss, tissue damages, metabolic disturbances, infections, neural and cognitive changes). In G.A.S., thymic involution, adrenal hyperplasia and increase of GC concentrations were the most representative elements of the organism response. The classical opinion proposed by Selye himself was that the rise in GC tone enhanced the defence reactions elicited by specific aggression. Abnormal adaptive responses to stress and, consequently according to this concept, excessive secretion of GC were supposed to favor the emergence of «diseases of adaptation» such as diffuse collagen disease, allergy, and rheumatic disease. Now in 1991, it is indeed difficult to reconcile these views about the etiopathogenic role of GC in «diseases of adaptation» together with their extended use in the therapeutic strategy of these diseases themselves! The new hypothesis proposed by Munck et coll. (15) is therefore compatible with the anti-inflammatory and immunosuppressive effects of GC, and states that the main physiological function of GC in stress is in fact to regulate the primary defence reactions and to prevent them from overshooting.

Another steroid component, *vitamin D*, was shown to modulate immune responses during the past few years (17). Anti-proliferative and differentiation-inducing effects of its active metabolite 1,25 $(OH)_2 D_3$ were evidenced in hematopoiesis, mainly upon the myeloid lineage, and this suggests that the hormone could be used in the treatment of myelo-dysplastic syndromes and myeloid leukemias. T-cell activation is accompanied by the expression of specific receptors for 1,25 $(OH)_2 D_3$, and the production of IL-2 signal (but not of the receptor) is inhibited at low concentrations. The synthesis and the release of GM-CSF as well as IFN-gamma induced after lectin-activation of T-cells are also inhibited by 1,25 $(OH)_2 D_3$. Besides these immunomodulatory effects of the vitamin D endocrine system, an autocrine/paracrine system seems to be implicated since 1,25 $(OH)_2 D_3$ was shown to be produced after hydroxylation of the precursor by human macrophages (18). This local system might be involved in the modulation of hematopoiesis and of regional inflammatory sites.

2° NEUROENDOCRINE EFFECTS OF CYTOKINES

One of the most recent focus of interest in the field of Neuroendocrine-Immunology was to investigate the means by which the immune system may communicate some information to the brain in physiological conditions (to distinguish from specific immune reactions in pathological disorders of CNS, such as demyelinating diseases). The first immuno-neuroendocrine feedback loop was simultaneously advanced by several authors and concerned the activation of the hypothalamo-pituitary-adrenal (HPA) axis by IL-1 related signals (19-21). Moreover, a disturbance of this regulatory circuit was proposed to be implicated in some autoimmune (22) and inflammatory arthritic disorders (23). However, the precise way by which IL-1 reaches the brain to activate corticotrophin-releasing factor (CRF)-producing neurones is still unknown and is highly discussed since, even in severe immuno-activating conditions such as toxic shock, circulating levels of IL-1 do not rise up at sufficient levels to make it a true «hormone» of the immune system (24). The recent description of neuronal IL-1 (25) and of its interactions with nerve growth factor (NGF) (26) further puts forward the possibility of a generalized activation of the IL-1 system in severe

infections and of specialized actions of IL-1 in neural plasticity or regenerative processes.

This is not the case for other cytokines like IL-6 or tumor necrosis factor (TNF), the concentrations of which may be rapidly and highly increased in infection stress (24, 27), up to levels (10^{-9} M) necessary to induce ACTH, GH, PRL and LH hypophysial release (28). A paracrine action at the anterior pituitary level must also be considered since a source of IL-6 was suggested for the hypophysial folliculo-stellate cells (29).

So, at the present time, there is strong evidence that immune-derived signals may trigger hypothalamic neuroendocrine functions and that a regulatory feedback loop is installed by this way which may prevent an overshooting of immune responses. The precise physico-chemical conditions of the cell-to-cell communication involved (endocrine or paracrine) remain however to be further explored.

3° THYMIC CRYPTOCRINE SIGNALLING AND THE RECOGNITION OF SELF NEUROENDOCRINE FUNCTIONS

The thymus is the primary lymphoid organ responsible for the shaping of the T-cell repertoire and for the induction of immune self-tolerance. In the human species, this fundamental role takes place mainly during the fetal life and, while congenital hypo- or aplasia of the thymus (like in Di George's syndrome) leads to irremediable death in children, this organ may be removed after birth without significative immune deficiency.

