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

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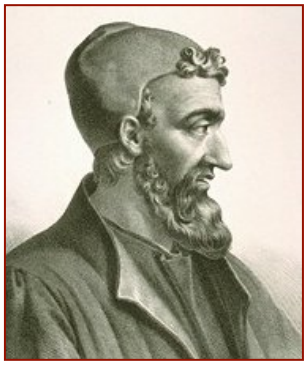
Research director of F.S.R.-NFSR of Belgium

International Symposium – Saint-Petersburg
23-26 June 2015

The moving place of the thymus in the history of medicine

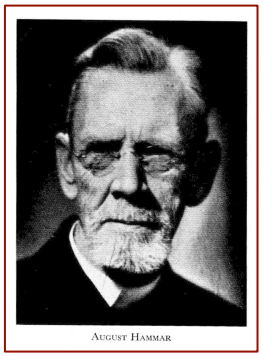


Claude Galen – 2nd father of Western medicine (129 – 210 AD)

Thymos (Θψμοσ) = physical association between breath and blood (soul, energy and courage).

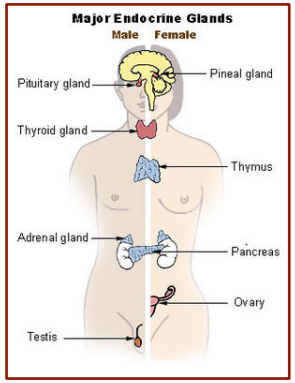
Refers to the spirited part of Plato's three constituents of psyche (with logical and appetitive).

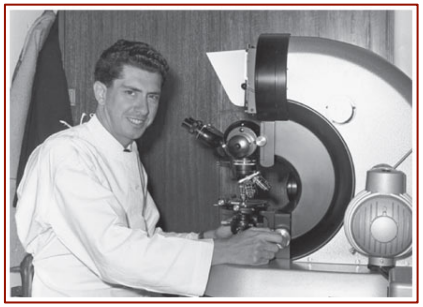
'Troubles thymiques' in French medical language
= mood disorders, *i.e.* bipolar and unipolar depression.



"The new views as to the morphology of the thymus gland and their bearing on the problem of the function of the thymus"

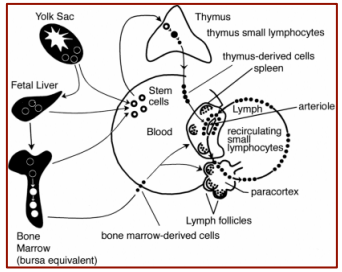
J August Hammar *Endocrinology* (1921) 5:43-73





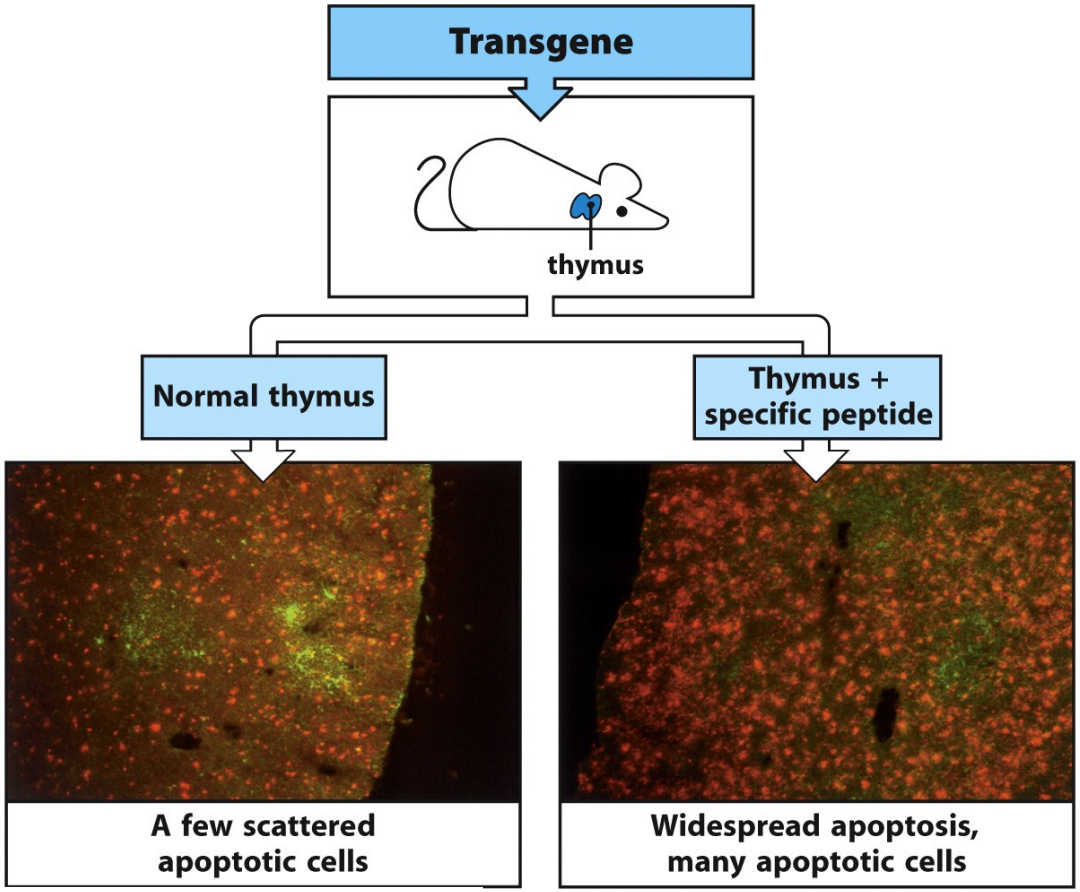
Jacques FAP Miller

Role of the thymus in murine leukaemia. *Nature* (1959) 183:1069.
Immunological function of the thymus. *Lancet* (1961) 2:748-9.

