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See also Architectonics, modular, of neural centers; Neuroanatomical research techniques; Neuroimaging; Mapping cerebral functional activity with radioactive deoxyglucose

Thymus gland, neuroendocrine-immunology

Vincent Geenen, Martin Wiemann and Henri Martens

"Autoimmune disease can be a depressing subject. In Shakespearean terms, 'it is a tale told by an idiot... signifying nothing'. In more modern metaphor, it is an error made at random in an enormous, delicately programmed computer. Nature has no other way of handling genetic error than by eliminating the faulty, and the physician handling autoimmune disease can expect no help from her." (Sir F. Macfarlane Burnet, 1972)

1. Introduction

In recent years, it became evident that close neuroendocrine-immune interactions play a fundamental role in embryonic development, as well as in the control of homeostasis. Moreover, a close relationship appears between those interactions and the prominent feature of the immune system, self-nonself discrimination. The neuroendocrine-immune cross-talk is initiated in the very first steps of T-cell development, within the thymus, the primary lymphoid organ. The present chapter illustrates the remarkable phylogenetic and ontogenetic continuity of neuroendocrine-immune interactions. As emphasized, an important physiologic implication of this continuity concerns the establishment of the immune central self-tolerance to the neuroendocrine system.

2. Structure and development of the thymus

In mammalian species, the thymus is a lymphoepithelial organ located in the anterior mediastinum, overlying the heart and the basal blood vessels. The thymus is divided into two lobes which are surrounded by a connective capsule which delineate pseudolobules by sending incomplete septa within the parenchyme.

Thymic epithelial cells (TEC) constitute the dominant cell population of the microenvironment. Embryologically, TEC derive from the endoderm of the third and fourth pharyngeal pouches. A contribution to thymic epithelium has also been reported for the ectoderm of the third branchial cleft and the cervical sinus. TEC development also depends upon an induction by the cephalic neural crest. Antigenic relationships have long been noticed between TEC from the subcapsular cortex and the medulla of thymic lobules, whereas the phenotype of TEC in the cortex is quite different. Thymic nurse cells (TNC) are very large epithelial cells located in the outer cortex which can enclose up to 50–100 immature T cells through a process known as "emperipolesis". Like most cells of the organism, TEC/TNC express class I major histocompatibility complex (MHC) molecules and display the intracellular machinery required for antigen presentation.

Macrophages are dispersed throughout thymic parenchyme; together with interdigitating (dendritic) cells located at the cortico-medullary junction, they are known to intervene in the presentation of antigens to immature T cells.

The thymus also receives a noradrenergic and a cholinergic autonomic innervation. A number of neuropeptides have been identified within thymic nerve fibers, mainly through immunocytochemistry.

3. Physiology of the thymus

The specific ability of the immune system to recognize the universe of foreign (nonself) antigens results from the rearrangements of gene segments coding for immunoglobulins (Igs) and for T-cell antigen receptors (TCR). T-cell differentiation is not a pure automatic genetically-driven process, but the thymic stromal network plays a pivotal role in the shaping of the peripheral T-cell repertoire. Within thymic microenvironment, a significant part of T-cell differentiation is mediated through cell adhesion molecules and exchanges of different cytokines, mainly interleukin-1 (IL-1), IL-6, IL-7, tumor necrosis factor (TNF) and leukemia-inhibitory factor (LIF).

The thymus recruits the peripheral T-cell repertoire in two distinct ways. On the one hand, the thymus induces central T cell tolerance through the negative selection of self-reactive T cells emerging from the random recombination of gene segments for TCR. The death by clonal deletion or by developmental arrest of self-reactive T cells is thought to follow a high affinity-binding of TCR to self-antigens presented by thymic MHC molecules. The terms "self-peptide" or "self-antigen" hereafter designate the short 8–10 amino acid sequences derived from endogenous proteins that are effectively presented to T cells by MHC-derived molecules expressed by TEC/TNC. Secondly, the thymus is responsible for the development and positive selection of the peripheral T-cell repertoire. This dual physiologic role of the thymus constitutes one major paradox in contemporary immunology and is difficult to accommodate within the current model of a unique trimolecular complex (TCR + complex MHC/self-antigen). It appears counterintuitive that two such opposite events as T-cell life and T-cell death are mediated by the same trimolecular complex.

As explained below, this paradox may be partially explained at the molecular level by the thymic repertoire of neuroendocrine (NE)-related precursors which may provide either accessory NE *signals* for T cell positive selection, or NE *self-antigens* for negative selection of self-reactive T cells.