At the onset of our investigations, we were interested to explore the hypothesis that neuroendocrine-immune interactions might occur during the early stages of T-cell differentiation. On the basis of ancient observations by Ott and Scott about the galactogogue actions of corpus luteum and thymic extracts (30) and given the expression of neurohypophysial (NHP)-related peptide signals in various peripheral organs (31), we demonstrated in human thymic extracts the coexistence of OT-like immunoreactivity (OT-LI) together with immunoreactive (ir) neurophysin (NP), the protein associated in the structure of several NHP precursors (32). By immunocytochemical methods, immunoreactivity characterizing various members of the NHP family was shown to co-localize in thymic epithelial cells (TEC) of the subcapsular cortex (SCC) and medulla from human, rat and murine thymuses. One specialized subpopulation of SCC, the thymic «nurse» cells (TNCs) (33), express an immuno-phenotype similar to that found in the diffuse neuroendocrine system (previously called APUD system) and contain ir-OT, ir-VP as well as ir-NP. Functional NHP receptors of the V_1- and/or OT-subtype are also detected on thymocytes and on an immature T-cell line ($RL_{12}NP$) derived from X-ray-induced thymic lymphoma in $C_{57}Bl/Ka$ mice. Consequently, physico-chemical conditions are encountered in the thymus for an effective epithelial-lymphoid communication through NHP-related signals according to the *cryptocrine* model (for a complete review, see 34). This new concept of cell-cell communication was recently advanced for testicular Sertoli cells and TNCs (35). This signalling differs from the paracrine model in that it involves exchange of chemical messages between specialized epithelial cells (Sertoli cells in the testis, TNCs or other TEC microenvironment in the thymus) and migratory differentiating elements (spermatids and immature T-cells, respectively). The mitogenic properties of NHP-related peptides upon various cell types (36), together with the high expression of NHP receptors by $RL_{12}NP$ cells, strongly suggest that the thymic peptide repertoire is implicated in T-cell positive selection and influences the biology of T-cell lymphoma (37).

Moreover, in thymic epithelium and in TNCs, cryptocrine signalling is closely linked to the recognition of self molecular structure and to the induction of self tolerance during the maturation of the T-cell system (38-41). This duality of

function (involvement in T-cell positive selection and self recognition) may be transposed from the cellular level to the molecules detected in TEC. On the basis of our studies, the immunodominant epitope of the NHP family present in the thymus seems to be located in the cyclic part of OT shared by the ancestral peptide vasotocin (VT) from which OT- and VP-precursor genes would have emerged by gene duplication. If further confirmed, a presentation by TEC major histocompatibility complex (MHC) proteins of this self epitope representative of the NHP family may induce a high affinity interaction and the subsequent deletion of T-cells bearing a randomly rearranged T-cell receptor offering the precise configuration for the MHC/self epitope complex. In favour of this working hypothesis, one should notice that autoimmune processes have never been described against hypothalamic OT-producing neurones, and this protective effect could contribute to the conservation of reproductive functions. Since the VP molecule exhibits a different amino acid residue in its cyclic part, a lower degree of protection would follow and, indeed, autoimmune hypothalamitis leading to diabetes insipidus has been described by some authors (42). Another indirect argument for a tolerogenic effect of the self NHP epitope may be found in the frequency and the titers of the antisera obtained after active immunisation against members of the NHP family (VP>OT>VT).

This model also applies remarkably well to other hormone or neural-related peptide families. For example, with regard to the insulin family, given the high homology between insulin and IGFs, the presentation of thymic IGFs (7) could contribute to the induction of immune tolerance for pancreatic endocrine function. A molecular breakdown in this process would obviously contribute to the emergence of autoimmune type I diabetes (insulin-dependent). As for NHP-related peptides, this postulated tolerogenic effect does not exclude the implication of IGFs in T-cell positive selection and this latter was recently attested by the demonstration of the contribution of IGF-mediated signalling in the development of T-cell lymphomas (43). Finally, in collaboration with the Laboratory of Molecular Neurobiology at the Karolinska Institute, we have demonstrated the intrathymic expression of the pre-protachykinin A (PPT-A) gene (44). Surprisingly, neurokinin A (NKA), and not SP, was the peptide encoded by this gene which was in fact detected in the neuroendocrine compartment of thymic epithelium. This observation was nevertheless in accordance with the reported mitogenic properties of NKA upon thymocytes, whereas the SP signal is detected in the thymic sensory innervation and the SP-receptor is expressed by cell components of the thymic bed vasculature. Once again, at the level of primary amino acid structure, NKA and SP are highly homologous peptides and share common immunodominant epitopes. Consequently, it seems reasonable and logical to postulate that, besides its accessory activation properties in T-cell differentiation, NKA represents the self tolerogenic peptide for the tachykinin family. Undoubtedly, the elucidation of the self protein/peptide gene repertoire expressed in the thymus should be a fruitful area of research for the future, in the same manner than the definition of the *self molecular identity of the cell* should lead to a better understanding of endocrine-specific T-cell mediated autoimmune disorders.

CONCLUSIONS

Neuroendocrine-Immunology is a rapidly evolving inter-disciplinary field and we do hope, by means of that global survey, to have somewhat contributed in the arousal of awareness for the clinical and pharmacological implications of these closely linked interactions.

In its infancy, the field was judged by many scientists to be too integrative or, inversely, to focus on too particular observations without physiological consequences. Many problems are still unresolved, some concepts and working

models have still to be submitted to scientific investigation. However, even unperfect, a rational model is valuable when it opens new avenues for the research enterprise and, afterwards, time and experimentation are making the rest...

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