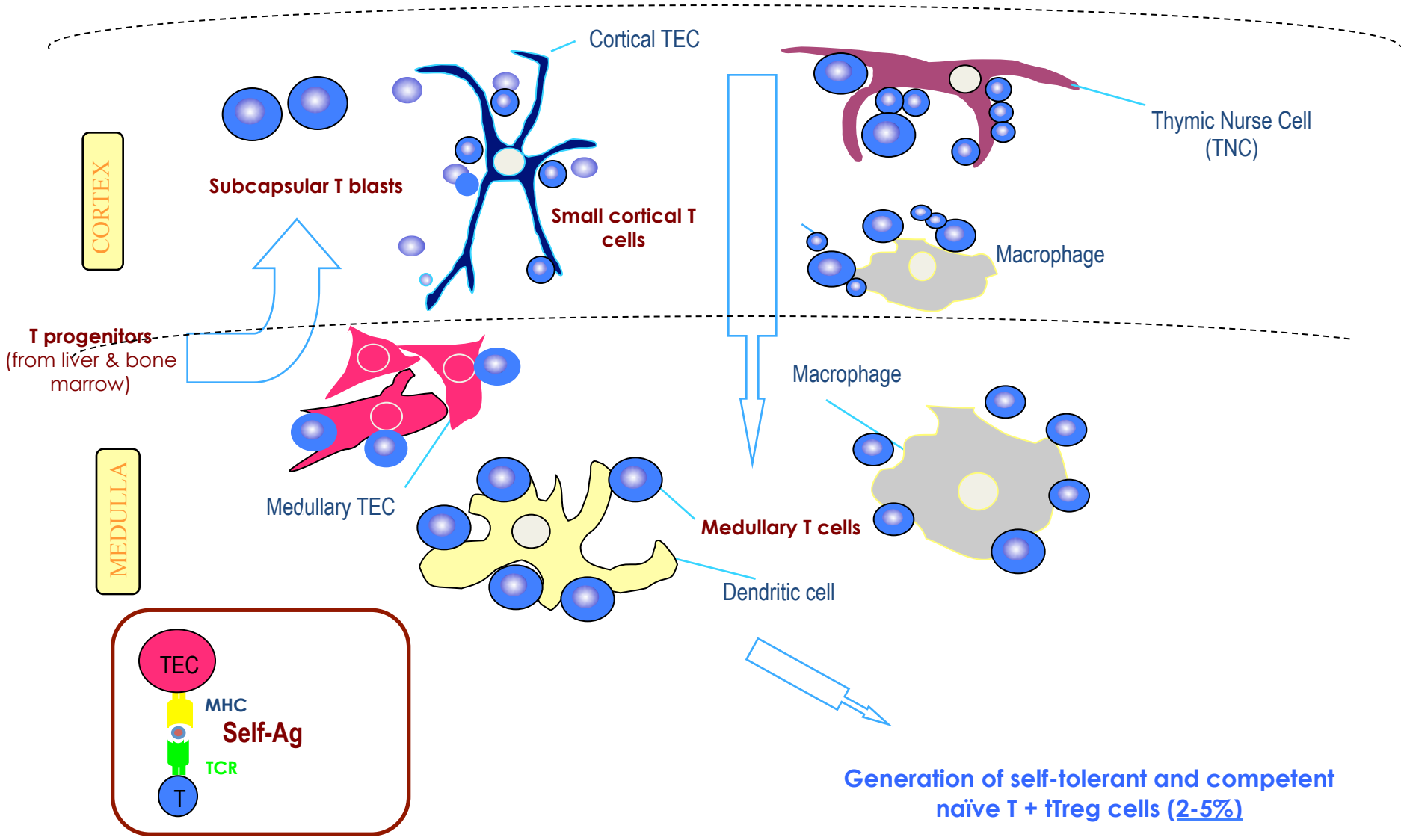


‘Central’ self-tolerance induction in the thymus

Ohki H, Martin C, Corbel C, Coltey M & Le Douarin NM *Science* 1987
Kappler JW, Roehm N & Marrack P *Cell* 1987
Kisielow P, Bluethmann H, Staerz UD, Steinmetz M & von Boehmer H *Nature* 1988

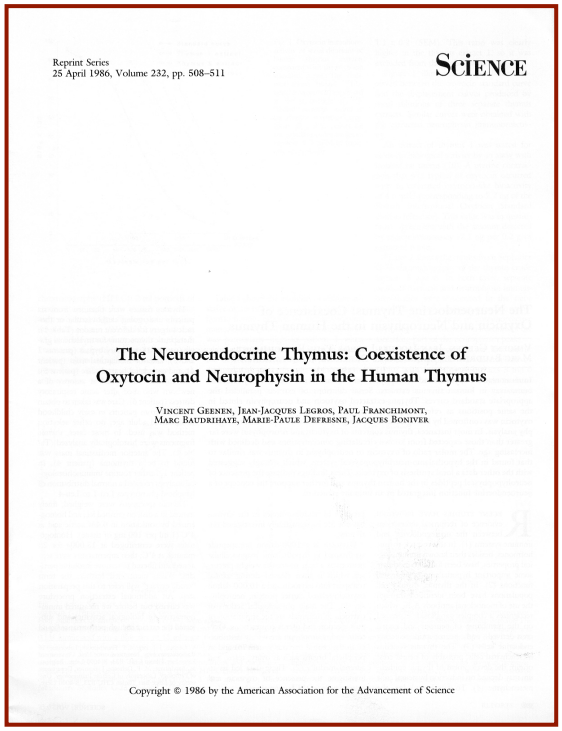


T-cell differentiation in the thymus



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



Reprint Series
25 April 1986, Volume 232, pp. 508–511

SCIENCE

The Neuroendocrine Thymus: Coexistence of Oxytocin and Neurophysin in the Human Thymus

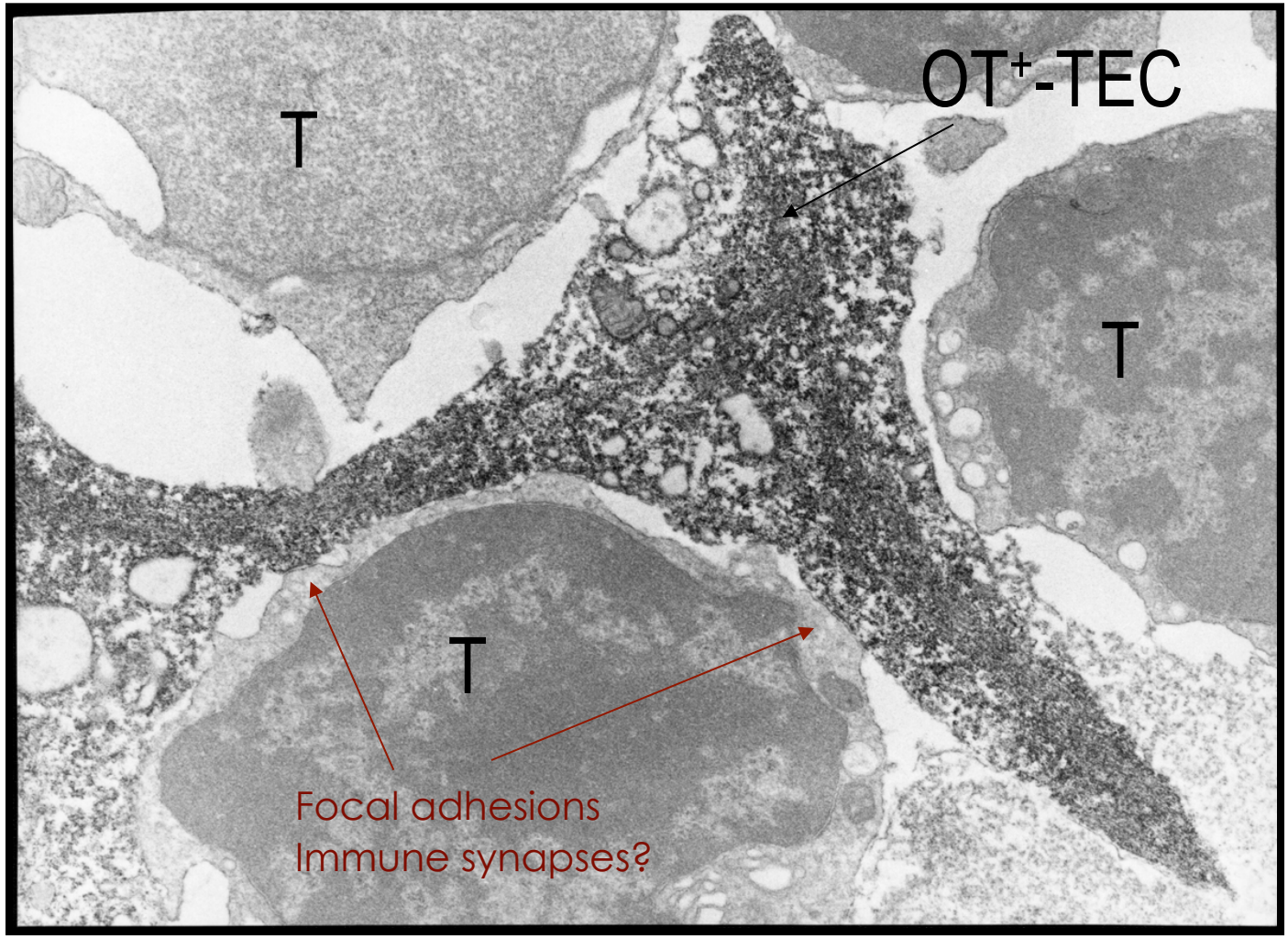
VINCENT GEENEN, JEAN-JACQUES LEGROS, PAUL FRANCHIMONT, MARC BAUDRIHAYE, MARIE-PAULE DEFRESNE, JACQUES BONIVER

