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At the Cutting Edge

Biosynthesis and paracrine/cryptocrine actions of 'self' neurohypophysial-related peptides in the thymus

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The observation by different groups of the thymic expression of neuropeptide-related genes has engendered considerable interest for two reasons. First, it supports the concept of an intimate dialogue between the neuroendocrine and immune systems at the level of early T-cell differentiation [1–5]. Secondly, it allows the possibility of novel strategies for immunomodulation by the development of specific neuropeptide agonists or antagonists. Though important questions remain to be solved, particularly of the precise biochemical identity of neurohypophysial-related peptides in the thymus from different species, their resolution will clearly play a key part in the current reappraisal of classical endocrinology.

Background

Two groups independently and simultaneously reported the coexistence of immunoreactive (ir) oxytocin (OT) and ir-neurophysin (NP) in the human thymus [6], and the presence of ir-vasopressin (VP) in rat and mouse thymus [7]. Soon afterwards, additional evidence in favour of intrathymic synthesis of neurohypophysial-related peptides was provided by positive dot blot hybridization of human thymic mRNA preparations with bovine OT and VP cDNA probes [8,9]. In addition, specific immunostaining with antisera against OT, VP and NP was found for epithelial cells in human thymic subcapsular cortex (SCC) and medulla [9,10].

One epithelial cell population derived from the SCC and the outer cortex, the thymic 'nurse' cells (TNCs), were found to contain ir-OT, -VP, -NP, and to express an immunophenotype similar to that found in various components of the diffuse neuroendocrine system [11]. As recently pointed out [12], TNCs are functionally related to testicular Sertoli cells, inasmuch as they are large epithelial cells in very close contact with developing elements (T-cells in TNCs, spermatids in Sertoli cells). The concept of 'cryptocrine' [12] was thus suggested to characterize the possibility of closed intercellular signalling in a specialized and possibly privileged microenvironment.

If this is the case, then neurohypophysial-related peptides might serve as chemical vectors of such cryptocrine information between TNCs and Sertoli cells and the cells they surround (lymphocytes, spermatids). The presence of oxytocin with both immunological and biological activity has been recently confirmed in the rat thymus [13,14]

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and in the human fetal thymus [15]. Secondly, rat thymic ir-OT concentrations have been observed to rise after lesions of the hypothalamic paraventricular nuclei or after various hormonal manipulations, further supporting a local intrathymic synthesis of an OT-like peptide.

Since neurohypophysial-related signals have been detected in the thymic microenvironment, it seemed logical to look for the expression of appropriate receptors by immature T-cells. Indeed, OT receptors have been detected in rat thymic membrane preparations [16] and on rat thymocytes [17]. In addition, VP receptors of the V_1 subtype have been described in an immature thymic lymphoma-derived murine T-cell line, RL12-NP [18]. Evidence for the functional integrity of these receptors was provided by the ability of various neurohypophysial-like peptides in RL12-NP cells to induce the breakdown of membrane phosphoinositides, and to increase cytoplasmic inositol trisphosphate, both of which were inhibited by a V_1 antagonist (Martens et al., submitted).

The involvement of neurohypophysial-related signals and receptors in T-cell differentiation is further supported by the reported mitogenic actions of these peptides upon different cell types, including rat bone marrow cells and thymocytes. The hydrolysis of phosphoinositides by receptors coupled to phospholipase C seems commonly to mediate mitogenic signals, as shown for the cloned 5-HT_{1c} and *mas* oncogene/angiotensin receptors [19]. Neurohypophysial-related peptides were also found to exert metabolic activities analogous to insulin upon adipocytes and thymocytes [20], and similar to interleukin-2, the T-cell growth factor, on cytotoxic splenocytes; this latter action appears to be mediated by a novel V_1-type VP receptor [21]. These actions, taken with the demonstrated involvement of neurohypophysial peptides in early cell activation [22], may even support the possibility that thymic OT and VP are implicated in the development of lymphoproliferative disorders [18]. Though a role for VP-like peptides in brain development and plasticity has been previously suggested [23], specific actions of VP/OT-like peptides in T-cell ontogeny, for example thymic T-cell positive and negative selection processes, have not yet been described.

The questions

When human thymus extracts are subjected to repeat Sep-pak purifications, we found concentrations of thymic ir-OT between 1.65 and 4.5 ng/g wet weight, and of ir-VP between 0.01 and 0.06 ng/g; total thymic ir-NPs ranged from 68 to 256 ng/g, and thymic ir-hNPI (human 'VP-associated' NP) from 34 to 90 ng/g (Geenen et al., unpublished observations). The molar ratio ir-OT + ir-VP/total ir-NP is thus different from the 1/1 observed in the hypothalamus, a discrepancy previously suggested to be the consequence of differences in extraction methodologies.

The complete characterization of human thymic neurohypophysial-related mRNAs is still lacking. Preliminary Northern blots with human VP or OT cDNA failed to reveal a signal intensity above background (Schmale, personal communication), or any evidence for a hybridization product smaller than hypothalamic OT mRNA [24].

More recently, we have produced and characterized monoclonal antibodies (Mabs) to OT [25]. One of them, O13, is very specific for OT, with the responsible epitope seemed to be located in the C-terminal tripeptide. Two others, O22 and O33, are probably directed against tyrosine-containing epitope(s) shared by VP and other members of the neurohypophysial peptide family. Using Mabs to OT and to VP, we have shown that stromal cells immunostain in the SCC and in the medulla of the human thymus. These cells are a subset of epithelial (cytokeratin$^+$) neuroendocrine cells (A2B5$^+$, NP$^+$), and also contain ir-interleukin-1 [26]. There are, however, two ways in which the immunostaining differs from that in the hypothalamus. First, OT and VP immunoreactivity co-localizes in the same cells; and, secondly, Mab O13 — which is highly OT-specific — does not label thymic epithelial cells.

