VIEWPOINT
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The thymic repertoire of neuroendocrine self-antigens: physiological implications in T-cell life and death

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During phylogeny and ontogeny, the thymus appears as a crucial meeting point between the neuroendocrine and immune systems; through cryptocrine intercellular communication, thymic neuroendocrine-related precursors can influence the early steps of the immune response, while T-cell precursors are educated to recognize the principal neuroendocrine families. Here we summarize the observations that support the dual role of the thymic repertoire of neuroendocrine-related polypeptide precursors in T-cell differentiation.

Although the thymus has long been considered to be a part of the endocrine system, it is difficult to apply the model of endocrine cell–cell signalling to the process of intrathymic T-cell differentiation. This might be due, at least in part, to the importance of the thymus as the primary lymphoid organ responsible for T-cell differentiation, such that its immune properties have overshadowed its endocrine role. However, these two functions are intimately linked, and neuroendocrine–immune interactions in T-cell education have important physiological and pathological implications.

The neurohypophysial hormone family

The neurohypophysial (NHP) hormones constitute a family of nonapeptides that are highly conserved throughout evolution[1] and all have cysteine residues in positions 1 and 6 forming a disulfide bridge. They can be divided in two lineages corresponding to the oxytocin (OT)-like and vasopressin (VP)-like peptides. Both of the lineages are thought to have arisen from the duplication of a single ancestral gene[1]. In mammalian vertebrates, OT-like peptides are implicated in the control of reproduction, whereas VP-like peptides regulate water homeostasis and also have some cardiovascular functions. All known NHP hormones are synthesized as larger precursors containing a neurophysin domain (± 10 kDa), the biological function of which remains unclear.

Thymic NHP-related signals

As early as 1910, Ott and Scott showed that thymic extracts could induce milk ejection (galactokinesis) when injected into goats. The causative agent was identified by du Vigneaud's group in the 1950s as OT. Subsequent studies have confirmed that thymic epithelial cells (TEC) from human and different animal species are a site of synthesis of peptides related to the NHP family, with marked dominance for those of the OT lineage[2–5]. Using various polyclonal and monoclonal antibodies (mAbs) against different epitopes of the NHP family, we confirmed that the dominant thymic NHP-related epitope belongs to the OT lineage although both *pro-OT* and *pro-VP* gene transcripts are detected at low levels within human and murine thymus extracts[6]. A striking example of the intimate neuroendocrine–immune interactions within the thymus is provided by thymic nurse cells (TNC), which synthesize NHP-related peptides and express on their surface markers of the neuroendocrine system such as A2B5 and neuron-specific enolase[7]. Primary cultures of TEC/TNC do not secrete NHP-related peptides and a recent ultrastructure study confirmed the presence of immunoreactive (IR)-OT in the cytosol, clear vesicles and perimembrane space of murine TEC/TNC, but not within large dense secretory granules such as those in the hypothalamo–NHP axis[8]. A nonclassical distribution of NHP-related mRNA and peptides has also been detected in eosinophils of the mouse spleen[9].

The term 'cryptocrine' has been introduced in endocrinology to describe a particular type of cell–cell interaction[10,11]. Cryptocrine cell–cell signalling occurs in microenvironments characterized by large 'nursing' epithelial cells (like TEC/TNC in the thymus or Sertoli cells in the testis) that enclose the cell populations (T cells and spermatids, respectively) that differentiate in close vicinity of these 'nurse' cells.

Thymic NHP peptide receptors

Functional NHP-type receptors are expressed by a variety of T-cell lines[12–15], with a predominance of V1-type receptors on pre-T cells (murine RL_{12}-NP cell line) and OT-type receptors on differentiated cytotoxic T cells (murine CTL-L_2 cells)[12]. Two recent studies also found an NHP peptide receptor of the V1b subtype in the human and rat thymus[16,17].

Within the thymic microenvironment, the physicochemical conditions are conducive to functional cryptocrine signalling between TEC/TNC and pre-T cells[12]. Moreover, NHP peptides increase tritiated

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thymidine ([^{3}H]TdR) incorporation into freshly isolated murine and human thymocytes[12] and induce the phosphorylation of the focal adhesion kinase p125^{FAK} (Ref. 18) (H. Martens *et al.*, unpublished). Thus, there are considerable data supporting thymic cryptocrine signalling mediated by NHP-related peptides.