FAMILY	THYMIC NEUROPEPTIDES
Neurohypophysial family	Oxytocin / OT (>> Vasopressin / VP)
Neuromedins	Neurotensin / NT
Tachykinin family	Neurokinin A
Natriuretic peptide family	ANP
Somatostatin family	Cortistatin
Insulin family	IGF-2 (> IGF-1 > Insulin)

Goxe B, Martens H & Geenen V *Immunol Today* (1996) 17:312-7

Geenen V et al. *Encyclopedia of Neuroscience 3rd Edition* (2003)

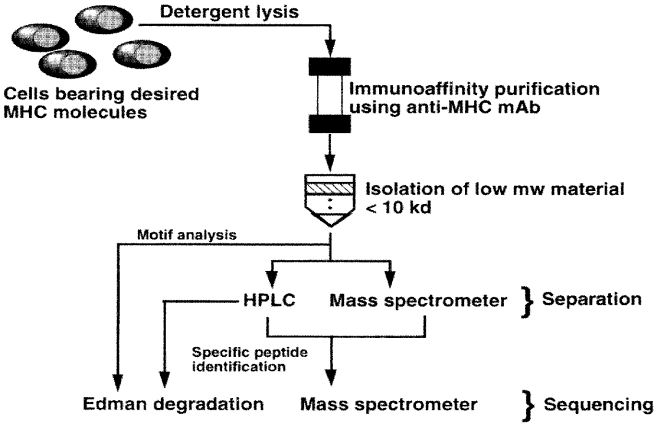
OT cryptocrine secretion in human TEC

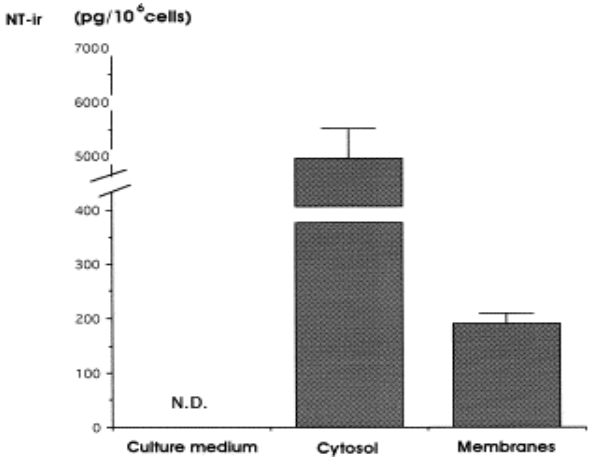


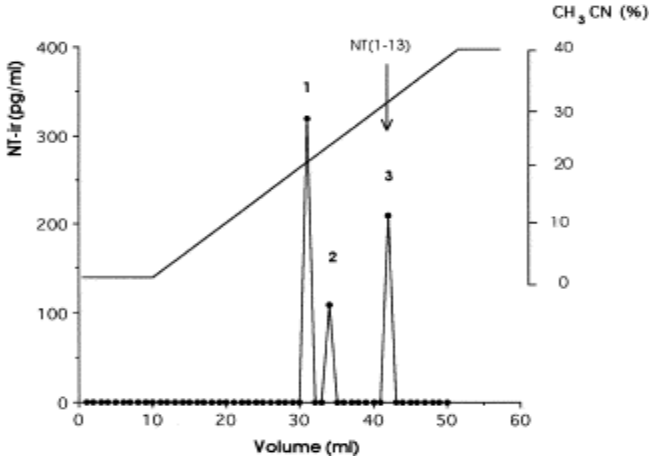
MHC-I presentation of neurotensin by human TEC

