J Mol Med (1995) 73:449–455

REVIEW

V. Geenen · B. Goxe · H. Martens · E. Vandersmissen
Y. Vanneste · I. Achour · O. Kecha · P.J. Lefebvre

Cryptocrine signaling in the thymus network and T cell education to neuroendocrine self-antigens

Received: 24 October 1994 / Accepted: 4 July 1995

Abstract Both during phylogeny and ontogeny the thymus appears as a nodal point between the two major systems of cell-to-cell signaling, the neuroendocrine and immune systems. This review presents the experimental observations which support a dual role in T cell selection played by the thymic repertoire of neuroendocrine polypeptide precursors. Through the mode of cryptocrine intercellular signaling thymic neuroendocrine-related precursors synthesized in thymic epithelial cells have been shown to influence the early steps in T cell differentiation. In addition, thymic neuroendocrine-related polypeptides are a source of self-antigens which are presented by the major histocompatibility system of the thymic epithelium. Preliminary data also suggest that the intrathymic T cell education to neuroendocrine self-antigens is not strictly superimposible to the antigen presentation by dedicated presenting cells. Insulin-like growth factor-II (IGF-II) was identified as one dominant member of the insulin family expressed by thymic epithelial and nurse cells. The intrathymic presentation of IGF-II or IGF-II derived self-antigens is under current investigation. If further confirmed, the central tolerogenic properties of IGF-II could be considered in the elaboration of a strategy for an efficient and safe prevention of insulin-dependent diabetes.

Key words Thymus · Cryptocrine signaling · Neuroendocrine self-antigens · Molecular evolution · Developmental biology · T cell tolerance

Abbreviations *IDDM* Insulin-dependent diabetes · *IGF* Insulin-like growth factor · *IGFBP* IGF-binding protein · *TCR* T cell antigen receptor · *MHC* Major histocompatibility complex · *OT* Oxytocin · *TEC* Thymic epithelial cell · *TNC* Thymic nurse cell · *VP* Vasopressin

V. Geenen (✉) · B. Goxe · H. Martens · E. Vandersmissen
Y. Vanneste · I. Achour · O. Kecha · P.J. Lefebvre
Department of Medicine-Endocrinology,
Institute of Pathology CHU B-23, University of Liège,
B-4000 Liège-Sart Tilman, Belgium

Introduction

Although the thymus has long been considered as a gland, there is still difficulty in applying the model of endocrine cell-to-cell signaling to the intrathymic process of T cell differentiation. This may be due to the importance of the thymus as a central organ of the immune system, so that its immune properties have overshadowed its endocrine role. These two functions are, however, intimately interlinked, and neuroendocrine-immune interactions in T cell education have important physiological and pathological implications.

Thymic development of T lymphocytes

The thymus shapes the T cell repertoire in two distinct ways. The most specific role of the thymus is the induction of central immunological self-tolerance which follows the negative selection of self-reactive T cells. The existence of such "forbidden" self-reactive T cell clones has never been demonstrated, but theoretically they could emerge during the random recombination of the gene segments coding for the chains of the T cell antigen receptor (TCR) [31]. The clonal deletion or developmental arrest of self-reactive T cells is thought to follow the high-affinity binding of TCRs that recognize self-antigens presented by thymic major histocompatibility complex (MHC) derived proteins. The generic terms self-antigen or self-peptide are used in this review to designate the short eight to ten amino acid sequences derived from endogenous proteins that are effectively presented to T cells by MHC-related molecules in the thymic stroma. Another role of the thymus concerns the developmental program and the positive selection of the peripheral T cell repertoire. This dual physiological function of the thymus remains a paradox in contemporary immunology [2, 5]. The objective of the present review is to show how neuroendocrine-related polypeptide precursors synthesized in the thymic stroma may explain, at least partially, the paradox of thymic physiology.

The neurohypophysial hormone family

The neurohypophysial hormones constitute a family of nonapeptides that have been highly conserved throughout evolution [1]. They can be divided in two lineages corresponding to the oxytocin (OT)-like and vasopressin (VP)-like peptides. Both of the lineages may have followed the duplication of one ancestral gene. These peptides all consist of nine amino acids with cysteine residues in positions 1 and 6 forming a disulfide bridge. In mammalian vertebrates OT-like peptides are implicated in the control of reproduction whereas VP-like peptides regulate water homeostasis as well as some cardio-vascular functions. All known neurohypophysial hormones are synthesized as larger precursors which all possess a 10-kDa neurophysin-like domain in their structure. Both at the peptide and genetic levels, members of this family have recently been demonstrated in molluscs and insects (for a complete review, see [19]).