Thymic OT as the self-antigen of the NHP family

In the thymus, cryptocrine signalling is intimately associated with the presentation of self-antigens to developing T cells. This action was long thought to be mediated by interdigitating thymic cells only, but there is now evidence that TEC/TNC are also actively involved in the thymic induction of immunological self-tolerance[19]. At the molecular level, major histocompatibility complex (MHC) class I molecules are involved in central tolerance since they present peptides derived from the processing of endogenous proteins to the T-cell receptor (TCR) on CD8$^+$ T cells[20]. The OT amino acid sequence CYIQNCPLG possesses appropriately positioned hydrophobic tyrosine (Y) and leucine (L) residues, which permit it to be anchored to the groove of some MHC class I molecules[21]. Therefore, it is hypothesized that in human and other mammalian species, thymic OT is the self-antigen of the NHP family.

The biochemical pathways leading to the presentation of OT by TEC/TNC

Human TEC surface membranes were purified by ultracentrifugation and dissolved in a non-ionic detergent. The solution was then run down an affinity column prepared with a mAb directed to human MHC class I molecules. Following SDS-PAGE, instead of the expected 45 kDa fractions (the molecular mass of MHC class I heavy chain), western blot analyses revealed a 55 kDa fraction that could be labelled both by anti-MHC class I mAb and by an antiserum against the highly conserved part of neurophysins. This protein was still present after mercaptoethanol and/or heat denaturation, and the antiserum to neurophysin did not recognize β-microglobulin. Given these data, we interpret the thymic membrane 55 kDa protein as a chimeric or a hybrid precursor including neurophysin- (10 kDa), as well as MHC class I heavy chain (45 kDa)-related domains. This 55 kDa protein differs from the hypothalamic OT precursor (16 kDa) and probably reflects a strong binding of the neurophysin domain to MHC class I heavy chain at a post-transcriptional level[22].

Until recently, the binding of OT or VP to neurophysins for transport along the NHP axis provided a useful model for studying the interaction of a short peptide with a larger protein. Several studies have pointed out the importance of the tyrosine residue in position 2 of OT and VP for this binding[23,24]. Analogous biochemical principles seem to rule the binding of antigens to the groove of some MHC class I molecules, including the presence of a tyrosine residue in position 2 (Ref. 21). So, by analogous binding, neurophysin *transports* OT along axons of the NHP axis while the neurophysin domain of the TEC membrane chimeric 55 kDa protein could be implicated in the *presentation* of thymic OT to pre-T cells.