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

= ELYENKPRRPYIL





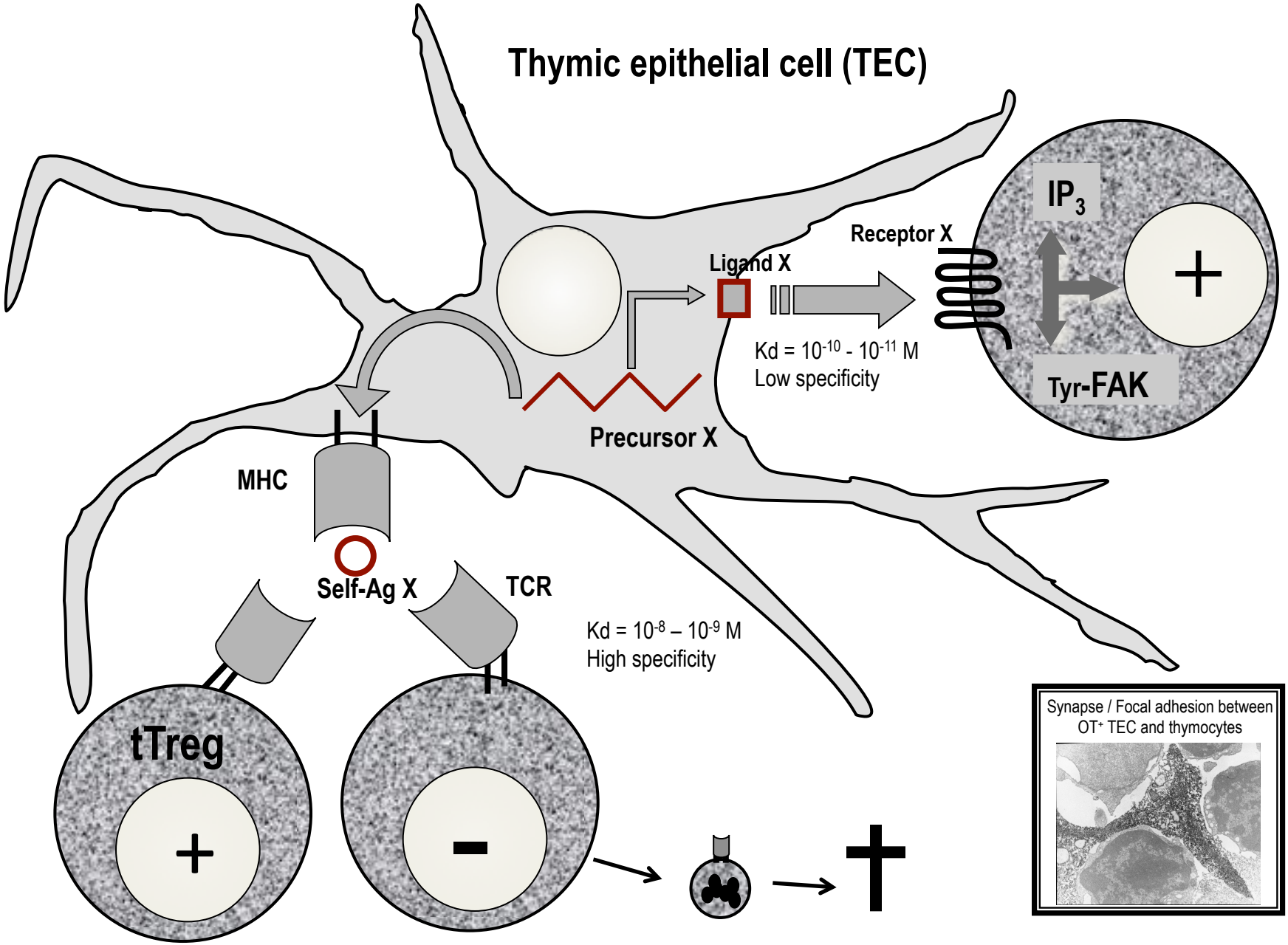


Vanneste Y et al. *J Neuroimmunol* (1997) 76:161-6

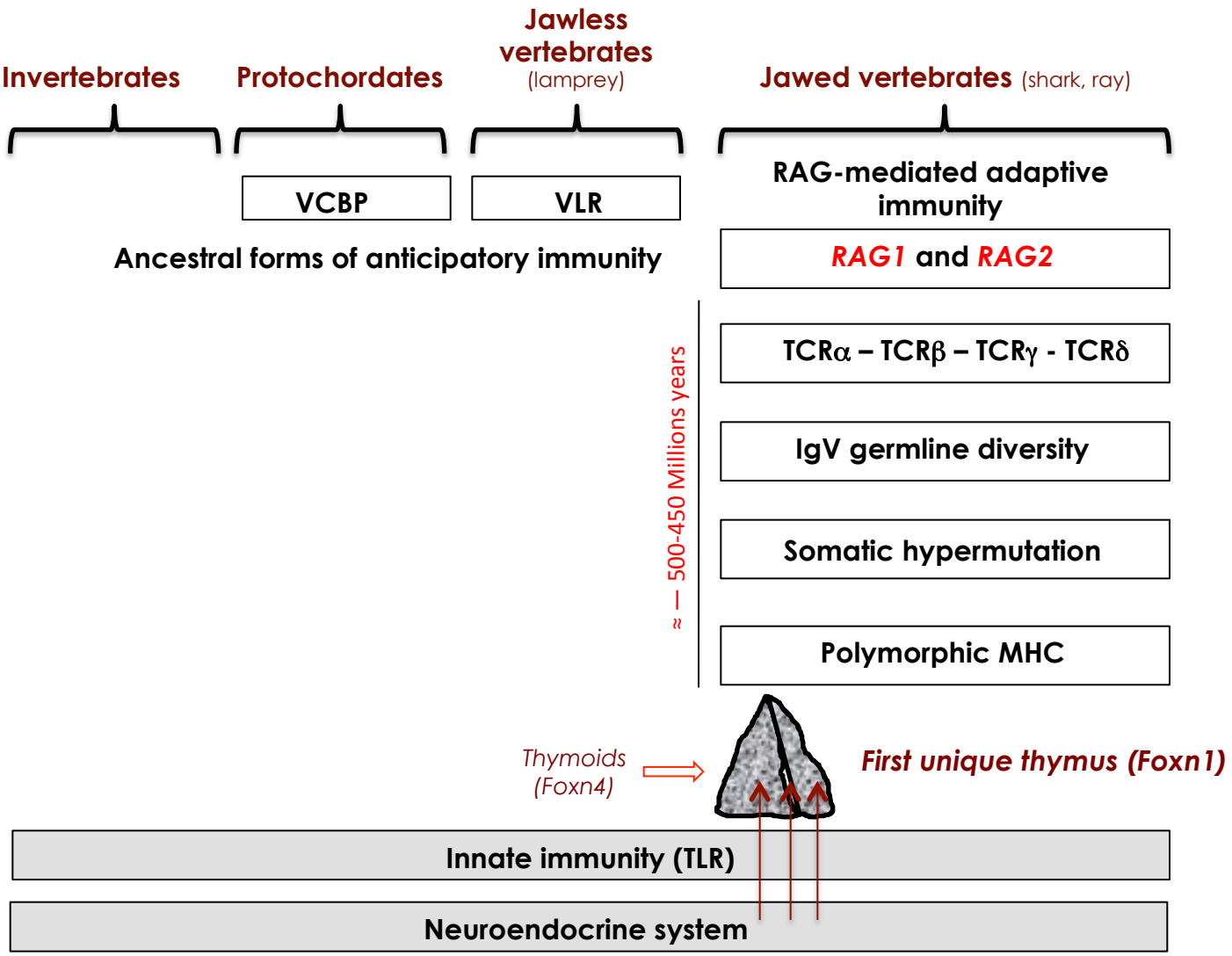
A paradigm shift: from thymic neuropeptides to '*neuroendocrine self-peptides*'

- 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.

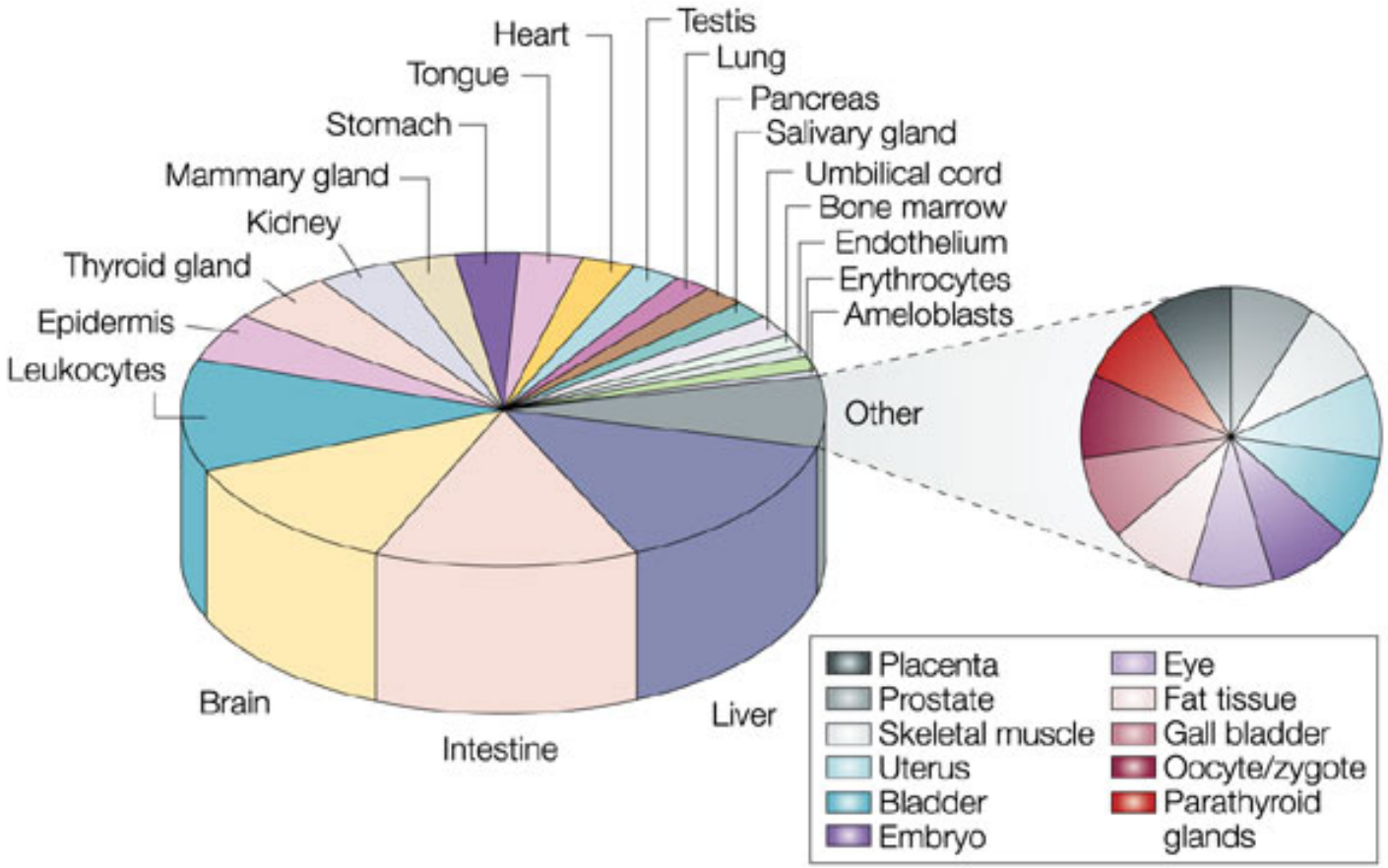
The dual role of neuroendocrine self-antigen precursors



