The central role of the thymus in the programming of immunological self-tolerance to neuroendocrine self: Implications for the pathogenesis of autoimmune diseases

Vincent Geenen
Research director of F.S.R.-NFSR of Belgium

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The moving place of the thymus in the history of medicine

Claude Galen – 2nd father of Western medicine (129 – 210 AD)

Thymos (Θυμός) = physical association between breath and blood (soul, energy and courage).
Refers to the spirited part of Plato’s three constituents of psyche (with logical and appetitive).
‘Troubles thymiques’ in French medical language = mood disorders, i.e. bipolar and unipolar depression.

“The new views as to the morphology of the thymus gland and their bearing on the problem of the function of the thymus”
J August Hammar Endocrinology (1921) 5:43-73

Jacques FAP Miller
‘Central’ self-tolerance induction in the thymus
Ohki H, Martin C, Corbel C, Coltey M & Le Douarin NM Science 1987
Kappler JW, Roehm N & Marrack P Cell 1987

Transgene

Normal thymus
A few scattered apoptotic cells

Thymus + specific peptide
Widespread apoptosis, many apoptotic cells
T-cell differentiation in the thymus

1. Negative selection of self-reactive T cells during fetal life
2. Generation of self-specific tTreg cells early after birth
Thymic neuropeptides: Organization of the repertoire

<table>
<thead>
<tr>
<th>FAMILY</th>
<th>THYMIC NEUROPEPTIDES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurohypophysial</td>
<td>Oxytocin / OT</td>
</tr>
<tr>
<td>family</td>
<td>(&gt;&gt; Vasopressin / VP)</td>
</tr>
<tr>
<td>Neuromedins</td>
<td>Neurotensin / NT</td>
</tr>
<tr>
<td>Tachykinin family</td>
<td>Neurokinin A</td>
</tr>
<tr>
<td>Natriuretic peptide</td>
<td>ANP</td>
</tr>
<tr>
<td>family</td>
<td></td>
</tr>
<tr>
<td>Somatostatin family</td>
<td>Cortistatin</td>
</tr>
<tr>
<td>Insulin family</td>
<td>IGF-2</td>
</tr>
<tr>
<td></td>
<td>(&gt; IGF-1 &gt; Insulin)</td>
</tr>
</tbody>
</table>

OT cryptocrine secretion in human TEC

Focal adhesions
Immune synapses?
MHC-I presentation of neurotensin by human TEC

Neurotensin (NT)  =  Glu - Leu - Tyr - Glu - Asn - Lys - Pro - Arg - Arg - Pro - Tyr - Ile - Leu

=  ELYENKPRRPYIL
A paradigm shift: from thymic neuropeptides to ‘neuroendocrine self-peptides’

- Dominant member of a neuroendocrine gene family expressed in the thymus.
- Highly conserved sequences throughout evolution of a family.
- Intrathymic transcription before expression in orthotopic tissues (*i.e.* OT).
- Importance for species preservation (*OT > VP*).
- **NO SECRETION** but processing through MHC pathways for antigen presentation.
The dual role of neuroendocrine self-antigen precursors
Integrated coevolution of the immune and neuroendocrine systems

- **Invertebrates**
- **Protochordates**
  - **Jawless vertebrates** (lamprey)
    - VLR
    - Ancestral forms of anticipatory immunity
  - **Jawed vertebrates** (shark, ray)
    - RAG-mediated adaptive immunity
      - RAG1 and RAG2
      - TCRα – TCRβ – TCRγ - TCRδ
      - IgV germline diversity
      - Somatic hypermutation
      - Polymorphic MHC

- Thymoids (Foxn4) → First unique thymus (Foxn1)

- Innate immunity (TLR)
- Neuroendocrine system

≈ 500-450 Million years
Intrathymic expression of tissue-restricted antigens

Nature Reviews | Immunology

Kyewski B et al. (2004)
The Origin of Organ/Cell-Specific Autoimmunity:
A Thymus Defect in Programming Self-Tolerance?
Ontogeny of gene expression in Balb/c thymus

Transcription of \textit{Insulin}-related genes in the thymus of BB rats

Kecha-Kamoun O et al., \textit{Diabetes Metabolism Research} (2001)
APS-I or APECED syndrome

- Very rare monogenic autosomal recessive disease (AI polyendocrinopathy)
- AIRE identified on 21q22.3 (positional cloning)
- 14 exons, transcription factor of 545 aa, > 45 mutations
- Maximal transcription in **thymic epithelium**

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### Table: Gene Expression Analysis

