Programing of the Autoimmune Diabetogenic Response in the Thymus during Fetal and Perinatal Life

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Abstract

The presentation of self-peptides in the thymus is responsible both for negative selection of self-reactive T cells emerging during stochastic TCR recombination in fetal life, as well as positive selection of self-specific regulatory thymic T (tTreg) cells during and after perinatal life. The combination of these two sequential processes programs central self-tolerance, a fundamental property of the adaptive immune system. A defect in intrathymic self-presentation, either genetic or acquired, is the earliest event in the pathogenesis of autoimmunity already during fetal development. This defect is necessary but not sufficient for the appearance of a classical autoimmune disease like type 1 diabetes (T1D). Environmental factors are required for activation of the diabetogenic autoimmune response that targets insulin-secreting B cells in pancreatic Langerhans’ islets. Based on epidemiological studies, viral infections have been suspected for a long time to be one of those environmental factors. In this Debate article, we present a series of experimental data that support the hypothesis that, following vertical transplacental transfer, viruses might infect the fetal thymus and disturb already in utero central self-tolerance orchestrated by this organ.

“It is with logic that we prove but it is with intuition that we discover.”

Henri Poincaré (1854-1912)

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Central Self-Tolerance of Neuroendocrine Functions

Following his formulation of the clonal selection theory, Frank Macfarlane Burnet already hypothesized that the thymus could be the site for the deletion of T-cell clones with reactivity to self and that such ‘forbidden’ T lymphocytes could play an essential role in autoimmunity (1). At the end of 80s, several groups independently demonstrated clonal deletion...
Type 1 Diabetes Development in the Fetal Thymus

of self-reactive T cells in the thymus (2-5), and the true biochemical nature of self-antigens encountered in this organ was then immediately questioned. In 1986, the hypothalamic neuropeptide Oxytocin (OT) was shown to be synthesized in thymic epithelial cells (TECs), including thymic ‘nurse’ cells (TNCs), from different species (6-8). Although functional specific neurohypophysial receptors are expressed by distinct thymic T-cell subsets (9,10), it is noteworthy that thymic OT is not secreted by TECs but that OT precursor is processed for antigen presentation by TEC major histocompatibility complex (MHC) proteins. This is also the case for the neurotensin (NT) precursor (11,12). Quite economically, TECs synthesize one dominant member per neuroendocrine family: OT for neurohypophysial peptides, NT for neuremendins, cortistatin for somatostatins, and insulin-like growth factor 2 (IGF-2) for the insulin family. Thus, the biochemical identity of neuroendocrine self can be defined as follows (see 13 for complete review):

- A hierarchy characterizes the profile of expression of neuroendocrine-related peptides in thymus epithelium: OT > Vasopressin (VP), IGF-2 > IGF-1 > Insulin. This hierarchy is very important since tolerance to a peptide is directly proportional to its concentration in the thymus (14).

- The dominant neuroendocrine self-peptide precursor includes sequences that are highly conserved during evolution of its family.

- A neuroendocrine self-peptide is important for individual (IGF-2) and species (OT) preservation.

- Some specific epigenetic regulation of their expression in the thymus may exist (for example, absence of parental imprinting of Igf2 in the thymus).

- And most importantly, neuroendocrine self-peptide precursors are not processed for classic secretion but for antigen presentation by thymic MHC proteins.

Thymus and Autoimmunity

In the early 2000s, the Heidelberg group of the late Bruno Kyewski reported the promiscuous gene expression (PGE) of tissue-restricted antigens (TRAs) in medullary (m) TECs and this mechanism was proposed to be a crucial key for our common knowledge of central immune self-tolerance and autoimmunity (15,16). However, contrary to neuroendocrine self-peptides, thymic TRAs do not behave as accessory signals binding to cognate receptors during T-cell differentiation, and PGE is a unique property of mTECs while neuroendocrine self-peptides are synthesized in cortical (c) and mTECs. The elegant studies performed by the Kyewski laboratory played an important role for the recognition by immunologists of the importance of thymus-dependent central self-tolerance (17-19). Moreover, an increasing number of studies pointed out a defect in the establishment of central self-tolerance as the earliest event in the programming of organ-specific autoimmune diseases (20,21). For example, Igf2 transcription is defective in the thymus of diabetes-prone BioBreeding rat, an animal model of human T1D (22).