Despite their high conservation during evolution the physiological role of the neurophysins remains obscure. In the so-called higher vertebrates, the neurophysins bind and transport the active nonapeptides OT and VP along axons of the hypothalamo-neurohypophysial neurons [3, 10, 28]. However, the expression of neurohypophysial genes in various species devoid of a neurohypophysial system strongly suggests another physiological role of neurophysins which remains to be deciphered.

OT as a cryptocrine signal in intrathymic T cell development

As early as 1910 Ott and Scott showed that thymic extracts can induce milk ejection when injected into the goat. It was only in the 1950's that the biochemical principle of milk ejection was identified as OT by du Vigneaud's group. Thymic epithelial and nurse cells (TEC/TNC) from various species synthesize neurohypophysial-related precursors, with a marked dominance of OT and its associated neurophysin [4, 21–23, 33, 37, 40, 41]. Using a 3′ RACE polymerase chain reaction protocol, the two neurohypophysial OT and VP genes were demonstrated to be transcribed in human and murine thymuses [27]. Since OT is the dominant peptide of the family expressed by TEC/TNC, the discrepancy in the data between the thymic cDNA and peptide levels supports the existence of posttranscriptional modifications. After several years of thorough research we arrived to the conclusion that the model of neurosecretion cannot explain the processing and the fate of neuropeptides synthesized in thymic epithelium [23]. The intrathymic *OT* gene expression and OT synthesis are not correlated with secretion of the nonapeptide or its neurophysin in the supernatant of primary cultures of TEC/TNC. A recent ultrastructural study has further demonstrated that ir-OT and neurophysin are expressed by TEC only (and not by immature T cells). Thymic ir-OT is not located in secretory granules but is diffuse in the cytosol, clear vacuoles, and the juxtamembranar space of murine TEC [49]. Such ultrastructural features were also recently reported for OT and VP synthesized by murine spleen eosinophil-like cells [32]. The model of cryptocrine cell-to-cell signaling has been advanced by Funder [18] to describe the transmembrane exchanges of chemical informations between large nursing cells and immature elements which migrate and differentiate at their contact. On the basis of the above findings we hypothesised that thymic OT mediates a cryptocrine-type signaling between TEC and pre-T cells.

As an important argument supporting an effective cryptocrine signaling in vivo in the thymus, neurohypophysial receptors are expressed in the rat thymus, by rat thymocytes (pre-T cells) [13, 16], by a murine pre-T cell line (RL12-NP), and by murine cytotoxic T cells [34, 47]. A molecular maturation of the neurohypophysial reception system expressed by T cells seems to occur in parallel with T cell differentiation since pre-T cells express neurohypophysial V_1-type receptors whereas cytotoxic T cells express neurohypophysial receptors of the OT-type [34]. These receptors are functional since they transduce neurohypophysial signals according to the rules established in other cellular systems. The interaction between neurohypophysial signals and their pre-T cell receptors is followed by the phosphorylation of focal adhesion kinases [43]. This event could play an important role in promoting the T cell interactions with the thymic microenvironment which are important for their developmental program (unpublished data). Thus the bulk of available data strongly suggest that thymic OT mediates a functional cryptocrine signaling which could serve as an accessory pathway in the positive selection of T cells. In medical pathology the neoplastic transformation of TEC (as seen in thymic carcinoma and in some thymomas) is sometimes associated with a clinical syndrome of water intoxication [42]. This syndrome may be due in fact to an overexpression of thymic ir-OT, leading to a release in the peripheral bloodstream and to a subsequent interaction with neurohypophysial V_2 receptors expressed by the kidney tubular cells.

OT as the self-antigen of the neurohypophysial hormone family

In the thymus cryptocrine signaling is intimately associated with presentation of the self-molecular structure to developing T cells. This action was long thought to be mediated by interdigitating thymic cells only, but there is increasing evidence that TEC/TNC are actively involved in the thymic induction of immunological self-tolerance [9]. Since OT and its neurophysin are coexpressed by TEC/TNC, we hypothesized a processing of the thymic neurohypophysial-related precursor that could be related to the one involved in antigen presentation. Following the appropriate methodology, human thymic stromal cell membranes were purified and solubilized in a nonionic

