Thymic Neuroendocrine Self-Antigens

Role in T-Cell Development and Central T-Cell Self-Tolerance

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ABSTRACT: **The repertoire of thymic neuroendocrine precursors plays a dual role in T-cell differentiation as the source of either cryptocrine accessory signals in T-cell development or neuroendocrine self-antigens presented by the thymic major histocompatibility complex (MHC) machinery. Thymic neuroendocrine self-antigens usually correspond to peptide sequences highly conserved during the evolution of one family. The thymic presentation of some neuroendocrine self-antigens is not restricted by MHC alleles. Oxytocin (OT) is the dominant peptide of the neurohypophysial family. It is expressed by thymic epithelial and nurse cells (TEC/TNCs) of different species. Ontogenetic studies have shown that the thymic expression of the OT gene precedes the hypothalamic one. Both OT and VP stimulate the phosphorylation of $p125^{FAK}$ and other focal adhesion-related proteins in murine immature T cells. These early cell activation events could play a role in the promotion of close interactions between thymic stromal cells and developing T cells. It is established that such interactions are fundamental for the progression of thymic T-cell differentiation. Insulin-like growth factor 2 (IGF-2) is the dominant thymic polypeptide of the insulin family. Using fetal thymic organ cultures (FTOCs), the inhibition of thymic IGF-2-mediated signaling was shown to block the early stages of T-cell differentiation. The treatment of FTOCs with an mAb anti-(pro)insulin had no effect on T-cell development. In an animal model of autoimmune type 1 diabetes (BB rat), thymic levels of (pro)insulin and IGF-1 mRNAs were normal both in diabetes-resistant and diabetes-prone BB rats. IGF-2 transcripts were clearly identified in all thymuses from diabetes-resistant adult (5-week) and young (2- and 5-days) BB rats. In marked contrast, the IGF-2 transcripts were absent and the IGF-2 protein was almost undetectable in ± 80% of the thymuses from diabetes-prone adult and young BB rats. These data show that a defect of the thymic IGF-2–mediated tolerogenic function might play an important role in the pathophysiology of autoimmune Type 1 diabetes.**

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THE DUAL ROLE OF THYMIC NEUROENDOCRINE SELF-ANTIGENS IN T-CELL DIFFERENTIATION

Before reacting against "non-self" infectious agents, the immune system must be able to tolerate the host molecular structure ("self"). The induction of immune self-tolerance is a multistep process that is initiated inside the thymus during fetal ontogeny (central self-tolerance) and also involves inactivating (anergizing)[1] mechanisms outside the thymus (peripheral self-tolerance).[2] The thymus is the primary lymphoid organ implicated in the development of immunocompetent and self-tolerant T lymphocytes.[3] Our experimental studies since 1985 have established that the thymus also constitutes one privileged meeting point between the two major systems of intercellular signaling, the neuroendocrine and immune systems.[4,5] The thymic parenchyme is the site of synthesis for protein precursors belonging to various neuroendocrine families. Thymic precursors not only provide accessory signals for T-cell growth and development, but they are also the source of neuroendocrine self-antigens which are presented to differentiating T cells. According to the theory of T-cell negative selection,[6–8] the intrathymic presentation of neuroendocrine self-antigens would induce the clonal deletion or developmental arrest of self-reactive T cells. Such self-reactive T cells randomly emerge during the recombination of gene segments coding for the chains of the T-cell receptor of antigen (TCR) and they are bearing one TCR specifically directed toward the complex CMH/self-antigen. The thymus is the major, if not the only one, lymphoid organ wherein permanently occurs a confrontation between the presentation of the self molecular structure and a pure random phenomenon with a potential toxic threat for the host organism. In physiological conditions, this confrontation leads to the deletion or the inactivation of such self-oriented toxicity. Even if other tolerizing mechanisms exist in the periphery, it is now well established that the thymus exerts the dominant tolerogenic control upon the immune system.