Integrated coevolution of the immune and neuroendocrine systems



Intrathymic expression of tissue-restricted antigens



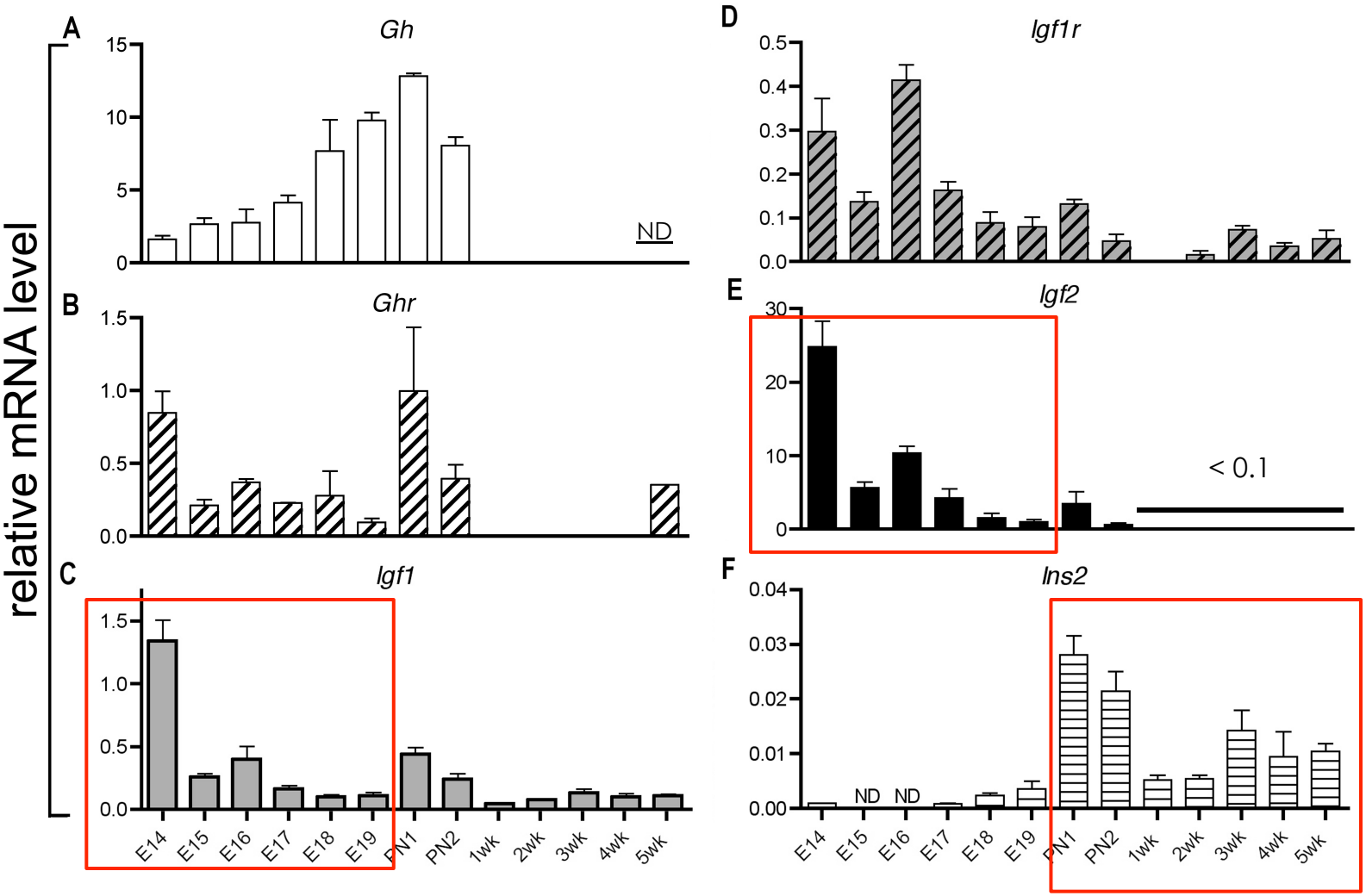
Nature Reviews | Immunology

Kyewski B et al. (2004)

The Origin of Organ/Cell-Specific Autoimmunity:

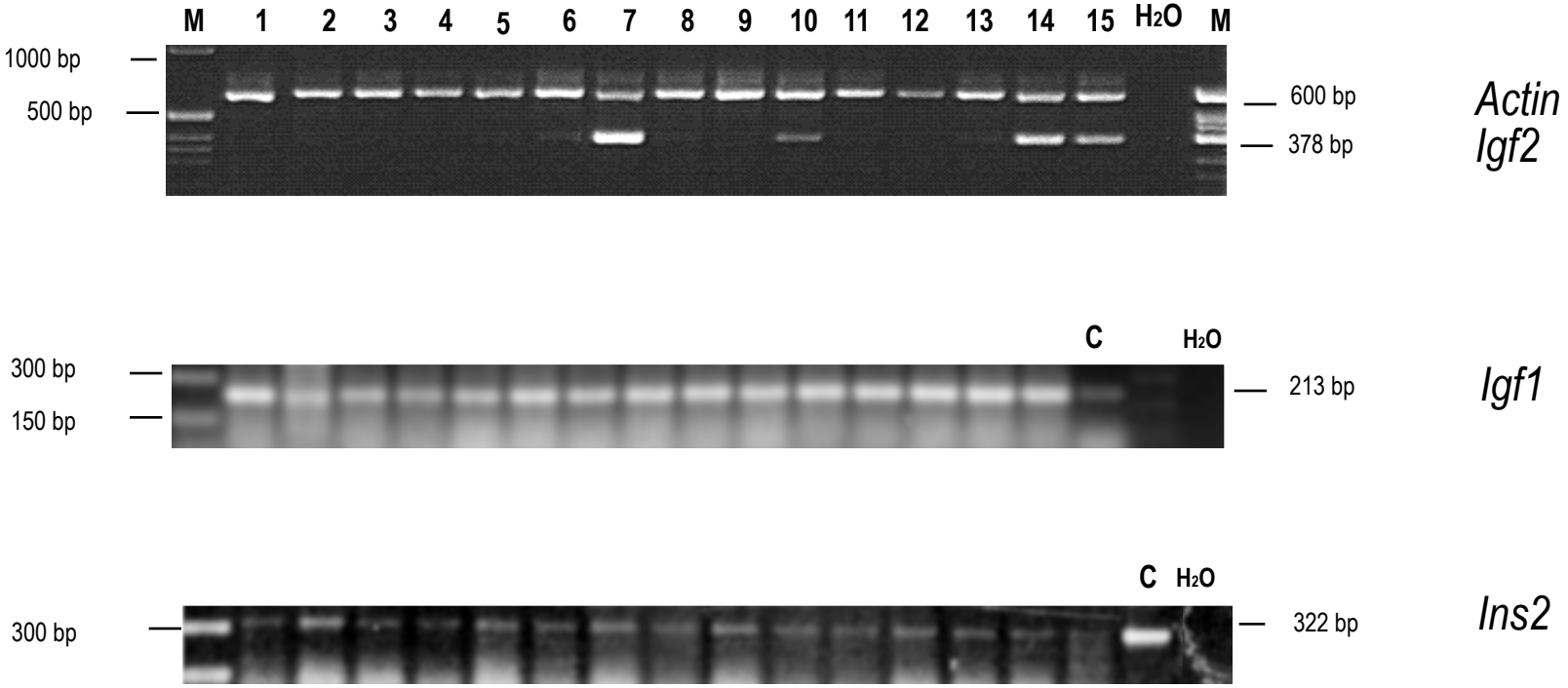
A Thymus Defect in Programming Self-Tolerance?