detergent. The solution was passed on an immunoaffinity column prepared with a monoclonal antibody directed to the monomorphic part of human MHC class I molecules (monoclonal antibody B9.12) [39]. The choice of this class I specific monoclonal antibody was justified by the fact that MHC class I proteins usually present antigenic sequences derived from endogenous proteins. To avoid MHC-antigen complex dissociation, the column was eluted with diethylamine in basic pH conditions. After analysis using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, instead of the expected 45-kDa fractions (MW of MHC class I heavy chains), immunoblot analyses revealed a 55-kDa fraction which could be labeled by monoclonal antibody B9.12 as well as by an antiserum against the central highly conserved region of neurophysins [26]. Since antineurophysin antiserum and monoclonal antibody B9.12 exhibit no cross-reactivity, the most plausible explanation for these data is that the thymic 55-kDa precursor is a chimeric or a hybrid protein bearing both a neurophysin-like (10-kDa) and a MHC class I heavy chain-related (45-kDa) domain [26, 27]. The MHC class I domain is most probably implicated in the membrane translocation of this chimeric/hybrid protein while its neurophysin domain could bind OT for presentation to pre-T cells. The precise biochemical mechanisms leading to the synthesis of this hybrid neurohypophysial/MHC class I protein remain of course to be further deciphered. Since the three exons of neurohypophysial genes are transcribed in the thymus, the origin of this protein should reside at a posttranscriptional level (such as a *trans*-splicing-like event), or even at a post-translational level (as the ATP-dependent covalent binding to ubiquitin of proteins targeted to proteolysis).

For another experimental argument for the role of thymic OT as the self-antigen of the neurohypophysial family we investigated the effects of the immune recognition of neurohypophysial antigens on the cytokine profile secreted by human TEC in primary cultures. Only antibodies directed to OT (but neither anti-VP antibodies nor different preparations of Igs) were able to stimulate the TEC production of interleukin 6 and leukemia-inhibitory factor as measured by specific EASIAs (Medgenix Diagnostics, Fleurus, Belgium; Martens et al., manuscript submitted).

The thymic repertoire of neuroendocrine self-antigens

Based upon these observations, a model (Fig. 1) has been advanced proposing that neuroendocrine-related thymic peptides ("X") engage into two distinct types of interactions with pre-T cells depending on their intervention as *signals* or as *self-antigens* of their respective family. This model can be easily applied to various neuroendocrine hormone families. A kind of economical principle appears in the organization of the thymic peptide repertoire. TEC are not the site of expression of all members of one given family, but a representative member is dominantly expressed by TEC/TNC (Table 1). From our studies, a difference also appears between neuroendocrine autoantigens expressed by the peripheral tissues tackled by an autoimmune response and their homologous self-antigens expressed in thymic epithelium. This difference is important to take into account since autoan-

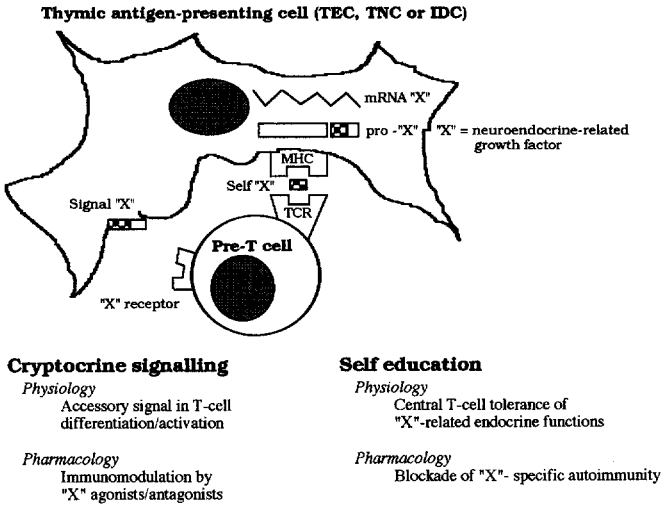

Fig. 1 Neuroendocrine-related polypeptide precursors ("X") synthesized in TEC/TNC exert two types of physiological actions in T cell differentiation. Through cryptocrine signaling they interact with neuroendocrine-type receptors ("X" receptor) expressed by target pre-T cells and may constitute accessory *signals* in the process of T cell development. In relationship with the thymic MHC system, the highly conserved sequences of neuroendocrine families are presented as *self-antigens* (self-"X") to pre-T cells and could induce the negative selection of T cells bearing a randomly rearranged TCR directed against their respective families. An efficient pharmacological manipulation of both types of interactions may be expected in the near future

Table 1 The organization of the thymic repertoire of neuroendocrine self-peptide precursors

Neuroendocrine families	Physiological functions	Neuroendocrine self-antigens
Neurohypophysial family		
VP	Water metabolism	OT
OT	Reproduction	
Insulin family		
Insulin	Glucose metabolism	IGF-II
IGF-I	Growth	
IGF-II	Fetal development	
Tachykinins		
SP	Pain, sensory innervation	NKA [17]
NKA	Growth, development	
NKB	?	
Parathormones		
PTH	Calcium metabolism	PTH-rP [30]
PTH-rP	Fetal development	
Calcitonins		
CT	Calcium metabolism	CGRP [11]
CGRP	Trophic factor, fetal development	