According to its nature as the source of either cryptocrine accessory signals or self-antigens, respectively, the thymic repertoire of neuroendocrine precursors recapitulates at the molecular level the dual physiological role of the thymus in T-cell positive and negative selection. The interaction of neuroendocrine self-antigens with their corresponding TCR implies a binding of moderate affinity (from 10^{-6} to 10^{-8} M), but with a high selectivity. On the other hand, cryptocrine signaling between thymic neuroendocrine-related peptides and their cognate neuroendocrine-type receptors expressed by pre-T cells involves a high-affinity binding (from 10^{-10} to 10^{-11} M), albeit with a low specificity.[9] Moreover, a hierarchy of dominance and an economical principle appear in the organization of the polypeptide repertoire expressed in the thymus. This is of high significance since self-tolerance primarily concerns dominant antigenic determinants of self-molecules.[10] This model concurs with the "avidity/affinity hypothesis" that has been proposed as another explanation of the thymic paradox in T-cell life and death.[11] According to this latter hypothesis, T lymphocytes are positively selected if their TCR is barely engaged with self-antigen at low concentrations (10^{-12} M), and are deleted if TCR is strongly engaged with self-peptide at high concentrations (10^{-6} M). However, since the affinity of a TCR for its cognate antigen is rather low (10^{-8} M at the maximum),[12] the intrathymic concentration of self-peptides is of crucial importance for determining positive or negative

T-cell selection. It therefore became a primary objective to define the nature and the amount of peptide/MHC combinations that contribute *in vivo* to positive or negative selection of a particular TCR in a normal thymus.

ONTOGENY OF THYMIC OT AND IGF GENE EXPRESSION

Although the two neurohypophysial genes, OT and vasopressin (VP), are expressed in human and murine thymuses,[13] at the peptide level OT is the dominant member of this family which is synthesized by TEC/TNCs in these species. Using RT-PCR, *in situ* hybridization, and immunocytochemistry (ICC), we recently investigated the ontogeny of neurohypophysial gene expression in the thymus of Balb/c mice. Transcripts of *proOT* and *proVP* are detected without any visible modulation in the thymus already from fetal day (FD) 14 until day 7 after birth. In the murine thymus, neurohypophysial transcripts are located in cells with an epithelial morphology and are absent in the lymphoid compartment.[14] Because of the microscopic size of thymic rudiments before FD 14, it was not possible to analyze earlier the thymic expression of the neurohypophysial genes. Nevertheless, the comparison with previous reports[15] shows that the transcription of neurohypophysial genes in the rodent thymus precedes their expression in the magnocellular neurons of the hypothalamic–neurohypophysial axis. At the peptide level, this difference is more evident since ir-OT is detected in the thymus on FD 15, whereas ICC labels ir-OT in the hypothalamus only on FD 20.[16] Thus, the expression of neurohypophysial genes in the murine thymus coincides with the appearance of T-cell progenitors and slightly precedes their hypothalamic transcription. This observation is highly significant with regard to the physiological role proposed for thymic OT both in T-cell lymphopoiesis and in central tolerance of the hypothalamo-neurohypophysial functions. The early expression of thymic OT is another experimental argument supporting a tolerogenic role of the thymic repertoire of neuroendocrine self-antigen precursors. Indeed, it is logical that the induction of central self-tolerance precedes the appearance of antigenic epitopes in the target organs susceptible to an autoimmune aggression.[17,18] Furthermore, the putative thymic deletion of OT-reactive T-cell clones will allow the immunomodulation by peripheral OT without the risk of inducing an autoimmune hypothalamitis. For example, a non-specific immune activation is usually observed in the postpartum, a period characterized by an increase of lactatory hormones (prolactin and OT) and an enhancement of the estrogen/progesterone ratio.

The components of the IGF axis have also been characterized in the human thymus. Human TEC expresses different members of this axis, with a predominance of IGF-2 and IGF-binding proteins (IGFBP) 2 to 6.[19,20] In the human and rat thymuses, IGF-1 expression is restricted to sparse cells with a macrophage-like morphology and distribution.[20,21] RT-PCR analyses of total RNA from murine fetal and postnatal thymuses revealed that IGF-1, IGF-2, IGF type 1 (IGF-1R) and type 2 (mannose-6-phosphate [M6P]/IGF-2R) receptors are expressed from FD 14 through seven weeks of age. Though RT-PCR conditions are not quantitative, a striking difference appeared between the IGF-2 signals and the others studied. Similar mRNA levels of IGF-1, IGF-1R, and M6P/IGF-2R were detected in all the fetal and postnatal murine thymuses. However, IGF-2 mRNA levels declined after birth, but weak signals were

still detected in seven-week old thymuses. By *in situ* hybridization, IGF-2 mRNAs were detected mainly in the epithelial component of the murine thymus. Therefore, the expression of insulin-related genes in the thymus also precedes their peripheral transcription, as in the pancreatic islet cells.