Ontogeny of gene expression in Balb/c thymus



Kermani H et al. *Neuroimmunomodulation* (2012) 19:137-47

Transcription of *Insulin*-related genes in the thymus of BB rats



Kecha-Kamoun O et al., *Diabetes Metabolism Research* (2001)

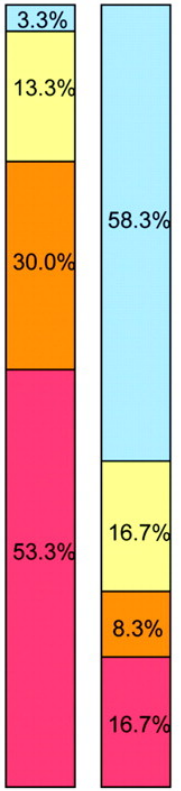
APS-I or APECED syndrome

- 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***

A

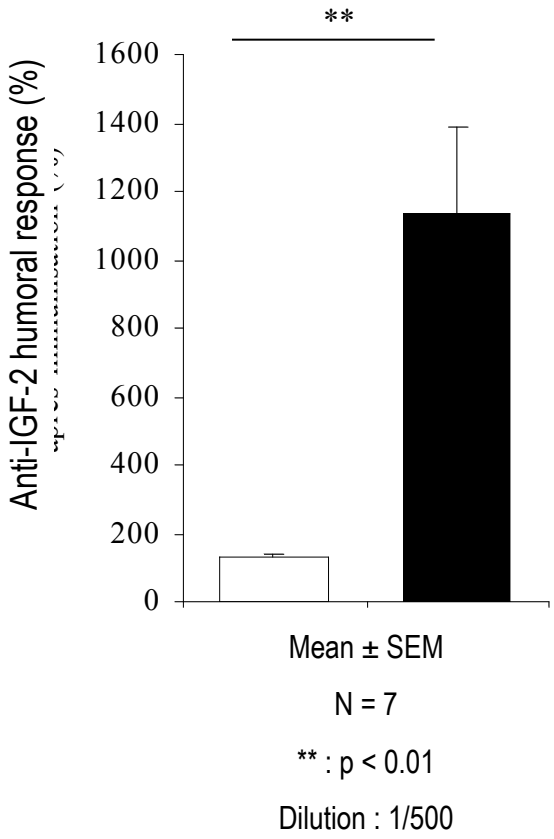
Probe name	Gene name	Tissue(s)	WT signal	KO signal	KO/ WT	t-test p-val	FPR Quad	FPR SAM
96030_at	casein alpha	mammary	75.62	1	0.013	0.0417	0.043	0.014
97180_f_at	hemoglobin y, beta-like embryonic chain	fetal erythrocytes	87.36	1.29	0.015	0.0803	0.279	0.014
100106_at	intestinal trefoil factor	intestinal goblet cells	74.59	1.99	0.027	0.0504	< 0.02	0.014
101820_at	neurotoxin homologue	granulocytes, monocytes	29.50	1	0.034	0.1105	< 0.02	0.014
94738_s_at	cryptdin,related sequence 2	Paneth cells	100.47	3.85	0.038	0.2561	0.164	-
101682_f_at	major urinary protein IV	lachrymal gland, parotid gland	26.49	1.2	0.045	0.0392	0.063	0.014
94153_g_at	salivary protein 1	salivary gland	22.62	1.06	0.047	0.0712	0.131	0.014
102998_at	cytochrome P450 1a2	liver, lung, duodenum	20.99	1.01	0.048	0.0868	0.043	0.014
101115_at	lactotransferrin	mammary gland, uterus	19.78	1	0.051	0.0320	0.043	0.014
92353_at	serine protease (BSSP)	hair follicles, brain	18.77	1	0.053	0.1070	1	-
100463_at	gamma-casein precursor	mammary gland	21.93	1.17	0.053	0.0596	0.071	0.014
92546_r_at	prostaglandin D	brain, epididymis	22.82	1.26	0.055	0.0001	0.063	0.014
96153_at	neutrophilic granule	granulocytes	28.46	1.72	0.060	0.0246	0.063	0.014
160899_at	Purkinje cell protein 4	brain, eye (lens)	32.67	2.09	0.064	0.0327	< 0.02	0.014
161815_f_at	major urinary protein I	liver	31.23	2.04	0.065	0.0704	0.043	0.014
98858_at	glucose dependent insulinotropic polypeptide	K cells of small intestine	27.16	1.78	0.066	0.0517	< 0.02	0.014
101910_f_at	major urinary protein 3	liver	21.02	1.47	0.070	0.0328	0.164	0.014
94775_at	oxytocin	brain	26.59	1.92	0.072	0.0334	1	-
101636_at	salivary protein 2	salivary gland	16.80	1.23	0.073	0.0382	1	-
98623_g_at	insulin-like growth factor II	embryo, choroid plexus and leptomeninges in adult	94.85	6.96	0.073	0.1179	< 0.02	0.014
99958_at	mast cell protease-2	mast cells	13.70	1.01	0.074	0.0248	0.043	0.014
94707_s_at	amelogenin	ameloblast cells	34.69	2.57	0.074	0.0328	< 0.02	0.014
103235_at	preproneuropeptide y	brain	19.54	1.47	0.075	0.0143	0.043	0.014
103887_at	S100 calcium binding protein A9	immature BM myeloid cells, monocytes, neutrophils	68.93	5.26	0.076	0.1529	0.279	0.014
162341_r_at	aldose reductase	many	19.37	1.48	0.076	0.0121	0.279	-
97889_at	fatty acid binding protein	intestine	37.46	3.04	0.081	0.0254	0.043	0.014
94045_at	α-1-microglobulin/bikunin precursor	liver	12.74	1.04	0.082	0.0781	0.279	0.014
100150_f_at	preproinsulin II	pancreatic islet beta cells	19.70	1.62	0.082	0.1692	< 0.02	0.014
100002_at	inter-alpha-inhibitor H3 chain	liver, brain	12.07	1	0.083	0.0266	0.043	0.014
98830_at	spermine binding protein	prostate	13.56	1.13	0.083	0.1047	0.131	-