Table 2 The insulin peptide family

	B domain	A domain
IGF-II		
Human	RPSETL*CGGELVDTL*QFVCGDRGFYF	*GIVEECCFRS*CDLALLETYCA
Cow		
Rat	S	
Mouse	G G S	
IGF-I		
Human	GPETLCGAELVDALQFVCGDRGFYFNKPT	GIVDECCFRSCDLRRLEMYCA
Cow		
Rat	P	
Mouse	P	
Insulin		
Lymnæa	PHRRGVCGSALADLVDFACSSSNQPAMV	NIVCECCMKPCTLSELRQYCP
Bombyx	QAVHTTCGRHLARTLADLCWEAGVD	GIVDECCLRPCSVAVLLSYC
Hagfish	RTTGHLCGKDLVNALTIACGVRGFFYDPTLM	GIVEQCCHKRCSIYNLQNYCN
Guinea pig	FVSRHLCGSNLVETLTSVCQDDGFFYIPKD	GIVDQCCTGTCTRHQLQSYCN
Rat	FVNQHLCGSHLVEALYLVCGERGFFYTPLT	GIVDQCCTSICSLYQLENYCN
Human	FVNQHLCGSHLVEALYLVCGERGFFYTPLT	GIVEQCCTSICSLYQLENYCN
Leydig I-L	PAQEAPEKLCGHHFVRALVRLCGGPRWSPEDG	NPARHCCLSGCTRQDLLTLCPH
Relaxin		
Porcine	NDFIKACGRELVRLWVEICGVWS	TLSEKCCEVGCIRKDIARLC

tigens are known to activate autoreactive immune cells whereas, at least theoretically, self-antigens should delete or anergize autoreactive cells. In the neurohypophysial family there is now good evidence that OT is the neurohypophysial self-antigen. Thus a strong immunological tolerance protects the OT lineage, more than the VP one, from an eventual autoimmune aggression. Indeed some cases of idiopathic diabetes insipidus have been shown to result from an autoimmune hypothalamitis oriented toward VP-producing neurons [29, 44]. Given the importance of the OT lineage in the control of the reproductive process at several levels (parturition, maternal behavior, lactation, and paracrine regulation of gonadal functions), a stronger tolerance of this lineage appears to be crucial for the preservation of the species. Therefore in the neurohypophysial family OT behave as the self-antigen while VP is strongly suspected to be the target autoantigen of autoimmune process. This conclusion is also supported by the frequence and the titers of antibodies induced by active immunization against neurohypophysial peptides (VP>OT>VT). An infiltration of the hypothalamo-neurohypophysial tract by inflammatory mononuclear cells has been observed repeatedly, both after active immunization against VP [15] and in spontaneous autoimmune diabetes insipidus [29]. Altogether, these data strongly support the idea that hypothalamic magnocellular neurons express on their surface antigenic markers specific of their neurosecretory activity.

In the insulin family many authors have shown that insulin is an autoantigen involved in the diabetogenic autoimmune process. Thymic epithelium is the site of a marked expression of IGF-II [25], although IGF-II is not secreted by primary cultures of human or rat TEC. A marked hyperplasia of the thymus has been observed in transgenic mice for the *Igf2* gene [48]. Following the alignment of amino-acid sequences of insulin-related members (Table 2) one may observe that the sequences of residues 7–15 in B domains and residues 1–10 in A domains are highly conserved throughout evolution of the insulin family. Therefore those sequences might be considered as self-antigens of insulin-related polypeptides. Interestingly, these sequences closely correspond to the target antigen of cytotoxic T cells oriented against insulin [45]. Such a correspondence between highly conserved sequences and dominant antigens has also been noticed recently by others [36]. We have identified and further characterized ir-IGF-II in the cytosol and membrane preparations from human thymuses (Achour et al., submitted). This result is surprising because pro-IGFs possess no transmembrane domain. We are currently investigating the mechanisms involved in the intrathymic presentation of IGF-II. A molecular defect in thymic T cell education to IGF-II or to IGF-II-derived self-peptides could play a role in the physiopathology of insulin-dependent diabetes (IDDM). IGF-II is very homologous but not identical to insulin, and this biochemical difference could elicit completely opposite immune responses. Indeed, while insulin has been shown to activate autoreactive cytotoxic T cells [45], IGF-II could program their tolerant state. These differences in the biochemical identity and immunological responses elicited by insulin-related antigens could be fundamental for the design of an efficient and secure prevention of autoimmune IDDM.