THE ROLE OF THYMIC OT AND IGF IN T-CELL DEVELOPMENT

After their migration from the fetal liver and then from bone marrow, immature T-cell progenitors receive from the thymic parenchyme various types of signals which regulate their differentiation program toward T-cell death or development. These signals are not strictly thymus-specific, but their local action is linked to their expression within a particular microenvironment and at a crucial step of T-cell differentiation. The ability of pre-T cells to respond to thymic OT and IGF-2 was demonstrated by a series of different approaches.

Functional neurohypophysial hormone receptors are expressed by immature T cells and by mature cytotoxic T cells.[22,23] These lymphocyte receptors are different from classic V1a/V2 receptors, and rather appear such as another V1 (V1b or V3?) subtype, as well as the OT type.[23,24] They are able to transduce OT and VP into a phospho-inositide turnover, and to mediate mitogenic effects of neurohypophysial-related peptides on freshly isolated human pre-T cells.[23] Moreover, in a line of pre-T cells derived from a murine thymic lymphoma (RL12-NP), OT and VP quickly stimulate the phosphorylation of $p125^{FAK}$, a tyrosine-kinase involved in focal adhesion, as well as other proteins implicated in this process like paxillin and a 130-kDa protein ($p130^{CAS}$?). Neurohypophysial peptide-induced $p125^{FAK}$ phosphorylations are blocked by a V1 antagonist (Manning compound).[25] As demonstrated by others, the role of $p125^{FAK}$ is crucial for T-cell differentiation.[26] The OT-mediated activation of $p125^{FAK}$ in RL12-NP cells suggests that thymic OT intervenes in T-cell selection, either as a promoter of focal adhesion itself, or as an anti-apoptotic inducer of a cryptocrine signaling between TEC and T cells leading to the proliferation and survival of early T-cell precursors.

There is increasing evidence that IGFs are implicated in the development and modulation of the immune response. Thymocytes express both types of IGF receptors (IGF-1R and M6P/IGF-2R).[27,28] Administration of IGF-1 stimulates thymus and spleen growth and T-cell proliferation and development and modulates the regeneration of T cells in a rat model of dexamethasone-induced apoptosis.[29,30] In addition, the thymus of IGF-2 transgenic mice contains high levels of IGF-2 mRNA and displays an increased cellularity, with a higher number of the $CD4^+$ T-cell subset.[31] We also examined the role of IGFs on murine T-cell development by evaluating the effect of anti-IGFs and IGF-receptors neutralizing Abs on the generation of thymocyte subpopulations in fetal thymic organ cultures (FTOCs).[32] Neither anti-IGF-1 nor anti-IGF-2 induced a significant change in the total cell number or the percentage of dead cells as measured by propidium iodide staining. FTOC treatment with anti-IGF-2 mAb, an anti-IGF-1R mAb, or an anti-M6P/IGF-2R polyclonal Ab induced a blockade of T-cell differentiation at the $CD4^-CD8^-$ (double negative) T cells, as shown by a significant increase in the percentage of $CD4^-CD8^-$ cells and a decrease in the percentage of $CD4^+CD8^+$ cells. In addition, the treatment

with anti-IGF-1R Ab blocked T-cell differentiation at the $CD4^+CD8^+$ stage as shown by a decrease in single positive subsets. Moreover, anti-IGF-2 Ab treatment induced an increase in $CD8^+$ single positive cells, suggesting that thymic IGF-2 has a role in determining differentiation into the CD4 or CD8 lineage. The total percentage of viable cells was not affected by any of the anti-IGF-R Abs tested. However, in FTOCs treated with anti-IGF-2R, there was a 31% decrease in the total cell number. This decrease was more important (81%) with the FTOC treatment by anti-IGF-1R. Although the (pro)insulin gene is slightly expressed in the thymus,[33] FTOCs treated with a specific anti-(pro)insulin mAb were unaffected neither in total cell number, nor in the main steps of T-cell differentiation.[32]