It is formally possible that what we are observing is cross-reaction with epitopes expressed by thymic epithelial cytokeratins; however, we believe that this probability is extremely low, given the coexistence in the same cells of immunoreactivity characterizing various members of the neurohypophysial peptide superfamily (polyclonal antisera against neurophysins and neurohypophysial peptides; Mabs O22, O33 against OT, and BER-

312 against VP) [26]. On the other hand, these observations clearly demonstrate molecular differences between hypothalamic and thymic OT, a difference which clearly needs to be further investigated.

OT- and VP-related peptides have been biochemically or immunologically described in a range of peripheral organs [27], even in the Brattleboro rat which exhibits a defect in the expression of the hypothalamic VP precursor gene. However, high levels of peripheral expression of such a neurohypophysial gene are found only in the ruminant corpus luteum [23], where OT is synthesized, and secreted, and functions as a true hormone, although with differential and tissue-specific regulation of expression. The hypothesis of variant or related genes for OT/VP-like peptides, preferentially expressed in peripheral tissues, has been advanced previously [27] and may be worthy of reconsideration given our present findings. This hypothesis received additional recent support with the observation of a neurophysin-related precursor in cell membranes of a small-cell lung carcinoma [28].

In the neurohypophysial gene family, vasotocin (VT) represents a good candidate as such a variant for different reasons. VT is a structural hybrid molecule formed by the OT hexapeptide cyclic moiety and the VP C-terminal tripeptide chain. Plausibly, a VT-precursor may represent the ancestral protein from which OT-and VP-precursors have emerged by gene duplication. Both bioactive and ir-VT have been detected in the pineal gland, and in fetal pituitary in different mammalian species [29–31]. Even though VT gene expression in mammals has not yet been reported, ir-VT has been described and carefully characterized by HPLC in ovine fetal blood, urine, and amniotic fluid [32], in human newborn plasma [33], as well as in ovine fetal thymic organs [34].

The polyclonal antiserum used in our first report on thymic OT displayed significant cross-reactivity with synthetic VT [35]. A more precise RP-HPLC procedure was reported to discriminate poorly between rat testicular and thymic ir-VP and synthetic VT [27]; these authors suggested that the VP/VT-like peptide may be neither peptide per se. Moreover, if VT were expressed in the human thymus, we would have an explanation why Mab O33, with an epitope in the cyclic region shared by OT and VT, labels some thymic epithelial cells, while Mab O13 does not.

There are also recent observations of considerable potential significance from comparative studies of neurohypophysial gene molecular evolution. The existence of two genes, coding for VT precursors (VT-1 and VT-2 types) and containing an NP-like sequence was recently described for teleost fish [36], suggesting a tetraploid hypothesis for the evolution of VP and OT. To complicate matters even futher, however, the authors pointed out that preliminary data support the existence of at least two other genes for VT-1 type precursors. There are thus several lines of evidence that VT may constitute a potential candidate as the member of the neurohypophysial gene family expressed in many peripheral organs; clearly, further biochemical and molecular biological studies are however needed to identify the factor beyond doubt.

Conclusions and perspectives

In terms of the predominant role of the thymus in human fetal life for the fundamental processes of the induction of self-tolerance and shaping of the T-cell repertoire, it seems plausible that the genes involved in peptidergic control of T-cell differentiation may be mainly expressed during embryonic life. We are thus currently investigating the hypothesis that preferential fetal expression of some hitherto unidentified VP/OT-related gene(s) intervenes in this closed endocrine–immune dialogue and contributes in this way to the process of T-cell differentiation.

Given the potential role of 'self' peptides [37] in the induction of immune tolerance, preferential synthesis and presentation of an OT-like peptide by thymic epithelium may also provide an explanation of the observation that whereas autoimmune processes have been implicated in some idiopathic forms of central diabetes insipidus (autoimmune 'hypothalamitis') [38], a similar mechanism has never been described against OT or OT-producing neurones. In addition, such a mechanism may contribute to fetal tolerance which is seen until the very late stages of pregnancy, and may also explain the known difficulty in raising antibodies of high titers against OT.

Over a decade ago, a variety of findings were reported supporting the concept of the thymus as a classical endocrine gland [39–41]. The next critical step in our understanding of T-lymphocyte differentiation may lie in the identification of 'self' peptides expressed in the thymic stroma, as well as the elucidation of their potential dual role in T-cell positive selection (through paracrine/cryptocrine signalling) and negatively, in the induction of tolerance. This latter may follow a full or partial presentation of epitopic 'self' peptide fragments in the groove of major histocompatibility complex (MHC) proteins on thymus epithelial cells, and a subsequent very high affinity interaction with a randomly rearranged T-cell receptor, thus offering a cognate configuration for MHC/'self' peptide association. If this is the case, a disturbance in this tolerogenic molecular mechanism may lead to autoimmune pathology.

We are well aware that this hypothesis is so far largely untested. On the other hand, we believe it to be worth consideration in the context of the contemporary scientific debate about the dual physiological role of thymic epithelium (including TNCs) in tolerogenicity and T-cell repertoire positive selection [42,43]. Importantly, to the extent to which they have been tested, the molecular and cellular mechanisms proposed are logically coherent and consistent with the empirical data available.

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