4. Cryptocrine signaling and T cell positive selection

As early as 1910, Ott and Scott reported that thymic and ovarian extracts induce milk ejection when injected into the goat. This galactogogue action present in such tissues could not be attributed to oxytocin (OT), as it was still unknown at that time. We have demonstrated that TEC/TNC from different species synthesize polypeptide precursors belonging to neurohypophysial (NHP), insulin and tachykinin families. TNC provide a striking example of an intimate NE-immune

microenvironment since their epithelial component (but not engulfed T cells) synthesizes NE-related peptides and expresses antigenic markers of the diffuse NE system. Classic secretory granules are, however, extremely rare or completely absent in TEC/TNC and, at least under basal conditions, primary cultures of human TEC do not secrete NE-related peptides. A recent ultrastructural study has further confirmed the presence of immunoreactive (ir)-OT in murine thymic epithelium, but not within secretory granules. The term "cryptocrine" was introduced in 1990 by J.W. Funder to describe a novel type of cell-to-cell signaling within specialized micro-environments (such as TNC or Sertoli cells), where mobile cells (T cells or spermatozoa lineages) differentiate in close contact with large epithelial nursing cells.

In the thymus, physico-chemical criteria and properties of NHP receptors expressed by immature T cells are further evidence to substantiate a possible cryptocrine signaling role in vivo mediated by NHP signals originating from TEC/TNC. Our observations have further suggested a molecular switch in the type of the NHP receptor, since pre-T cells express V1-type receptors, whereas mature cytotoxic T cells predominantly express OT-type receptors. The interaction between NHP signals and receptors mediates a mitogenic effect, as shown by the increase of [^{3}H]TdR incorporation in serum-free cultures of human and murine thymocytes (pre-T cells) treated with various peptides of the NHP family. Furthermore, in murine pre-T cells NHP-related signals induce phosphorylation of the p125^{FAK} focal adhesion kinase, recently identified by J.T. Parsons (University of Virginia at Charlottesville, USA) (unpublished results). The activation of focal adhesion-related proteins in pre-T cells could be crucial for their interaction with TEC and for their subsequent developmental program. Taken together, these observations clearly suggest that NHP-related signals synthesized in TEC/TNC mediate a functional cryptocrine signaling in the thymic network.

5. Self-antigens of neuroendocrine protein families

Intrathymic cell-to-cell signaling is associated with the presentation of the molecular structure of self to developing T-cells. Using various polyclonal and monoclonal antibodies (mAbs), we have demonstrated that ir-OT is the dominant NHP peptide expressed by human TEC/TNC. Some ir-vasopressin (VP) is on occasion detected in the same cellular subpopulations of thymic epithelium, but at concentrations 50-100-fold lower. The cyclic OT sequence CYIQNCPLG is highly conserved among members of the NHP family, and moreover possesses Y and L residues in appropriate positions for anchorage to the groove of some MHC class I molecules. Based on these biochemical properties, we have advanced the hypothesis that OT is the dominant self-antigen of the NHP family.

In collaboration with the late H. Persson, we have shown that neurokinin A (NKA) encoded by the *PPT-A* gene is the dominant peptide of the tachykinin family in rat TEC. This expression is steroid-dependent, in that adrenalectomy markedly enhanced levels of PPT-A mRNA in rat thymus. Ir-IGF-II is the dominant peptide of the insulin family expressed by human and rat thymic epithelium. No ir-IGF-II can be detected in the supernatants of rat TEC cultures; further questioning the existence of a classic secretory pathway in the thymic epithelium. Interestingly, an isolated thymic overgrowth has been repeatedly observed in IGF-II transgenic mice. The sequence CGGELVDTL from the IGF-II B domain is highly conserved throughout the insulin family, and also possesses L residues in appropriate positions for anchorage to some MHC class I molecules. This sequence may thus be considered as a major self-antigen of the B domains of whole insulin family.

6. Thymic presentation of the OT self-antigen

Thymic ir-OT is thought to be synthesized as a 55-kDa large precursor which represents about 2% of the total thymic NHP-related ir-material. This precursor is translocated into cell membranes of the thymic stroma. It possesses a neurophysin domain (10 kDa), like all known NHP precursors, and is retained by an affinity matrix coupled with a mAb (B9.12) directed against the monomorphic determinant of human MHC class I molecules (45 kDa). The precursor can be precipitated by B9.12, and a 55-kDa band subsequently labelled on Western blots by an antiserum directed against the central highly conserved domain of neurophysins. These observations strongly support the coexistence of both neurophysin and MHC class I-related domains within the structure of the thymic OT precursor. The MHC class I domain could thus be involved in the membrane targeting of the precursor, and the neurophysin domain in presentation of the OT self-peptide to pre-T cells. The identification of the thymic NHP-related precursor as a chimeric or hybrid protein bearing neurophysin- and MHC class I-related domains strongly suggests the existence of post-transcriptional mechanisms which remain to be deciphered.