THYMIC PRESENTATION OF NEUROENDOCRINE SELF-ANTIGENS

The synthesis of OT in TEC/TNCs is not coupled to the classic secretion of the nonapeptide and its precursor-associated binding neurophysin in the supernatant of TEC primary cultures. In the murine thymus, ir-OT is not located in secretory granules, but is diffuse in the cytosol, in vesicles of the endoplasmic reticulum, and in close association with cytokeratin filaments.[34] Similar ultrastructural features have also been described for ir-OT and ir-VP synthesized by murine spleen eosinophil-like cells.[35] Those independent observations repeatedly questioned the classic model of neurosecretion which was established for OT and VP in the hypothalamo-neurohypophysial tractus. They further suggested a processing of the OT precursor that differs in the thymus compared to the situation in the hypothalamo-neurohypophysial axons. As discussed above, the thymic function is associated with the presentation of self-antigens to developing T cells. This action was long thought to be mediated by thymic macrophages and dendritic/interdigitating (IDC) cells only, but TEC/TNCs also are actively involved in the induction of central self-tolerance.[36–38] Thus, we hypothesized a processing of thymic proOT that could be related to antigen presentation instead of a classic neurosecretion.

Using affinity-chromatography with a mAb directed against the monomorphic part of human MHC class I molecules,[39] we identified in TEC/TNC plasma membranes a 55-kDa protein that was labelled both by anti-MHC class I mAb and anti-neurophysin antibodies.[40] Since anti-neurophysin Abs do not cross-react with either MHC class I proteins, nor with β_2-microglobulin, this 55-kDa membrane protein may represent a hybrid protein including both a neurophysin domain (10 kDa) and an MHC class I heavy chain-related domain (45 kDa). The precise biochemical mechanisms underlying the formation of this hybrid neurohypophysial/MHC class I membrane protein are still to be further deciphered. Some preliminary hypotheses may be advanced, however. The origin of this protein could reside at the posttranscriptional level (such as a *trans*-splicing phenomenon) or at the posttranslational level (such as the ATP-dependent binding of ubiquitin to protein targeted to proteolysis). Following this putative explanation, the MHC class I domain would be implicated in membrane targeting of this hybrid protein, whereas neurophysin binds OT for presentation to pre-T cells. Other authors have shown the translocation of a 45-kDa neurophysin-like material in the cell membranes of cancer cells, and have provided strong arguments supporting the behavior of neurohypophysial-related

peptides as candidate tumoral antigens.[41,42] Thus, both in the hypothalamo–neurohypophysial axis and in the thymus, the neurophysin part of the OT precursor fulfills the same function: binding of the active nonapeptide OT and tranport to the external limits of neurons or TEC/TNCs. The tyrosine residue in position 2 of OT and VP plays an important role in their binding to neurophysin.[43] Interestingly, the tyrosine residue in the same position plays a crucial role in the binding of antigens to some MHC class I alleles for their presentation.[44] The particular features of thymic OT-mediated T-cell education to the neurohypophysial family can be related to the observation of a dissociation between thymic T-cell education to self and peripheral T-cell recognition of antigens.[45] Selective advantages appear from this model of thymic neuroendocrine–self-antigen presentation. A first advantage is the absence of a tight allelic restriction in thymic T-cell education to a neuroendocrine family. Such an allelic restriction of central T-cell tolerance was hardly conceivable and our data indicate that it is not the case in reality. Concerning the presentation of thymic OT, though MHC class I molecules are involved in the process, it is the invariant neurophysin domain of the hybrid membrane 55-kDa protein that binds OT for presentation to pre-T cells. Another advantage resides in the presentation to pre-T cells of the structure characteristic of the neurohypophysial family.

The antigenic behavior of thymic OT was further demonstrated by another type of experiments. The immunological recognition of OT by specific mAbs at the outer surface of human TEC plasma membrane induced a marked secretion of the cytokines interleukin-6 and leukemia inhibitory factor in the supernatant of TEC cultures.[46] Given the nature of the epitopes recognized by anti-OT mAbs, we could conclude that the molecule OT is fully processed at the level of the TEC plasma membrane. The absence of biological effects following the treatment of TEC cultures with anti-VP mAbs further confirms that thymic OT behaves as the self-antigen representative of the neurohypophysial hormone family.