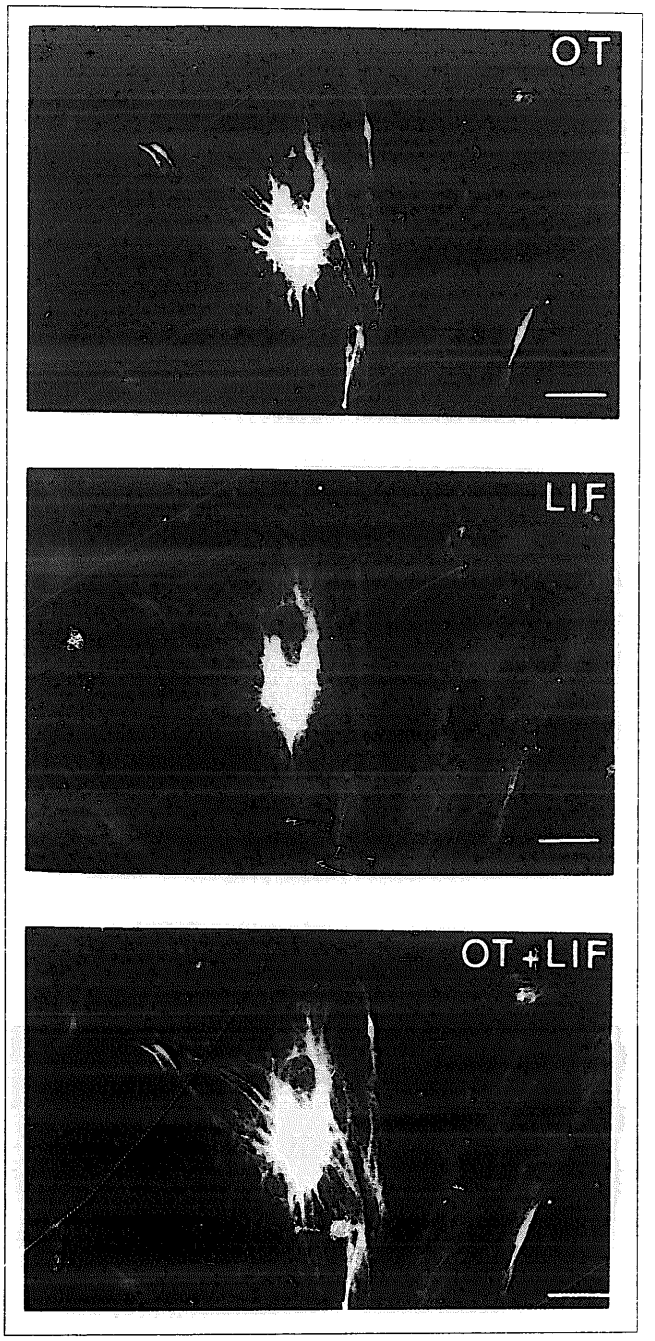

Fig. 1. *Colocalization of IR-OT with IR-LIF in human TEC after 21-day culture. Immunofluorescence staining was obtained with anti-OT (green) mAb O33 and anti-LIF (red) antiserum. The lower frame is a superimposed image of OT and LIF immunostaining. Yellow-orange indicates an overlap of IR-OT and IR-LIF. Bar is 10 μm. Abbreviations: IR, immunoreactive; LIF, leukaemia inhibitory factor; OT, oxytocin.*

Although thymic MHC class I pathways are involved in the process, it appears that TEC/TNC T-cell education to OT is not restricted in an allelic fashion in contrast with the peripheral presentation of alloantigens by dedicated antigen-presenting cells.

Table I. The organization of the thymic repertoire of neuroendocrine-related self-peptide precursors

Function	Endocrine factors	Paracrine/ autocrine factors	Thymic repertoire of neuroendocrine-related self-peptide precursors	Refs
Glucose metabolism	Insulin	IGF-I IGF-II	IGF-II	
Calcium homeostasis	PTH Calcitonin	PTHRP CGRP	PTHRP CGRP	41 42
Water homeostasis	ADH/VP	?	OT	
Reproduction (parturition, lactation, gonadal functions and maternal behaviour)	OT	?	OT	
Natrium metabolism	Natriuretic peptides		ANP	43

Abbreviations: ADH, anti-diuretic hormone; ANP, atrial natriuretic peptide; CGRP, calcitonin gene-related peptide; IGF-I, insulin-like growth factor 1; OT, oxytocin; PTH, parathyroid hormone; PTHRP, PTH-related peptide; VP, vasopressin.

Another selective advantage resides in the potential presentation of the characteristic cyclic structure of the NHP family to pre-T cells. In addition to our findings, a recent study also suggests a distinction between thymic T-cell education and antigen-presenting functions[25].

Furthermore, our most recent results show that OT is colocalized in human cultured TEC with interleukin 1β (IL-1β), IL-6 and leukaemia-inhibitory factor (LIF) (Fig. 1). Oxytocin is specifically recognized at the outer surface of human TEC by mAbs O13 and O33 directed against the linear C-terminal and the cyclic part of the OT molecule, respectively; this recognition markedly enhanced the secretion of IL-6 and LIF (but not IL-1β) by primary cultures of human TEC, while the addition of mAbs to VP did not exert any effect[44]. These data provide strong evidence of the full processing of OT expressed at the outer surface of the TEC membrane, and support the hypothesis of thymic OT as the self-antigen of the NHP family.

Self-antigens of tachykinin and insulin families

Neurokinin A (NKA) is the peptide of the tachykinin family encoded by the preprotachykinin A (*PPT-A*) gene in human and rat TEC (Ref. 26). Thymic *PPT-A* expression was shown to be glucocorticoid dependent since adrenalectomy of Sprague-Dawley rats markedly enhanced the levels of thymic *PPT-A* mRNA (A. Ericsson and V. Geenen, unpublished). In contrast to other members of the tachykinin family, NKA also exerts mitogenic effects on murine thymocytes. This IL-1-type bioactivity of NKA suggests an interaction with specific receptors expressed by pre-T cells that could be another accessory pathway in T-cell maturation. The amino acid sequence of NKA (HKTNSFVGLM) has the same C-terminal epitope as other members of the tachykinin family. This epitope possesses a leucine residue in position 9 that could be used in the binding to some MHC class I molecules; thus NKA can be predicted to be a T-cell epitope.