For a long time the binding of OT or VP to neurophysins has been a useful model for the study of interactions between small peptides and larger proteins. Very interestingly, a striking analogy exists in the binding of antigenic peptides to MHC class I proteins. So, just as neurophysins transport OT and VP neuropeptides along the axons of the NHP tract, the neurophysin-like domain of the thymic OT precursor might present OT as the self-antigen of the NHP family. These studies also illustrate that the intrathymic T-cell education to a NE self-antigen is different from the presentation of auto- or alloantigens found in the periphery in the case of dedicated antigen-presenting cells. This specific pathway offers two important selective advantages. Firstly, though MHC processing pathways are involved, thymic T-cell education to a NE self-antigen is not restricted in an allelic fashion established as the case for peripheral immunocompetent cells with dedicated antigen-presenting cells. Secondly, through a binding to the neurophysin domain of the hybrid precursor, the cyclic structure of the NHP peptide family can be presented to immature T cells.

7. Central T cell tolerance of neuroendocrine functions

Since the dominant thymic peptide of the NHP family is OT, it is logical to conclude that the OT-mediated NE functions are better tolerated by the immune system than VP-mediated ones. While OT is the tolerogenic self-antigen of the NHP family in the human species, a number of observations suggest that VP may be an autoantigen involved in autoimmune processes. Given the importance of OT in the control of reproduction at separate levels (parturition, maternal behavior, lactation, and paracrine regulation of gonadal or uterine functions), a strong tolerance of the OT lineage appears to be crucial for the preservation of species. By contrast, a mononuclear infiltration of the neurohypophysial infundibulum has been reported following the experimental breakdown of immunological tolerance to VP. Spontaneous breakdown of NHP immunological tolerance might also intervene in the pathogenesis of autoimmune hypothalamitis with a secondary "idiopathic" diabetes insipidus. Such observations support the concept of differentiated NE cells expressing antigenic markers independent of their neurosecretory activity. Membrane expression of NE cell-specific antigens would clearly be crucial for imprinting their immunological identity.

Through thymic IGF-II, self-peptides of the insulin family may also be presented to the developing T-cell system. This is of high physiological significance in terms of the central

T-cell tolerance of pancreatic islet β cells, the most obvious function of which is the synthesis and endocrine secretion of insulin. Since the breakdown of the immunological tolerance to pancreatic islet β cells is more and more implicated as a major etiologic event in type 1 diabetes mellitus, a defect in the thymic presentation of IGF-II-derived self-peptides could play an important role in the molecular mechanisms of autoimmune insulin-dependent diabetes mellitus.

Thus, from the literature available, the thymus more and more appears as a privileged nodal point between the major systems of cell-to-cell signaling and recognition, the neuroendocrine and the immune systems. Further knowledge of the mechanisms implied in thymic T-cell education to neuroendocrine self should greatly help the elaboration of novel strategies for the treatment of autoimmune endocrine disorders. Already, the prevention of such disorders may be considered on the basis of the tolerogenic properties of thymic neuroendocrine-related self-antigens.

8. Evolutionary aspects

During the evolution of the endocrine system, various forms of cell-to-cell signaling have appeared, from the primitive stages of *autocrine* signaling (when a cell is alone) and *cell adhesion* (involved during early embryonic development), passing through the more differentiated steps of *paracrine* and *(neuro)endocrine* signaling, up to the most advanced forms of *synapses* in neuronal networks. The *cryptocrine* cell-to-cell communication evidenced in the thymus can be presumed to be a primitive, intermediate step between cell adhesion and paracrine-type signaling. In the past recent years, mainly by means of recombinant DNA technology, intercellular signaling molecules have been shown to be organized in several families composed of distinct members responsible for particular types of cell-to-cell signaling (Table 1).

On the other hand, the immune system has primarily evolved to protect the integrity of self against aggression from nonself infectious invaders. Given the common peptide nature of most

Table 1. The organization of the thymic repertoire of neuroendocrine (NE)-related precursors

Function	Endocrine factors	Paracrine/autocrine factors	Thymic repertoire of neuroendocrine-related self-peptide precursors
Glucose metabolism	Insulin	Insulin-like growth factor-I (IGF-I)	ir-IGF-II
		Insulin-like growth factor-II (IGF-II)	
Calcium homeostasis	Parathormone (PTH)	PTH-related peptide (PTH-rP)	ir-PTH-rP
	Calcitonin	Calcitonin gene-related peptide (CGRP)	ir-CGRP
Water homeostasis	Anti-diuretic hormone (ADH/VP)	?	ir-OT
Reproduction [Parturition–Lactation–Gonadal functions –Maternal behavior]	Oxytocin (OT)	?	