Neurotensin (NT) and somatostatin have been extracted from the chicken thymus, especially after hatching, and have been characterized both immunochemically and chromatographically.[47] Ir-NT is expressed at the cell surface of human TEC, and cultured human TECs contain ± 5 ng ir-NT/10^6 cells, of which 5% is associated with plasma cell membranes. HPLC analysis of ir-NT present in human TEC revealed a major peak of ir-NT corresponding to intact NT_{1-13}. Using an affinity column prepared with the same anti-MHC class I Ab, NT-related peptides were retained on the column and were eluted together with MHC class I proteins.[48] With regard to the thymic presentation of NT, there is no physical constraint for a non-covalent binding to MHC since this neuropeptide is a linear peptide (in contrast to cyclic OT and IGF-2). In addition, the C-terminal sequence of NT includes tyrosine, isoleucine and leucine residues, which can all be used in the anchorage to most of the MHC class I alleles. Given these characteristics, it is logical to postulate that NT and NT-derived C-terminal fragments could behave as natural ligands for a majority (if not all) of MHC class I alleles. This hypothesis is also in agreement with the high degree of conservation of NT-related C-terminal region throughout evolution.[49]

Neurokinin A (NKA) is the peptide of the tachykinin family encoded in human and rat TEC by the preprotachykinin A (PPT-A) gene.[50] Thymic *PPT-A* expression appears to be glucocorticoid-dependent since adrenalectomy of Sprague-Dawley rats markedly enhances thymic expression of *PPT-A* (and *NPY*) mRNAs (Ericsson

and Geenen, unpublished observations). Interestingly, NKA exerts IL-1-like mitogenic effects on murine thymocytes,[51] suggesting that tachykinin receptors are expressed by immature T cells and are implicated as an accessory pathway in T-cell maturation and positive selection. The amino-acid sequence of NKA shares the same C-terminal epitope with other members of the tachykinin family, and the leucine residue in position 9 could be used in the binding to some MHC class I alleles, thus making NKA the self-antigen of the tachykinin family. The other tachykinin encoded by *PPT-A,* substance P (SP), is not detected in TEC, but is present in sensory nerve fibers of the thymus.[52] Thymic-specific receptors for SP are associated with the vasculature in the medulla, where they could control local blood flow and vascular permeability.[53]

For IGFs, the role of binding and transport proteins is ensured by IGFBPs. IGFBPs have co-evolved with IGFs, but are not intrinsic part of IGF precursors, and are encoded by distinct genes. These proteins play a prominent role in regulating the bioavailability and distribution of IGFs.[54,55] Interestingly, some IGFBPs are in close relationship with cell plasma membranes (through binding to integrins or the extracellular matrix), but their relationship with MHC as well as their potential implication in thymic IGF presentation to immature T cells deserves to be further investigated.

A DEFECT OF THYMIC FUNCTION IN AUTOIMMUNE TYPE 1 DIABETES

The development of an autoimmune disease affecting the neuroendocrine system may be viewed as a failure to develop or to maintain self-tolerance of cellular and molecular components which are constitutively expressed by neuroendocrine cells. Three types of factors are usually thought to be implicated in the pathogeny of autoimmune diseases: (1) The immune effectors are CD4 and CD8 auto-reactive T cells which are specifically oriented against a given target cell or molecule. These auto-reactive T cells are usually thought to result from a spontaneous breakdown of peripheral T-cell tolerance. (2) A series of extra- and intra-MHC genes are related to different autoimmune diseases. Some of these genes intervene in the presentation of target auto-antigens to auto-reactive T lymphocytes, but others certainly not. (3) An environmental factor is involved and establishes a link between the target auto-antigens and auto-reactive T cells. A molecular mimicry between target auto-antigens and microorganisms was shown to intervene at this level.[56] The involvement of microbial superantigens has also been proposed to activate peripheral auto-reactive T cells.[57]