This novel view of the thymus as the primary rampart against autoimmunity culminated with the identification by positional cloning of the Autoimmune Regulator (AIRE) gene (23). Autoimmune polyendocrinopathy candidiasis and ectodermal dystrophy (APECED) or Autoimmune Polyglandular Syndrome-1 (APS-1) is an autosomal recessive monogenic autoimmune disease affecting children already in very early life. The three major symptoms are hypoparathyroidism, adrenal deficiency and chronic mucocutaneous candidiasis. Other endocrine autoimmune diseases may be associated such as type 1 diabetes, vitiligo, autoimmune thyroiditis, premature gonadal failure, alopecia, pernicious anemia. A defect in negative selection of autoreactive T cells was demonstrated in Aire−/− mice (24). These mice have a phenotype much less severe than APECED children but they also develop autoantibodies and lymphocytes infiltrates in peripheral organs (25). Most importantly, this autoimmune phenotype was linked to a marked decrease in intrathymic transcription of several TRAs and neuroendocrine self-peptides such as OT, IGF-2 and neuropeptide Y (25). The molecular actions of AIRE have been investigated in a huge number of following studies but our current knowledge in this field is out of the scope of this debate article. It is nevertheless important to mention that another factor, the fasciculation and elongation protein zeta family zinc finger-2 protein, FEZF2, also intervenes as another transcriptional modulator of the intrathymic expression of different neuroendocrine self-peptides and TRAs (26,27).

These studies have also important implications from the evolutionary point of view. Since their appearance in early forms of life, the neuroendocrine and innate immune systems coevolve without any sign of neuroendocrine autotoxicity/autoimmunity. Some 500 millions years ago, the emergence in sharks and rays of two transposon-like recombination-activating genes was responsible for the development of the adaptive immune system and its progressive inherent risk of autoimmunity. The appearance of the adaptive immune system exerted an evolutionary pressure so potent that, according to Paul Ehrlich’s prediction of ‘horror autotoxicus’, a unique thymus also appeared in sharks and rays with the specific role of orchestrating immune self-tolerance. Through transcription of dominant neuroendocrine representative precursors in TECs, the thymus so ensured a harmonious and peaceful coevolution of the neuroendocrine system and adaptive immunity. Far from being only the lymphoid organ responsible for T-cell development as long thought, the thymus now appears first as a cemetery for developing T cells; it has been estimated
that, from 100 hundred T-cell progenitors migrating through
the thymus, only 3% are leaving this organ (28) either as
self-tolerant and competent T cells against foreign non self-
antisgens or, mainly after birth, as self-reactive Treg cells that
inactivate peripheral forbidden self-reactive T-cell clones
having fortuitously escaped negative selection in the thymus.
This novel knowledge on the unique and essential role of
thymus-dependent central self-tolerance is currently paving
the way for the development of innovative treatments against
autoimmune diseases such as the inverse self-vaccination
against T1D which aims at restoring T-cell tolerance to islet B
cells using T1D-related self-antigens (29,30).

**Viral Etiology of T1D**

Intrathymic programing of an autoimmune response is
necessary but not sufficient for the appearance of a clinical
autoimmune disease and several strong arguments favor
the intervention of environmental factors for the initiation of
this type of chronic disease. The concordance index of T1D
in monozygotic twins is less than 50%, and there is an impressive
North-South gradient in T1D incidence with a maximum in
Scandinavian countries. The environmental agents that are
suspected include viral infections, anti-tumor immunotherapy,
endocrine disruptors and sex steroids, nutrition and vitamin
D deficiency, gut microbiota, and even stressful events (in
particular for autoimmune thyroiditis).

The viral etiology of T1D has been nicely summarized in
a recent review (31) and the question of an in-utero viral
infection in T1D pathogenesis has already been proposed (32).
Since more than 20 years, we have been investigating the
hyposis that an infection of the thymus by the entrovirus
coxsackie B4 (CV-B4) could interfere with the establishment of
central self-tolerance to islet B cells.

There are various mechanisms by which CV-B4 can contribute
to T1D development through interplay between the virus,
and innate and adaptive immunity (33-35). CV-B4 can infect
pancreatic cells, especially islet B cells and ductal cells and
CV-B4 infection persists in these cells as reported by our
team (35-37). CV-B4 can infect monocytes and macrophages,
resulting in the production of interferon alpha (IFN-α) and
inflammatory cytokines that play a role in activating an
autoimmune response against B cells (38-42). The infection of
monocytes with CV-B4 relies on enhancing antibodies, which
increase the viral load in the host and the pathogenic effect of
the virus (43-47).

**Thymus Dysfunction Induced by CV-B4**

As reminded above, CV-B4 targets B cells, and this is most
probably involved in T1D pathogenesis. However, the
development of this autoimmune disease needs the activation
of preexisting T lymphocytes directed towards B cell antigens.
Therefore, it was hypothesized that CV-B4 could disturb the
tolerance to B cells at a central level.

