Le Thymus en 2013 :

D'un organe 'vestigial' à la tolérance immunitaire vis-à-vis du Soi et à l'auto-immunité

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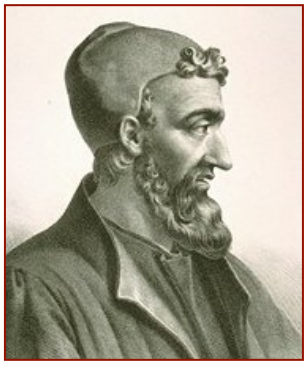
d'Histoire de la recherche biomédicale à l'ULg

Présentation à l'ARMB – 26 octobre 2013

The moving place of the thymus in the history of medicine

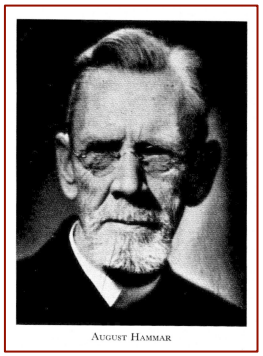


Claude Galen – 2nd father of Western medicine (129 – 210 AD)

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

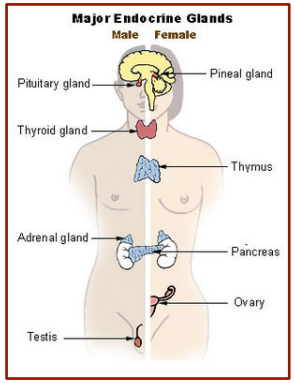
Refers to the spirited part of Plato's three constituents of psyche (with logical and appetitive).

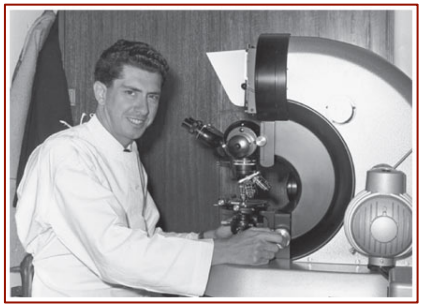
'Troubles thymiques' in French medical language
= mood disorders, *i.e.* bipolar and unipolar depression.



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

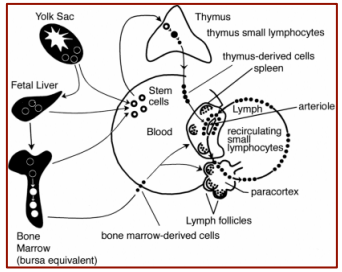
J August Hammar *Endocrinology* (1921) 5:43-73



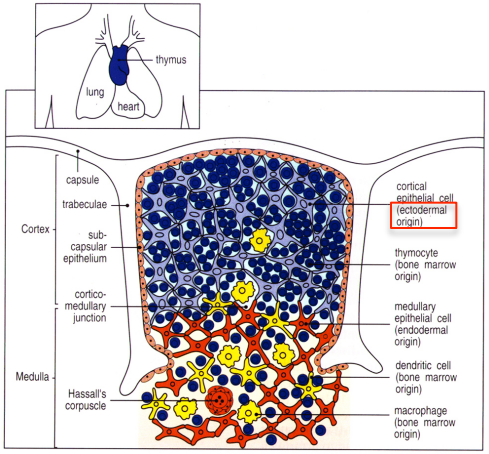


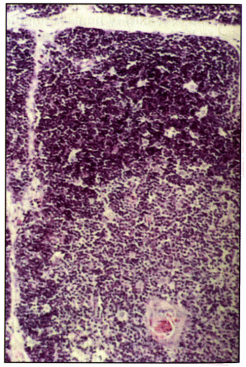
Jacques FAP Miller

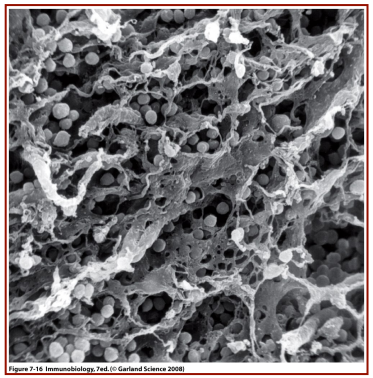
Role of the thymus in murine leukaemia. *Nature* (1959) 183:1069.
Immunological function of the thymus. *Lancet* (1961) 2:748-9.