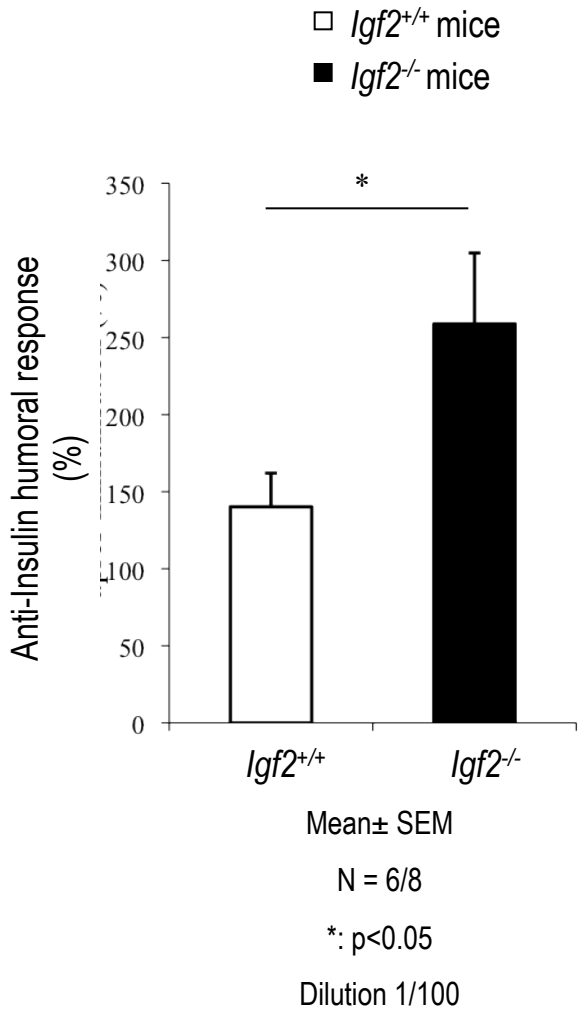
B



Anderson MS et al., *Science* (2002) 298:1395-1401

Contribution of *Igf2* expression to immunological tolerance toward INS

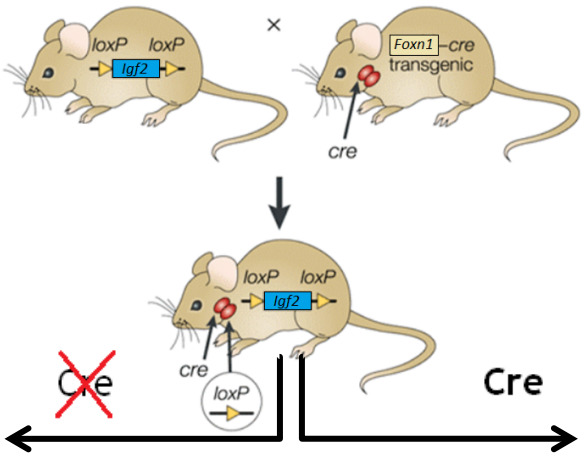




Hansenne I et al. *J Immunol* (2006) 176:4651-7

Specific deletion of *Igf2* in thymic epithelium – Development of *Igf2* $^{\Delta Thy}$ mouse

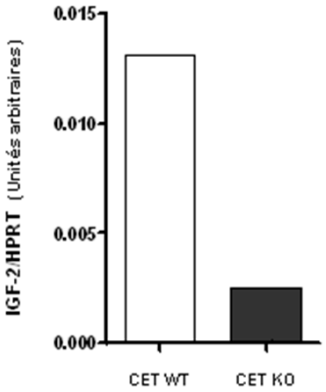
(Pr M. Constancia, University of Cambridge)



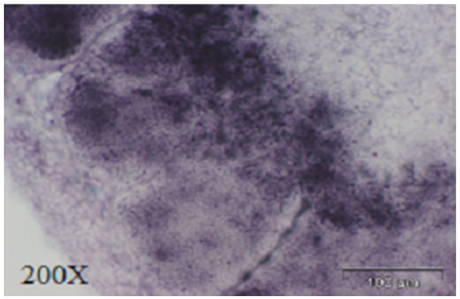
(Pr G. Holländer, University of Basel)