A new physiological role for neurophysin and other binding proteins?

For a long time the binding of neurohypophysial nonapeptides to their associated neurophysins for their axonal transport has been a useful model for understanding the interactions between small peptides and larger proteins

[1, 3, 10, 28]. Interestingly, the residue tyrosine in position 2 of OT and VP had been shown to play an important role in this binding [28]. Interestingly, the residue tyrosine in the same position has been shown to play a crucial role in the binding of antigens to some MHC class I alleles [35]. According to our data, even if MHC class I pathways are implicated in the process, the thymic neurophysin domain seems to be the final step in the presentation of OT by TEC/TNC to immature T cells. A very close functional analogy thus exists for neurophysins between, on one hand, the binding and transport of OT along the neurohypophysial axons until the nerve endings of the posterior pituitary and, on the other, the binding and presentation of the self-antigen OT to immature T cells at the TEC membrane. Other authors also have observed a dissociation between thymic T cell education to self and T cell recognition of antigens [46]. The major implication of this new physiological role of neurophysin in T cell education is that the central T cell tolerance to the neurohypophysial self-antigen OT is *not* tightly restricted by MHC class I alleles. Another selective advantage resides in the potential presentation to pre-T cells of the *structure* characteristic of neurohypophysial-related peptides. Because of the ring structure a classic presentation by MHC class I alleles is indeed excluded [38]. The absence of a tight MHC allelic restriction of central T cell tolerance to neuroendocrine self-antigens has important implications for the prevention of autoimmune endocrine diseases. At last, the role of neurophysin in the presentation of the neurohypophysial self-antigen is more appropriate with their high conservation throughout evolution.

In the insulin-related peptide system, the role of binding and transport proteins is assumed by IGF-binding proteins (IGFBPs). In contrary to neurophysins, IGFBPs are not part of IGF precursors but are encoded by separate genes. Interestingly, some IGFBPs are anchored to cell membranes, but the hypothesis of a relationship with MHC has never been explored. The putative presentation of IGF-II by a membrane-anchored IGFBP could explain why it is detected in membranes but not in the supernatants of TEC cultures. This hypothesis is currently being investigated in our department.

Developmental and evolutionary aspects

Both in ontogeny and phylogeny a continuum of interactions appears between the neurohypophysial family and the Ig/MHC/TCR superfamily. At the cellular level in the thymus TNCs have been shown to constitute a crucial microenvironment in which such interactions take place [22]. This continuum of neuroendocrine-immune interactions culminates at the biochemical level with the identification in thymic membranes of a hybrid neurohypophysial/MHC class I-related 55-kDa protein. Although the posttranscriptional mechanisms leading to this protein remain to be further explored, the existence of this hybrid protein argues strongly for a common ancestral origin of two families implicated in cell-to-cell signaling and molecular recognition. It is also noteworthy that the diversification of both families occurred at about the same time as the emergence of early vertebrates. With regard to the Ig superfamily, its extreme diversification is catalyzed by the recombinases (RAG 1 and 2) recently identified [7]. Since the existence of neurohypophysial precursors has been established in molluscs and insects, it is tempting to speculate that some biochemical properties of this family [14] may have used as structural guides during further evolution of the Ig/MHC/TCR superfamily.

The immune system has evolved primarily to protect the integrity of self against aggression from nonself-infectious invaders. Given the common peptide nature of most allo-, auto-, and self-antigens, the immune system must be educated to recognize and to tolerate the molecular structure of self. Although peripheral tolerogenic pathways are being increasingly established [20], the thymus is recognized as playing the central role in allowing T cells to recognize self-antigens. Since differentiation of the whole T cell repertoire involves recombination at random of gene segments coding for the antigen receptor (TCR) chains, the emergence of self-reactive T cells may naturally follow this highly hazardous biological phenomenom. The thymus thus exerts a radical "anti-hazard" constraint by purging the immune system of self-reactive T cells which otherwise could represent a serious threat for survival. In the same global perspective, pathological autoimmunity can be considered as the tribute paid by mammalian species for the higher complexity and efficiency of their immune defenses.

If a defect exists in the molecular mechanisms ruling intrathymic presentation of neuroendocrine self-antigens, self-reactive or intolerant T cells would migrate continuously from the thymus and could play a major role in autoimmune diseases. As early as in 1962 Burnet and Mackay [12] hypothesized that the thymus plays a homeostatic role by deleting forbidden self-reactive lymphocyte clones. The therapeutic benefit of thymectomy in a variety of autoimmune diseases (such as IDDM or myasthenia gravis) can be explained by the removal of the defective thymic self-censorship. Since insulin per se is not expressed in TEC/TNC, the presence of anti-insulin autoantibodies and autoreactive T cells in normal individuals is not surprising. It is well known, however, that the pathogenetic power of anti-insulin immune effectors is low, although they constitute good markers and perhaps predictors of the autoimmune process tackling islet β cells [6]. At this point one can reasonably ask: In the case of a defect in thymic IGF-II presentation, what could be the pathogenetic role of IGF-II-reactive T cells in the development of IDDM?