Although the relationship between lymphoepithelial structures and autoimmunity had been suspected by Burnet and Mackay in 1962,[58] the question of a defective central T-cell self-tolerance in the pathophysiology of autoimmune diseases has not been intensively investigated. Also, Burnet repeatedly proposed that the emergence of "forbidden" self-reactive T-cell clones should play a major role in the pathophysiology of autoimmunity. In this perspective, it has been shown that neonatal thymectomy prevents the emergence of diabetes in an animal model of autoimmune type 1 diabetes, the Bio-Breeding (BB) rat.[59] In clinical practice also,

thymectomy usually induces a significant improvement in patients suffering from autoimmune myasthenia gravis, especially when a thymoma (hyperplasia of thymic epithelium) is associated.[60] In both cases, the benefit of thymectomy can be explained by the removal of the defective thymic censorship. Such a trouble in central self-tolerance would be responsible for a continuous release and enrichment of the peripheral T-cell pool with intolerant and self-reactive lymphocytes. The development of diabetes is prevented by the transplantation of thymus from diabetes-resistant to diabetes-prone BB rats.[61] The transplantation of the thymus from NOD mice to diabetes-resistant mouse strains was also shown to induce diabetes in the recipients.[62] While bone marrow transplantation is rather ineffective in preventing autoimmune diseases of MRL/+ mice, thymus transplantation is a crucial factor for their prevention. [63] A defective process of thymic T-cell negative selection has been suggested on the basis that the thymus of diabetes-resistant BB rats contains thymocytes predisposed to auto-reactivity.[64] Another argument is the observation that grafts of pure thymic epithelium from NOD mouse embryos to newborn C57BL/6 athymic mice induced CD4 and CD8 T-cell–mediated insulitis and sialitis.[65] At the histological level, a defect in thymic function could be linked to a disorganization of the microenvironment, such as the giant perivascular spaces observed in the NOD mouse thymus,[66] and the epithelial defects of BB rat thymus.[67] Recently, we examined the G75 elution profiles of ir-IGFs in the thymus from Wistar Furth (WF) normal rats and from diabetes-resistant and diabetes-prone BB rats. A peak of ir-IGF-2 above 10 ng/ml was observed in the G75 profile of WF thymus extracts; a peak around 1.5 ng/ml was eluted from diabetes-resistant BB rat thymic extracts, however IGF-2 concentrations were almost undetectable in diabetes-prone BB rats.[68] IGF-2 transcripts were not detected by RT-PCR in the thymus of 11/15 diabetes-prone BB rats, but were clearly identified in the thymus of 15/15 diabetes-resistant BB rats. The defect of thymic IGF-2 expression was evidenced at different ages of the diabetes-prone BB rat. The expression of proinsulin and IGF-1 genes was normal in the thymus of diabetes-prone and diabetes-resistant BB rats. The defect of IGF-2 expression was tissue-specific since IGF-2 transcripts were detected in the brain and liver of diabetes-prone BB rats.[69] Taken together, these observations show a genetically determined defect of *IGF2* expression in the thymus of diabetes-prone BB rats. They also strongly support that a defect in the central T-cell self-tolerance of the insulin family is involved in the pathophysiology of autoimmune type 1 diabetes, at least in this animal model. A recent study has shown that the thymic and pancreatic expression of *IGF2* is not polymorphic enough to explain the human susceptibility to type 1 diabetes, which is associated with *IDDM2,*[70] the genetic locus that includes the contiguous IGF2 and insulin genes. It must be pointed out, however, that the imprinting of *IGF2* could partially explain why the susceptibility to the disease is higher in children from diabetic fathers than those from diabetic mothers.[71,72]

NEUROENDOCRINE SELF VERSUS AUTO-ANTIGENS

In the neurohypophysial family, the bulk of experimental data shows that OT is the self-antigen of the neurohypophysial hormone family. A strong immunological tolerance protects the OT lineage, more than the VP one, from an autoimmune

aggression. Indeed, some cases of so-called "idiopathic" central diabetes insipidus result from an autoimmune hypothalamitis oriented toward VP-producing neurons. Given the implication of the OT lineage at different levels of the reproductive process, a stronger tolerance of this lineage is important for the preservation of the species. Thus, in the neurohypophysial family, while OT behaves as the self-antigen, VP is suspected to be the target auto-antigen of the autoimmune process. As discussed previously, this conclusion is also supported by the frequences and the titers of Abs induced by active immunization against neurohypophysial peptides (VP >> OT). An infiltration of the hypothalamo-neurohypophysial tract by inflammatory mononuclear cells can be observed, both after active immunization against VP,[73] and in autoimmune "idiopathic" diabetes insipidus.[17,18]