<table>
<thead>
<tr>
<th>Probe name</th>
<th>Gene name</th>
<th>Tissue(s)</th>
<th>WT signal</th>
<th>KO signal</th>
<th>KO/WT</th>
<th>t-test p-value</th>
<th>FPR Quad</th>
<th>FPR SAM</th>
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<tbody>
<tr>
<td>66330_at</td>
<td>casin alpha</td>
<td>mammary</td>
<td>75.82</td>
<td>1</td>
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<td>0.0417</td>
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<td>97180_f_at</td>
<td>hemoglobin y, beta-like embryonic chain</td>
<td>fetal erythrocytes</td>
<td>87.36</td>
<td>1.29</td>
<td>0.016</td>
<td>0.0803</td>
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<td>0.014</td>
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<tr>
<td>100100_at</td>
<td>intestinal trefoil factor</td>
<td>intestinal goblet cells</td>
<td>74.59</td>
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<td>0.027</td>
<td>0.5064</td>
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<td>101620_at</td>
<td>neurotoxin homologue</td>
<td>granulocytes, monocytes</td>
<td>29.50</td>
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<td>0.034</td>
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<td>94738_s_at</td>
<td>cryptdin-related sequence 2</td>
<td>Paneth cells</td>
<td>100.47</td>
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<td>0.2561</td>
<td>0.164</td>
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<tr>
<td>101682_f_at</td>
<td>major urinary protein IV</td>
<td>lacrimal gland, parotid gland</td>
<td>26.49</td>
<td>1.02</td>
<td>0.045</td>
<td>0.0392</td>
<td>0.063</td>
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<td>94153_g_at</td>
<td>salivary protein 1</td>
<td>salivary gland</td>
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<td>0.0712</td>
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<td>102986_at</td>
<td>cytochrome P450 1a2</td>
<td>liver, lung, duodenum</td>
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<td>0.048</td>
<td>0.0688</td>
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<td>101115_at</td>
<td>lactotransferrin</td>
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<td>19.78</td>
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<td>0.3230</td>
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<td>92553_at</td>
<td>sense protein (BSP1)</td>
<td>hair follicles, brain</td>
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<td>0.053</td>
<td>0.1270</td>
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<tr>
<td>100463_at</td>
<td>gamma-casemorphin precursor</td>
<td>mammary gland</td>
<td>21.63</td>
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<td>92546_r_at</td>
<td>prostaglandin D</td>
<td>brain, epidermis</td>
<td>22.62</td>
<td>1.26</td>
<td>0.051</td>
<td>0.0603</td>
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<td>0.014</td>
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<td>96153_at</td>
<td>neutrophilic granule</td>
<td>granulocytes</td>
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<td>Funicular protein 4</td>
<td>brain, eye (iris)</td>
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<td>0.0327</td>
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<td>161815_f_at</td>
<td>major urinary protein I</td>
<td>liver</td>
<td>31.23</td>
<td>2.04</td>
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<td>0.0704</td>
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<tr>
<td>89858_at</td>
<td>glucose dependent insulinotropic polypeptide</td>
<td>X cells of small intestine</td>
<td>27.16</td>
<td>1.78</td>
<td>0.066</td>
<td>0.5517</td>
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<td>101919_f_at</td>
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<td>liver</td>
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<td>0.0329</td>
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<td>brain</td>
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<td>1.92</td>
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<td>101563_at</td>
<td>salivary protein 2</td>
<td>salivary gland</td>
<td>16.80</td>
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<tr>
<td>98623_g_at</td>
<td>insulin-like growth factor II</td>
<td>embryo, choroid plexus and rhombencephalon in adult</td>
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<td>6.96</td>
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<td>mast cells</td>
<td>13.70</td>
<td>1.01</td>
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<td>94707_s_at</td>
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<td>ameloblast cells</td>
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<td>0.074</td>
<td>0.0328</td>
<td>&lt; 0.02</td>
<td>0.014</td>
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<tr>
<td>103335_at</td>
<td>pregnaneyneuropetide y</td>
<td>brain</td>
<td>19.54</td>
<td>1.47</td>
<td>0.075</td>
<td>0.0513</td>
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<td>103387_at</td>
<td>S100 calcium binding protein A9</td>
<td>immature B/M myeloid cells, monocytes, neutrophils</td>
<td>66.93</td>
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<td>162341_r_at</td>
<td>aldose reductase</td>
<td>many</td>
<td>19.37</td>
<td>1.48</td>
<td>0.076</td>
<td>0.0121</td>
<td>0.279</td>
<td>-</td>
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<td>97869_at</td>
<td>fatty acid binding protein</td>
<td>intestine</td>
<td>37.48</td>
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<td>94045_at</td>
<td>o-1-microglobulin/bikunin precursor</td>
<td>liver</td>
<td>12.74</td>
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<td>0.279</td>
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<tr>
<td>100150_f_at</td>
<td>preproinsulin II</td>
<td>pancreatic islet beta cells</td>
<td>19.70</td>
<td>1.62</td>
<td>0.063</td>
<td>0.1962</td>
<td>&lt; 0.02</td>
<td>0.014</td>
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<tr>
<td>100002_at</td>
<td>inter-alpha-inhibitor H3 chain</td>
<td>liver, brain</td>
<td>12.07</td>
<td>1</td>
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<td>0.0266</td>
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<td>88830_at</td>
<td>spermine binding protein</td>
<td>prostate</td>
<td>13.56</td>
<td>1.13</td>
<td>0.083</td>
<td>0.1047</td>
<td>0.131</td>
<td>-</td>
</tr>
</tbody>
</table>