Pharmacological implications

The existence of cryptocrine signaling between thymic OT and functional neurohypophysial receptors expressed by T cells led us to investigate the possibility of a pharmacological manipulation of T cell activity by OT recep-

tor antagonists. Using the methodology of human whole blood cell cultures, we have shown that novel OT hexapeptide antagonists (developed by Merck Sharp & Dohme Research Laboratories) inhibit the production of interleukin-1β and interleukin-6 elicited by T cell activation with anti-CD3 [24]. The design of specific OT immune receptor antagonists could offer a therapeutic benefit in certain circumstances, such as the postpartum period, during which an enhancement of nonspecific immune reactivity is determined by the immunostimulatory lactation-inducing hormones prolactin and OT.

The treatment of autoimmune disorders remains highly nonspecific, although significant progress has been accomplished in recent years. We are now waiting for the design of innovative therapeutic procedures based on the mechanisms of self-tolerance and on the ways to reinduce immunological tolerance once it has been broken. The experimental feasibility of this tolerogenic approach has been known since 1953 [8]. If the prevention of autoimmune diabetes insipidus is not a primary goal, given the low occurrence of the disease and its easy treatment, the situation is radically different with regard to the cure and prevention of IDDM. Preliminary approaches using insulin as a prophylactic agent have provided some promising results in children at high risk for IDDM. However, one may logically question both the duration and the practical aspects of such a preventive approach. The identification of thymic IGF-II as a potential source of self-antigens for the whole insulin family should allow more definitive and safer tolerogenic strategies for IDDM prevention (such as vaccination procedures based on the negative selective effect of self-antigens). From our studies and those of others, it is also increasingly apparent that the tolerogenic properties of TEC are important and rather underestimated. Undoubtedly in the near future these physiological properties should be more exploited both in transplantation and in the prevention of autoimmune diseases.

Acknowledgements This manuscript is dedicated to the memory of our Colleague and Friend, Paul Franchimont, with our greatest respect and our warmest gratefulness. Deceased on 9 August 1994, at the age of 60 years, Paul Franchimont was a pioneer of radio-immunology in Europe and he founded the laboratory where most of the present studies have been performed. The stage of advancement of this work is greatly indebted to Paul Franchimont's encouragement and support since its very early initiation. These studies were supported by the Fund for Scientific Medical Research of Belgium (grants 3.4562.90, 7.4611.91, and 7.4548.93) and by the Juvenile Diabetes Foundation International. V. Geenen is Research Associate Professor of the National Fund of Scientific Research (Belgium). B. Goxe is PhD of the University of Paris VI and is supported by the Association de la Recherche contre le Cancer (France).