It was decided to investigate first whether CV-B4 is able to
infect human TECs. It was observed that CV-B4 persisted in
primary cultures of human TECs, which resulted in an increase
in the secretion of the cytokines IL-6, LIF, and GM-CSF in
the supernants (48). In a second step, CV-B4 E2 was able
to infect human fetal thymic organ cultures (FTOC). Double
positive CD4+CD8+ thymocytes were the principal target cells
of infection and were progressively depleted. MHC class I
expression by thymocytes was also markedly upregulated
by CV-B4 infection (49) but this was not dependent on IFNa,
which is known to be a major player in T1D pathogenesis (50).

Then, the infection of thymus with CV-B4 in an in-vivo model
was developed. Outbred mice (Swiss mice) were inoculated
with CV-B4 p.o. Viral RNA was detected in thymus, spleen and
blood up to 70 days after inoculation (51). These observations
prompted us to study further the impact of CV-B4 on mouse
thymus. It was shown that the diabetogenic CV-B4 E2 strain
disturbs T-cell differentiation in murine FTOC (52). The next
purpose was to investigate the result of a persistent infection
of TEC with CV-B4 on genes of the insulin family using a
murine TEC line. This cell line expressed IGF2 and IGF1 but
not insulin. A persistent infection with CV-B4 of this TEC line
resulted in a dramatic decrease in Igf2 transcription and
IGF-2 production in long-term cultures, while Igf1 transcripts
were much less affected (53). This is noteworthy since IGF-2
is the self-peptide of the insulin family involved in central
tolerance of the insulin family (see above). Thus an impaired
expression of thymic Igf2 might result in a defect of negative
selection of self-reactive thymocytes as well as a decrease in
the generation of self-reactive Treg cells. The combination
of these two mechanisms so would promote an autoimmune
attack against B cells (54). Molecular pathways responsible
for the CV-B4-induced decrease of thymic Igf2 expression in
a murine TEC line are under current investigation (55).

Since the thymus negative selection already operates
during fetal life, we investigated whether CV-B4 E2 can
reach the fetal thymus during the course of an in-utero
infection. Pregnant Swiss albino mice were inoculated with
the diabetogenic CV-B4 E2 strain. In-utero CV-B4 E2 infection
of offspring thymus was shown by detection of viral RNA and
infectious virus in the organ (56). Moreover, CV-B4
infection of the fetal thymus induced significant changes in
the percentage of thymic T-cell subsets as analyzed by
flow cytometry. Infection at 10 days of gestation blocked
the double negative (DN) to double positive (DP) transition
and then the DP to single positive (SP) differentiation, in
particular to SP8 (56). In this murine model, it thus appears
that CV-B4 infection during pregnancy can promote a
disturbance of T-cell differentiation. Whether CV-B4 infection on fetal thymus may impact the central development of autoimmune is currently investigated in our laboratories. Another consequence of the thymus infection by CV-B4 might be the progressive induction of immune tolerance towards this virus, which will render CV-B4 more virulent and cytotoxic towards islet B cells.

Conclusions (table 1)

An increasing number of studies support the idea that a dysfunction of the thymus programs the development of different organ-specific autoimmune responses such as the childhood autoimmune T1D. Such dysfunction is either genetic (AIRE and FEZF2 mutations) or acquired following thymus viral infection. Besides T1D, it may now be assumed that the majority of organ-specific autoimmune diseases are determined during fetal and perinatal period although the intervention of environmental factors is required later in life for the clinical appearance of such diseases.

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Table 1. Thymus dysfunction in the development of organ-specific autoimmune response

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<th>Thymus physiology</th>
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<td>• Negative selection of self-reactive T cells (already in fetal life)</td>
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<td>• Positive selection of self-reactive TReg cells (starting from perinatal period)</td>
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<th>Defect of thymus-dependent central tolerance during fetal and perinatal life</th>
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<td>Viral infection of the thymus</td>
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<td>• Release of ‘forbidden’ self-reactive T-cell clones (already in fetal life)</td>
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<td>• Decrease in positive selection of self-reactive TReg cells (starting from perinatal period)</td>
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<th>Bridge between self-reactive T cells and peripheral target antigens</th>
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<td>Role of environmental factors (viruses, anti-tumor immunotherapy, endocrine disruptors and sex steroids, gut microbiota, diet, vitamin D deficiency, stress...)</td>
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Disclosure

The authors Vincent Geenen and Didier Hober have nothing to disclose.

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