We have recently shown that insulin-like growth factor II (IGF-II) is one peptide of the insulin family that is expressed by human and rat TEC/TNC (Ref. 27). IR-IGF-I is also detected in human and rat thymus extracts but in lower concentrations. Although IR-IGF-II is expressed by TEC/TNC, it is not secreted into the supernatant of human or rat primary TEC cultures. Interestingly, overexpression of IGF-II in transgenic mice was shown to induce thymic hyperplasia and to increase thymic cellularity[28]. Our recent studies have shown that IR-IGF-II can be detected in confocal microscopy at the outer surface of human TEC membranes and thus can be presented to pre-T cells during their differentiation (I. Achour *et al.*, unpublished). The question of a central T-cell tolerance of the insulin family is of major physiological significance since it could lead to T-cell tolerance of many components of the pancreatic islet β cell, the site of synthesis and endocrine secretion of insulin, and the target of diabetogenic insulitis.

T-cell tolerance of neuroendocrine families: implications in pathogeny of autoimmune endocrine diseases

Since the dominant thymic peptide of the NHP family belongs to the OT lineage, it is logical to conclude that the OT-mediated neuroendocrine functions are better tolerated than the VP-mediated ones. Thus, a strong immunological tolerance protects the OT lineage, rather than the VP one, from autoimmune aggression. Indeed, some cases of idiopathic diabetes insipidus have been shown to result from an autoimmune hypothalamitis specifically oriented toward VP-producing neurons[29,30]. Given the importance of the OT lineage in the control of several levels of the reproductive process (parturition, maternal behaviour, lactation and paracrine regulation of gonadal functions), a stronger tolerance of this lineage is expected to be crucial for the preservation of the species. So, in the NHP family, OT behaves as the self-antigen, while VP could be the prospective