References

1. Acher R (1993) Neurohypophysial peptide systems: processing machinery, hydroosmotic regulation, adaptation and evolution. Regul Pept 45:1–13
2. Allen PM (1994) Peptides in positive and negative selection: a delicate balance. Cell 76:593–596
3. Ando S, McPhie P, Chaiken IM (1987) Sequence redesign and the assembly mechanism of the oxytocin/bovine neurophysin I biosynthetic precursor. J Biol Chem 262:12962–12969
4. Argiolas A, Gessa GL, Melis MR, Stancampiano R, Vaccari A (1990) Effects of neonatal and adult thyroid dysfunction on thymic oxytocin. Neuroendocrinology 52:556–559
5. Ashton-Rickardt PG, Tonegawa S (1994) A differential-affinity model for T-cell selection. Immunol Today 15:362–366
6. Bach JF (1994) Insulin-dependent diabetes as an autoimmune disease. Endocr Rev 15:516–542
7. Bartl S, Baltimore D, Weissman IL (1994) Molecular evolution of the vertebrate immune system. Proc Natl Acad Sci USA 91:10769–10771
8. Billingham RE, Brent L, Medawar PB (1953) Actively acquired tolerance of foreign cells. Nature 172:603–605
9. Bonomo A, Matzinger P (1993) Thymic epithelium induces tissue specific tolerance. J Exp Med 177:1153–1164
10. Breslow E, Burman S (1990) Molecular, thermodynamic, and biological aspects of recognition and function in neurophysin-hormone systems: a model system for the analysis of protein-peptide interactions. Adv Enzymol 63:1–67
11. Bulloch K, Radjocic T, Yu R, Hausman J, Lenhard L, Baird S (1993) The distribution and function of calcitonin gene-related peptide in the mouse thymus and spleen. Prog Neuroendocrinimmunol 4:186–194
12. Burnet FM, Mackay IR (1962) Lymphoepithelial structures and autoimmune disease. Lancet II:1030–1033
13. Caldwell J, Musiol IM, Walker CH, Pedersen CA, Mason GA (1993) Effects of mating and estrogen on thymic oxytocin receptors. Ann NY Acad Sci 689:573–577
14. Capra JD, Cheng KW, Friesen HG, North WG, Walter R (1974) Evolution of neurophysin proteins: the partial sequence of human neurophysin-I. FEBS Lett 46:71–74
15. Cau P, Rougon-Capuzzi G (1979) Autoimmune alterations in the neurohypophysis of rabbits immunized against vasopressin. Brain Res 177:265–271
16. Elands J, Resink A, de Kloet ER (1990) Neurohypophysial hormone receptors in the rat thymus, spleen, and lymphocytes. Endocrinology 126:2703–2710
17. Ericsson A, Geenen V, Robert F, Legros JJ, Vrindts-Gevaert Y, Franchimont P, Brené S, Persson H (1990) Expression of preprotachykinin A and neuropeptide Y messenger RNA in the thymus. Mol Endocrinol 4:1211–1218
18. Funder JW (1990) Paracrine, cryptocrine, acrocrine. Mol Cell Endocrinol 70: C21-C24
19. Gainer H, Wray S (1993) Cellular and molecular biology of oxytocin and vasopressin. In: Knobil E, Neill JD (eds) The physiology of reproduction, 2nd edn. Raven Press, New York, pp 1099–1129
20. Geenen V, Kroemer G (1993) Multiple ways to cellular immune tolerance. Immunol Today 14:573–575
21. Geenen V, Legros JJ, Franchimont P, Baudrihaye MF, Defresne MP, Boniver J (1986) The neuroendocrine thymus: coexistence of oxytocin and neurophysin in the human thymus. Science 232:508–511
22. Geenen V, Defresne MP, Robert F, Legros JJ, Franchimont P, Boniver J (1988) The neurohormonal thymic microenvironment: Immunocytochemical evidence that thymic nurse cells are neuroendocrine cells. Neuroendocrinology 47:365–368
23. Geenen V, Robert F, Martens H, Benhida A, Degiovanni G, Defresne MP, Boniver J, Legros JJ, Martial J, Franchimont P (1991) At the cutting edge. Biosynthesis and paracrine/cryptocrine actions of "self" neurohypophysial-related peptides in the thymus. Mol Cell Endocrinol 76: C27-C31
24. Geenen V, Martens H, Robert F, Vrindts-Gevaert Y, De Groote D, Franchimont P (1992) Immunomodulatory properties of cyclic hexapeptide oxytocin antagonists. Thymus 20:217–226
25. Geenen V, Achour I, Robert F, Vandersmissen E, Sodoyez JC, Defresne MP, Boniver J, Lefèbvre PJ, Franchimont P (1993) Evidence that insulin-like growth factor-II (IGF-II) is the dominant thymic peptide of the insulin superfamily. Thymus 21:115–127