Contribution of Igf2 expression to immunological tolerance toward INS

**Anti-IGF-2 humoral response (%)**

- Igf2+/+ mice
- Igf2−/− mice

N = 7
** : p < 0.01
Dilution : 1/500

**Anti-Insulin humoral response (%)**

- Igf2+/+ mice
- Igf2−/− mice

N = 6/8
*: p<0.05
Dilution 1/100

Specific deletion of *Igf2* in thymic epithelium – Development of *Igf2*\(^{Δ\text{Thy}}\) mouse

(Pr. M. Constancia, University of Cambridge)

(Pr. G. Holländer, University of Basel)

![Diagram showing the process of specific deletion of *Igf2* in thymic epithelium](image)

**IGF-2/IGFBP-3**

<table>
<thead>
<tr>
<th>Condition</th>
<th>IGF-2/IGFBP-3 (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CET WT</td>
<td>0.005</td>
</tr>
<tr>
<td>CET KO</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Thymus *Igf2*\(^{fl/fl}\) 20 μm

Thymus *Igf2*\(^{Δ\text{Thy}}\) 20 μm
• Despite its ubiquitous expression, *Igf2* deletion in the sole thymus leads to loss of tolerance toward IGF2.

• *Igf2* deletion in the sole thymus also lowers the level of immunological tolerance toward INS (central cross-tolerance between IGF2 and INS).
Humoral response to IGF-2 in T1D patients

**Method**
Specific and sensitive radio-binding assay using $^{125}$I-IGF-2

**Quantification**
Standard curve of a monoclonal antibody anti-human IGF-2 (CBL82)

A thymus defect in autoimmune neuroendocrine diseases

Thymus physiology
- AIRE-regulated transcription of neuroendocrine self-peptides in thymus epithelium.
- Deletion of T cells with high affinity for MHC/neuroendocrine self-peptide complexes.
- Selection of CD4+ CD25+ Foxp3+ tTreg, specific of neuroendocrine self-peptides.

Thymus physiopathology
- Absence or decrease in expression/presentation of neuroendocrine self-peptides in the thymus (APECED/APS-1, Graves' disease, Down syndrome, BB rat, etc.)
- Enrichment of T-cell repertoire with ‘forbidden’ self-reactive effector T cells (Teff).
- Decrease in selection of tTreg with specificity to neuroendocrine self-peptides.

Bridge between self-reactive Teff and target auto-antigens
- Role of environmental factors (viruses, diet, vitamin D deficiency, stress...)

The Role of Environment in T1D Pathogenesis
Coxsackievirus CVB4, thymus and T1D pathogenesis

Background

- Coxsackievirus B4 infection of murine fetal thymus organ cultures. 

- Persistent infection of human thymic epithelial cells by Coxsackievirus B4. 

- Coxsackievirus B4 infection of human fetal thymus cells. 

- Prolonged viral RNA detection in blood and lymphoid tissues from Coxsackievirus B4 orally-inoculated Swiss mice. 

Question: Does thymus infection by CVB4 interfere with programming of central self-tolerance toward insulin family?
Igf2 transcription and IGF-2 synthesis in a murine mTEC line

Conclusions

• The presentation of neuroendocrine self-peptides in the thymus ensured an integrated and harmonious evolution between the neuroendocrine and adaptive immune systems.

• A thymus dysfunction in programming central self-tolerance plays a primary role in the development of a specific autoimmune response directed against neuroendocrine organ/cell-restricted antigens.

• Resulting from this thymus defect, repertoire enrichment with self-reactive T cells and depletion of self-specific tTreg cells is a condition necessary but not sufficient for appearance of autoimmune endocrine diseases.
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