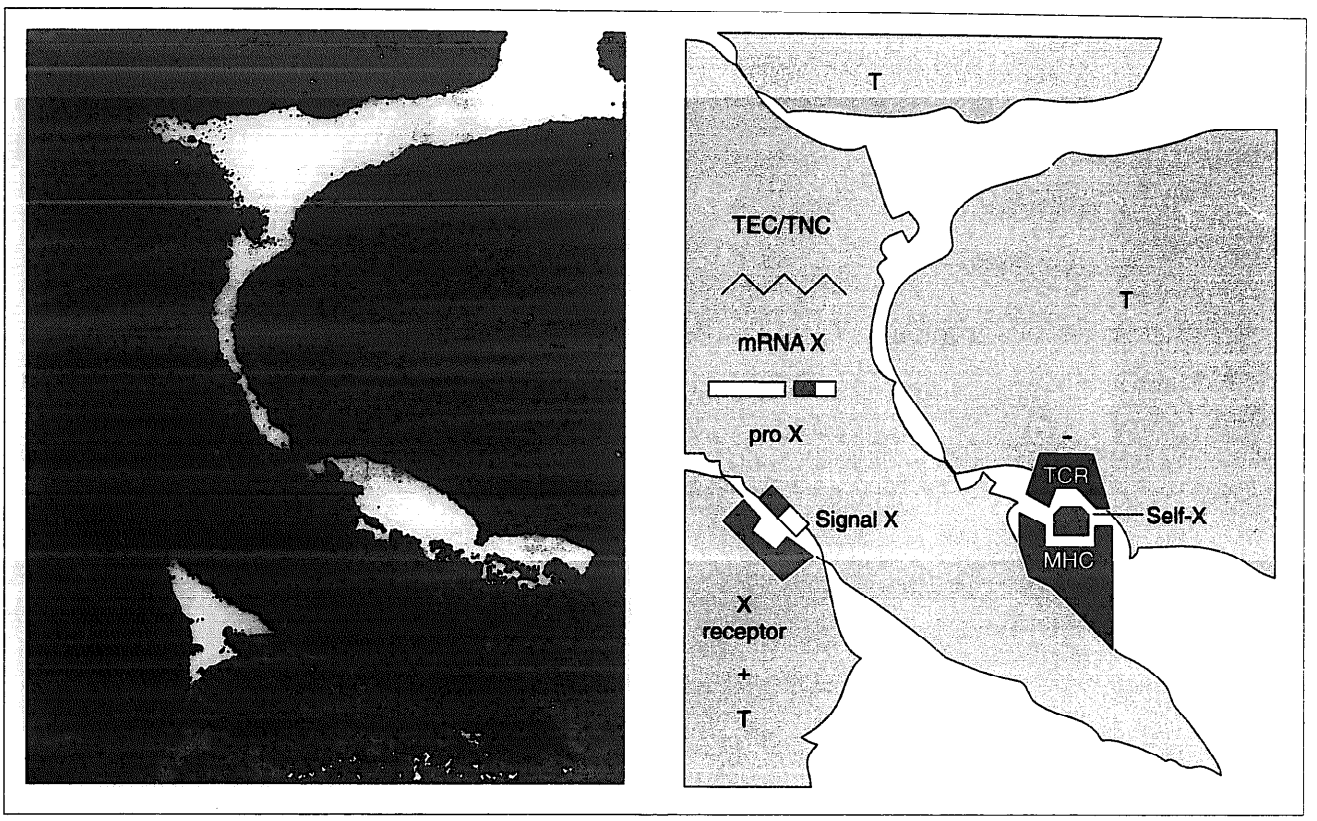

Fig. 2. *Ultrastructural image from the murine thymic cortex. Pre-embedding labelling with polyclonal anti-OT K31 at 1:700 (G. Jirikowsky, Munich) and peroxidase-anti-peroxidase. Diffuse IR-OT appears in the cytosol of a TEC/TNC process of the subcortical area with ramifications among packed thymocytes (T). Some points of focal adhesion can be observed between TEC and T cells: T cells appear as though they were 'suckling' the IR-OT-producing TEC/TNC. The economical principle of the model is based upon the homology of peptide sequences between endocrine signals and related thymic peptides that are synthesized by TEC/TNC and presented to the differentiating T cells. This homology strongly supports a dual role for the thymic repertoire of neuroendocrine precursors (pro X). First, they constitute a tolerogenic source of neuroendocrine self-antigens (self-X). These self-antigenic sequences are highly conserved throughout evolution of their family. On the other side, thymic neuroendocrine-related precursors are the source of signals (signal X) that provide accessory pathways in the process of T-cell positive selection following their interaction with neuroendocrine-type receptors (X receptor) expressed by developing T cells. Abbreviations: IR-OT, immunoreactive oxytocin; MHC, major histocompatibility complex; TCR, T-cell receptor; TEC, thymic epithelial cells; TNC, thymic nurse cells.*

target autoantigen of autoimmune processes. An infiltration of the hypothalamo–NHP tract by inflammatory mononuclear cells has been repeatedly observed, either after active immunization against VP (Ref. 31) or in spontaneous autoimmune diabetes insipidus[30]. Together, these data support the idea that hypothalamic neurons express some surface antigens representative of their neurosecretory activity.

The breakdown of immunological tolerance of endocrine pancreatic islet β cells is becoming increasingly recognized as a major etiopathogenic event in the emergence of autoimmune insulin-dependent diabetes mellitus (IDDM)[32]. This breakdown is thought to be followed by an autoimmune cascade of events leading finally to the disappearance of insulin-secreting islet β cells; consequently insulin is more often accepted as a major autoantigen of the diabetogenic autoimmune process. The sequence 7–15 (CGSHLVEAL) from the B domain of bovine insulin has been identified as a target autoantigen for H-2K^b-restricted cytotoxic T cells[33]. However, the biochemical identity between this insulin-derived autoantigen and the corresponding sequence of IGF-II (CGGELVDTL) is not complete. This difference in amino acid sequence could be important for the nature of the T-cell responses (activation vs. tolerogenic effect) elicited either by insulin- or IGF-II-derived peptide sequences. As a further argument for its tolerogenic properties, the production of IGF-II-specific antibodies is known to be more difficult than those specific for IGF-I (Ref. 34). Thymic IGF-II expression in diabetes-prone and diabetes-resistant BB rats is under current investigation to elucidate its precise role in the pathogenesis of IDDM.

Evolutionary aspects of neuroendocrine–immune interactions

During the evolution of the endocrine system, various forms of cell–cell signalling have appeared: (1) the primitive stages of