The fundamental principle is based upon homology of peptide sequences between peripheral NE signals and related tissue growth factors that are synthesized by TEC/TNC, and then presented to the differentiating T cell system. This homology suggests that the thymic peptide repertoire might constitute a tolerogenic source of *NE self-antigens*. These self-antigens are dominantly conserved throughout evolution of their family. On the other side, thymic NE precursors are also the source of *signals* which provide accessory pathways in the process of T cell positive selection following their interaction with NE-type receptors expressed by developing T cells.

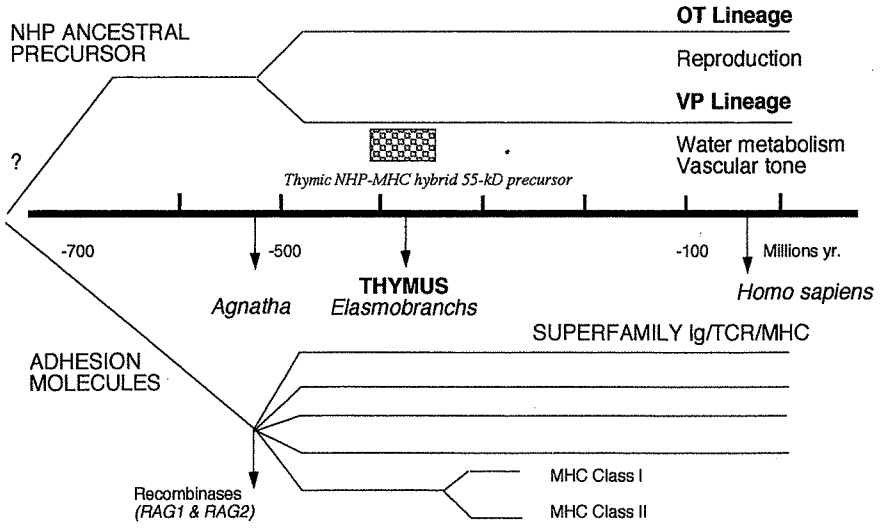

Figure 1. A simplified overview of parallel evolution and interactions between the NHP and Ig/MHC/TCR families. The thymic NHP-related precursor could have appeared after the duplication of the ancestral common NHP precursor, before or at the same time as the thymus. Most probably it preceded duplication of MHC-derived proteins in class I and class II which also occurred in the elasmobranches. The high degree of diversification and the potent properties of molecular recognition characterizing most of the members of the Ig superfamily originate from recombination at random of segment genes coding for Igs or TCRs. This complex process of extreme diversification has been shown to be under the control of recombination-activating enzymes encoded by two separate genes (*RAG1* and *RAG2*).

allo-, auto-, and self-antigens, the immune system must have been educated to recognize and to tolerate the molecular structure of self. Even though peripheral tolerogenic pathways are increasingly established, the thymus is clearly recognized as playing the central role in educating T cells to recognize self-antigens. Since differentiation of the whole T cell repertoire involves recombination at random of gene segments coding for the TCR chains, the emergence of self-reactive T cells may naturally follow this highly hazardous biological phenomenon. The thymus thus exerts a radical anti-hazard constraint by purging the immune system of self-reactive T cells which otherwise represent a serious threat for survival. In the same global perspective, pathological autoimmunity may be considered as the tribute paid by mammalian species for the very potent efficiency of their immune defences.

A phylogenetic continuum may also be envisioned from our findings. The evolution of the NHP family, in particular the coexistence of a NHP nonapeptide and a neurophysin domain within all known precursors, did not precede the ancestral origins of the Ig/MHC superfamily, but well its extreme diversification (Figure 1). It is noteworthy that the thymic NHP-related precursor contains both neurophysin- and MHC class I-related domains. This last point nicely illustrates the high degree of connectedness which exists between two families of proteins involved in molecular recognition, and strongly argues for their putative common ancestral origin (Figure 1).

9. Relevant information

European Immune Network (EBIN), chairman Dr. Cobi Heijnen (Wilhelmina Ziekenhuis, Utrecht, The Netherlands): c.heijnen@wkz.azu.nl

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See also Immune system–central nervous system interactions; Immune system: neural control; Oxytocin; Vasopressin and oxytocin; Neuroendocrinology

Update (April 1998)

The behavior of thymic OT as the self-antigen of the neurohypophysial family was further confirmed by the observation that the recognition of OT (but not VP) by specific mAbs at the outer surface of TEC plasma membrane activates the secretion of cytokines (IL-6 and LIF) in the supernatant of human TEC cultures (Martens et al., 1996).

Neurotensin-related peptides were found to be presented by MHC class I molecules of the human thymus (Vanneste et al., 1997)

Neurohypophysial peptides stimulate the phosphorylation of focal adhesion kinases such as p125FAK, a coprecipitated 130-kDa protein (probably p130CAS), as well as paxillin, a 68-kDa protein located at focal adhesion sites (Martens et al., 1998)

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