26. Geenen V, Vandersmissen E, Cormann-Goffin N, Martens H, Legros JJ, Degiovanni G, Benhida A, Martial J, Franchimont P (1993) Membrane translocation and relationship with MHC class I of a human thymic neurophysin-like protein. Thymus 22:55–66
27. Geenen V, Vandersmissen E, Martens H, Goxe B, Kecha O, Legros JJ, Lefèbvre PJ, Benhida A, Rentier-Delrue F, Martial JA (1995) Cellular and molecular aspects of thymic T-cell education to neurohypophysial principles. In: Saito T, Yoshida S, Imura H (eds) (1995) Proceedings of the First Joint World Congress of Neurohypophysis and Vasopressin. Elsevier, Amsterdam (in press)
28. Griffin JH, Alazard R, Cohen P (1973) Complex formation between bovine neurophysin-I and oxytocin, vasopressin and tripeptide analogs of their NH_2-terminal region. J Biol Chem 248:7975–7978
29. Imura H, Nakao K, Shimatsu A, Ogawa Y, Sando, T, Fujisawa I, Yamabe H (1993) Lymphocytic infundibuloneurohypophysitis as a cause of central diabetes insipidus. N Engl J Med 329:683–689
30. Kramer S, Reynolds FHJr, Castillo M, Valenzuela DM, Thorikay M, Sorvillo JM (1991) Immunological identification and distribution of parathyroid hormone-like protein polypeptide in normal and malignant tissues. Endocrinology 128: 1927–1937
31. Kruisbeek AM (1993) Development of αβ T cells. Curr Opin Immunol 5:227–234
32. Kumamoto K, Matsuura T, Amagai T, Kawata M (1995) Oxytocin-producing and vasopressin-producing eosinophils in the mouse spleen: immunohistochemical, immuno-electron-microscopic and in situ hybridization studies. Cell Tiss Res 281:1–10
33. Markwick AJ, Lolait SJ, Funder JW (1986) Immunoreactive arginine vasopressin in the rat thymus. Endocrinology 119: 1060–1064
34. Martens H, Robert F, Legros JJ, Geenen V, Franchimont P (1992) Expression of functional neurohypophysial peptide receptors by immature and cytotoxic T-cell lines. Prog NeuroEndocrinImmunol 5:31–39
35. Maryanski JL, Romero P, van Pel A, Boon T, Salemme FR, Cerrottini JC, Corradin G (1991) The identification of tyrosine as a common key residue in unrelated H-$2K^d$ restricted antigenic peptides. Int Immunol 3:1035–1042
36. Mertz AKH, Daser A, Skurnik M, Weissmüller KH, Braun J, Appel H, Batsford S, Wu P, Distler A, Sieper J (1995) The evolutionary conserved ribosomal protein L23 and the cationic urease §-subunit of *Yersinia enterocolotica* O:3 belong to the immunodominant antigens in *Yersinia*-triggered reactive arthritis: implications in autoimmunity. Mol Med 1: 44–55
37. Moll UM, Lane BL, Robert FR, Geenen V, Legros JJ (1988) Abundant occurrence of oxytocin-, vasopressin- and neurophysin-like peptides in human thymic epithelial cells. Histochemistry 89:385–390
38. Rammensee HG, Falk K, Rötzschke O (1993) Peptides naturally presented by MHC class I molecules. Annu Rev Immunol 11:213–244
39. Rebaï N, Malissen B (1983) Structural and genetic analyses of HLA class I molecules using monoclonal xenoantibodies. Tissue Antigens 22:107–117
40. Robert F, Geenen V, Schoenen J, Burgeon E, De Groote D, Defresne MP, Legros JJ, Franchimont P (1991) Colocalization of immunoreactive oxytocin, vasopressin and interleukin 1 in human thymic epithelial neuroendocrine cells. Brain Behav Immun 5:102–112
41. Robert F, Martens H, Cormann N, Benhida A, Schoenen J, Geenen V (1992) The recognition of hypothalamo-neurohypophysial functions by developing T cells. Dev Immunol 2:131–140
42. Robertson GL (1987) Posterior Pituitary. In: Felig P, Baxter JD, Broadus AE, Frohman LA (eds) Endocrinology and metabolism. McGraw-Hill, New York, pp 338–385
43. Schaller MD, Borgman CA, Cobb BS, Vines RR, Reynolds AB, Parsons JT (1992) $p125^{FAK}$, a structurally distinct protein-tyrosine kinase associated with focal adhesions. Proc Natl Acad Sci USA 89:5192–5196
44. Scherbaum WA, Bottazzo GF (1983) Autoantibodies to vasopressin cells in idiopathic diabetes insipidus: evidence for an autoimmune variant. Lancet I:897–901
45. Sheil JM, Shepherd SE, Klimo GF, Paterson Y (1992) Identification of an autologous insulin B chain peptide as a target antigen for H-$2K^b$-restricted cytotoxic T lymphocytes. J Exp Med 175:545–552
46. Simpson E, Robinson PJ, Chandler P, Millrain MM, Pircher HP, Brändle D, Tomlinson P, Antoniou J, Mellor A (1994) Separation of thymic education from antigen presenting functions of major histocompatibility complex class I molecules. Immunology 81:132–136
47. Torres BA, Johnson HM (1988) Arginine vasopressin (AVP) replacement of helper cell resuirement in IFN-g production. Evidence for a novel AVP receptor on mouse lymphocytes. J Immunol 140:2179–2183
48. Van Buul-Offers SC, de Haan K, Reijnen-Gresnigt MG, Meinsma D, Jansen M, Oei SL, Bonte EJ, Sussenbach JS, Van den Brande JL (1995) Overexpression of human insulin-like growth factor-II in transgenic mice causes increased growth of the thymus. J Endocrinol 144:491–502
49. Wiemann M, Ehret G (1993) Subcellular localization of immunoreactive oxytocin within thymic epithelial cells of the male mouse. Cell Tissue Res 273:79–87