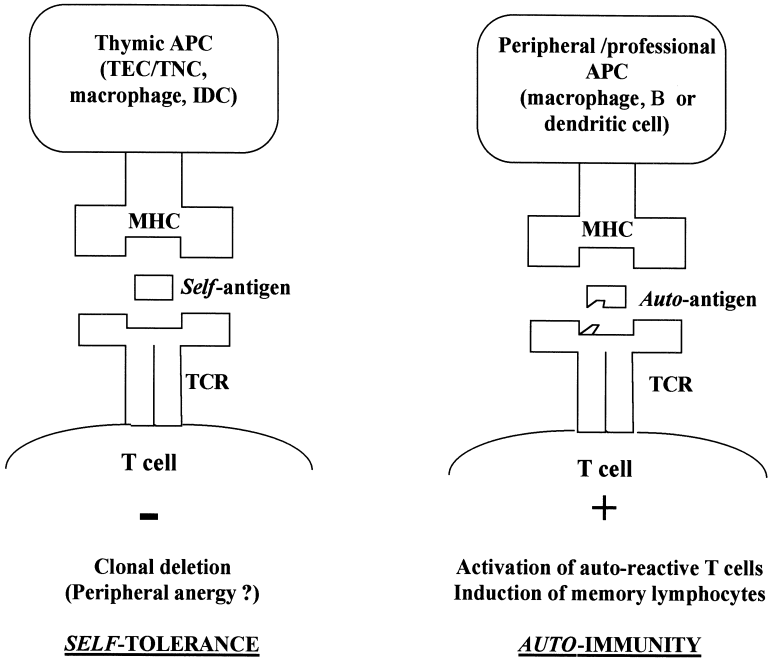

FIGURE 1. The opposite immune responses elicited by the thymic presentation of a self-antigen and the peripheral presentation of an auto-antigen. Thymic antigen-presenting cells (APC) are thymic epithelial and nurse cells (TEC/TNC), macrophages and interdigitating cells (IDC) or denditic cells. Neuroendocrine self-antigens correspond to peptide sequences of a precursor that have been highly conserved during evolution of their corresponding family (i.e., OT for the neurohypophysial peptides, IGF-2 for the insulin family, NKA for the tachykinins). They are very homologous to peripheral related auto-antigens (i.e., VP for the neurohypophysial peptides, insulin for the insulin family), although they are not identical. This biochemical difference between neuroendocrine self- and auto-antigens results in opposite immune responses with a deletion of self-reactive T-cell clones in the thymus (and anergy at the periphery?), and an activation of auto-reactive T cells and induction of memory lymphocytes at the periphery.

Insulin is one important auto-antigen tackled by various auto-reactive components of the immune system both in animal and human type 1 diabetes.[74,75] Moreover insulin is the specific marker of the pancreatic islet endocrine β cells. Oral, intranasal and parenteral administration of insulin or insulin-derived dominant auto-antigens have been shown to inhibit the occurrence of diabetes in animal models of type 1 diabetes.[76,77] However, one cannot exclude the risk of priming or triggering autoimmunity by peripheral administration of an auto-antigen.[78] Reprogramming self-tolerance that has not been installed or that is broken in autoimmune diseases is a very rational strategy for the prevention of devastating diseases such as multiple sclerosis, rheumatoid arthritis or type 1 diabetes. Such reprogramming could be based upon the tolerogenic properties of the thymic epithelium. Instead of the classic immunogenic vaccination (with immune activation and induction of memory/immunocompetent cells), the novel form of tolerogenic vaccination (or negative vaccine, so to use the phrase proposed by Nossal)[79] should provoke the deletion or the anergy of auto-reactive T lymphocytes (FIG. 1). The induction of T-cell tolerance following peptide vaccination has already been obtained with synthetic peptides representing cytotoxic CD8 epitopes of T cells oriented against tumor antigens or viruses.[80] According to Claude Bernard's principles of experimental medicine, the hope appears now that the correction of the defective central self-tolerance could prevent the appearance of autoimmune type 1 diabetes. The tolerogenic vaccination represents a very attractive strategy for preventing autoimmune diseases, the heavy tribute paid by the humans for the efficiency and complexity of their system of immune defenses.

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