Igf2 $^{fl/fl}$ Control

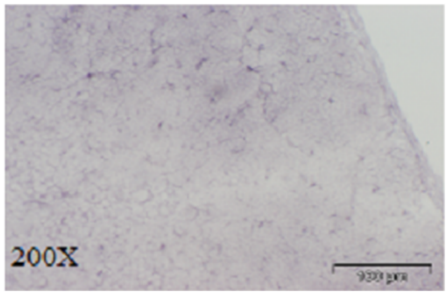
Igf2 $^{\Delta Thy}$



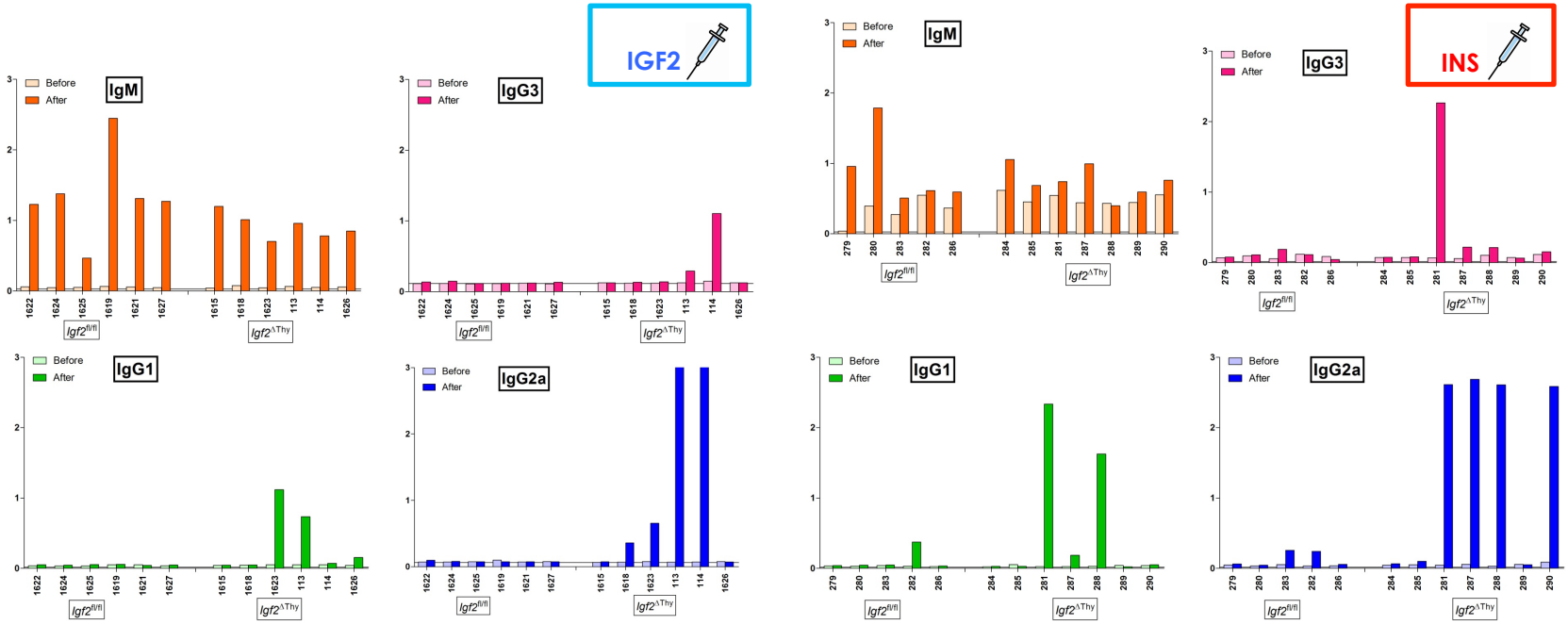
Thymus *Igf2* $^{fl/fl}$ 20 µm



Thymus *Igf2* $^{\Delta Thy}$ 20 µm



Titres and isotypes of Ig's after immunization with IGF2 or INS



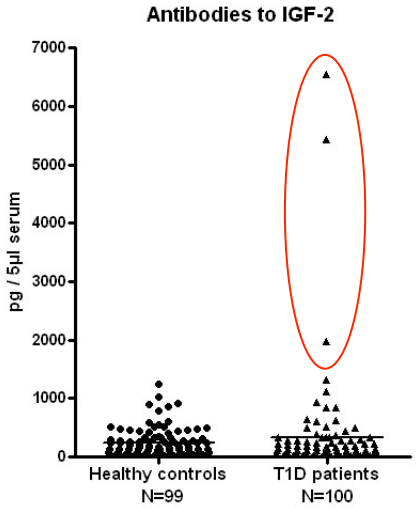
- Despite its ubiquitous expression, *Igf2* deletion in the sole thymus leads to loss of tolerance toward IGF2.
- *Igf2* deletion in the sole thymus also lowers the level of immunological tolerance toward INS (central cross-tolerance between IGF2 and INS).

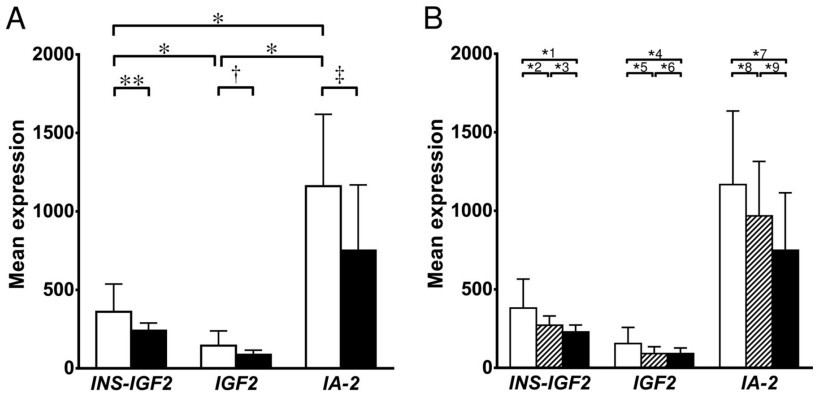
Bodart G et al. *Submitted*

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)





Kanatsuna N et al. *J Biol Chem* (2013) 288:29013-23

A thymus defect in autoimmune neuroendocrine diseases

Thymus physiology

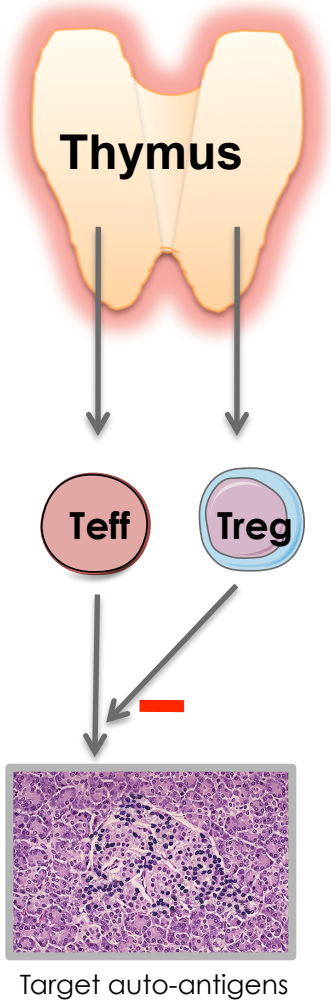
- AIRE-regulated transcription of neuroendocrine self-peptides in thymus epithelium.
- Deletion of T cells with high affinity for MHC/neuroendocrine self-peptide complexes.
- Selection of CD4+ CD25+ Foxp3+ tTreg, specific of neuroendocrine self-peptides.

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…)



Geenen V et al. *Front Neuroscience* (2013) 7:1-12

The Role of Environment in T1D Pathogenesis

Coxsackievirus CVB4, thymus and T1D pathogenesis

Background

- Coxsackievirus B4 infection of murine fetal thymus organ cultures.
  F. Brilot *et al*. *J Med Virol* (1998) 80:659-666.

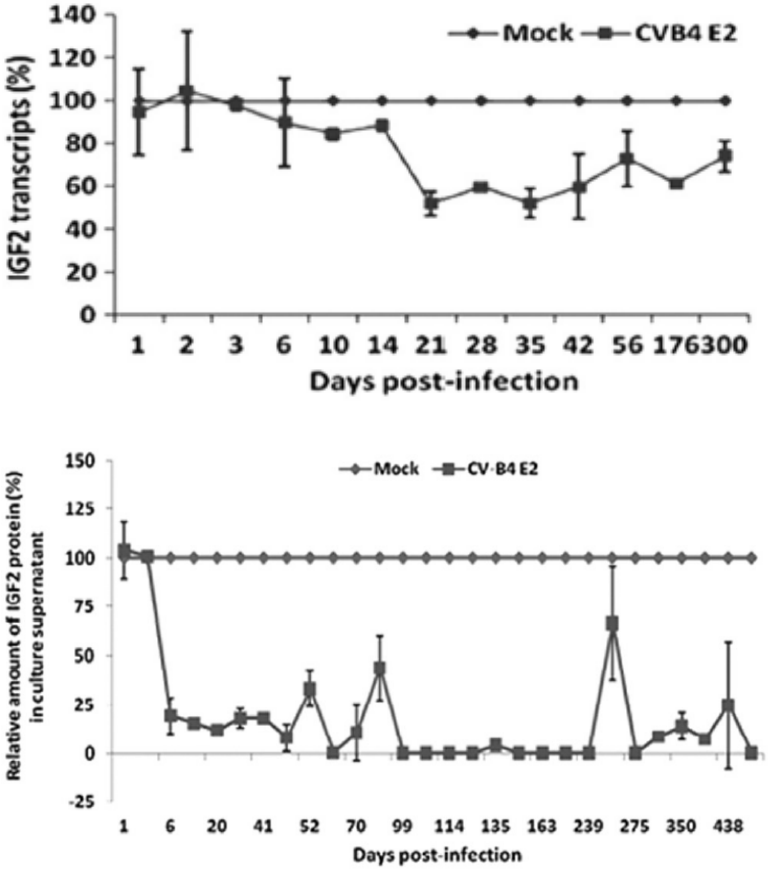
- Persistent infection of human thymic epithelial cells by Coxsackievirus B4.
  F. Brilot *et al*. *J Virol* (2002) 76:5260-5265.

- Coxsackievirus B4 infection of human fetal thymus cells.
  F. Brilot, V. Geenen, D. Hober & C. Stoddart, *J Virol* (2004) 78:9854-9861.

- Prolonged viral RNA detection in blood and lymphoid tissues from Coxsackievirus B4 orally-inoculated Swiss mice.
  H. Jaïdane *et al*. *Microbiol Immunol* (2006) 50:971-974.

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



Jaïdane H et al. *J Virol* (2012) 86:11151-62

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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Didier Hober, MD, PhD

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