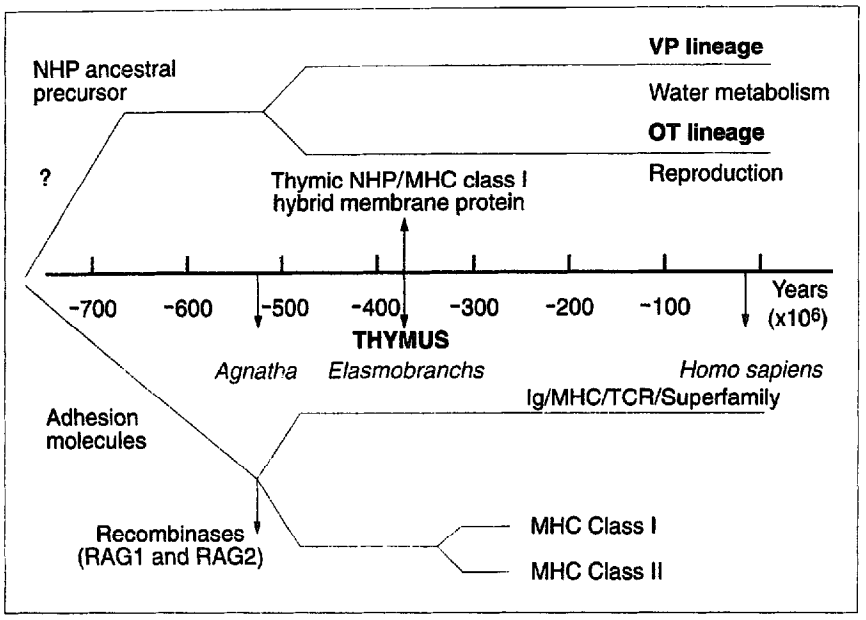

Fig. 3. *Parallel evolutions and interactions between the NHP and Ig/MHC/TCR families. The thymic OT chimeric 55 kDa precursor could have appeared after the duplication of the common ancestor of the neurohypophysial family, before or at the same time as the thymic organ. It most probably preceded duplication of MHC-derived proteins in class I and class II, which occurred with the first elasmobranchs. The extreme degree of diversification and the potent properties of molecular recognition characterizing most of the members of the Ig/MHC/TCR superfamily originate from random recombination of segment genes coding for Igs or TCRs. Abbreviations: Ig, immunoglobulin; MHC, major histocompatibility complex; NHP, neurohypophysial; OT, oxytocin; TCR, T-cell receptor; VP, vasopressin.*

autocrine signalling (for cells in isolation) and intercellular adhesion (which followed the first cell divisions in the conceptus); (2) the more differentiated steps of paracrine and (neuro)endocrine signalling; and (3) the most advanced forms of synapses in neural networks which have allowed development of cognitive functions. It is assumed that the cryptocrine mode occurs at the rather primitive stage between cell adhesion and paracrine exchanges of soluble signals[35]. In parallel with these distinct structural levels, the components of the genome coding intercellular messengers have evolved to deal with organizational systems of increasing complexity and have been progressively organized into separate families, each containing distinct members in charge of the different types of cell–cell signalling.

Conversely, the immune system has evolved to protect the integrity of self against aggression from infectious nonself. Because of the common peptidic nature of many alloantigens and self-antigens, the immune system must have been educated to recognize and to tolerate the molecular structure of the internal body. Despite the increasing attention paid to peripheral pathways of T-cell tolerance[36], the thymus is clearly the primary organ purging the immune system of self-reactive T cells, which could represent a potential threat to survival. The thymic repertoire of neuroendocrine-related self-peptide precursors constitutes an original model that underlines, at the molecular level, the dual role of the thymus in T-cell differentiation (Table 1). According to this model, thymic neuroendocrine-related polypeptide precursors are either the source of accessory signals for positive selection of T cells, or the source of self-peptides able to induce the negative selection of self-reactive T cells bearing a randomly rearranged TCR oriented against their respective family (Fig. 2). This model concurs with recent reports that have shown that a single peptide may mediate both positive and negative selection of T cells depending on the avidity or affinity of TCR for the peptide used[37,38].

The hypothesis of a phylogenetic continuum also arises from these observations (Fig. 3). As discussed earlier, the NHP family is highly conserved throughout evolution, even in invertebrates. By contrast, the expansion of the immunoglobulin (Ig)/MHC/TCR superfamily began at the level of the primitive vertebrates some 550 million years ago at the latest[39]. Thus, the foundations of the NHP family preceded diversification of the Ig/MHC superfamily. The classical feature of neurophysins (two variable sides connected to a greater central constant region) has already been described with the identification of their primary structure[40], and this constitutes another structural relationship with members of the Ig/MHC superfamily. Moreover, beyond the observation that some structural motifs of the NHP family might have served as guides for further development of the Ig/MHC superfamily, it is noteworthy that the thymic OT self-antigen precursor apparently contains both a neuroendocrine- and an MHC class I-related domain. This illustrates the intimacy of cooperation between a classical neuroendocrine family and the Ig/MHC/TCR superfamily, as well as a plausible common ancestral origin of these two families implicated in intercellular signalling and molecular recognition.

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