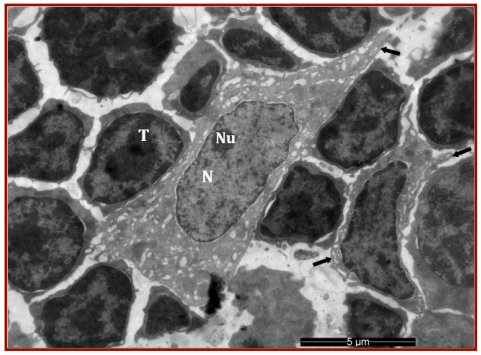


Cell populations in the thymus & Developmental biology







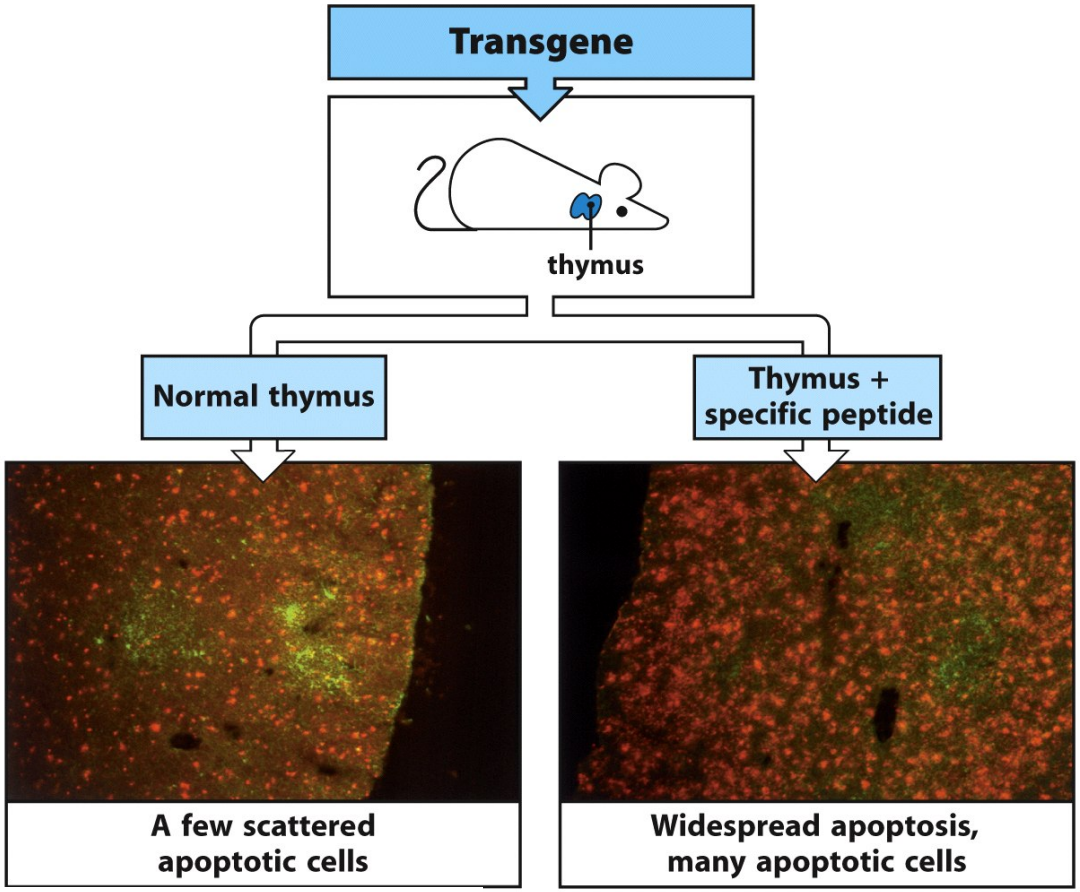


HOXA3 At ED10.5 expression in the 3rd pharyngeal pouch endoderm and neural crest mesenchyme. Absence of thymus and parathyroid hypoplasia in *Hoxa3*$^{-/-}$ mice.

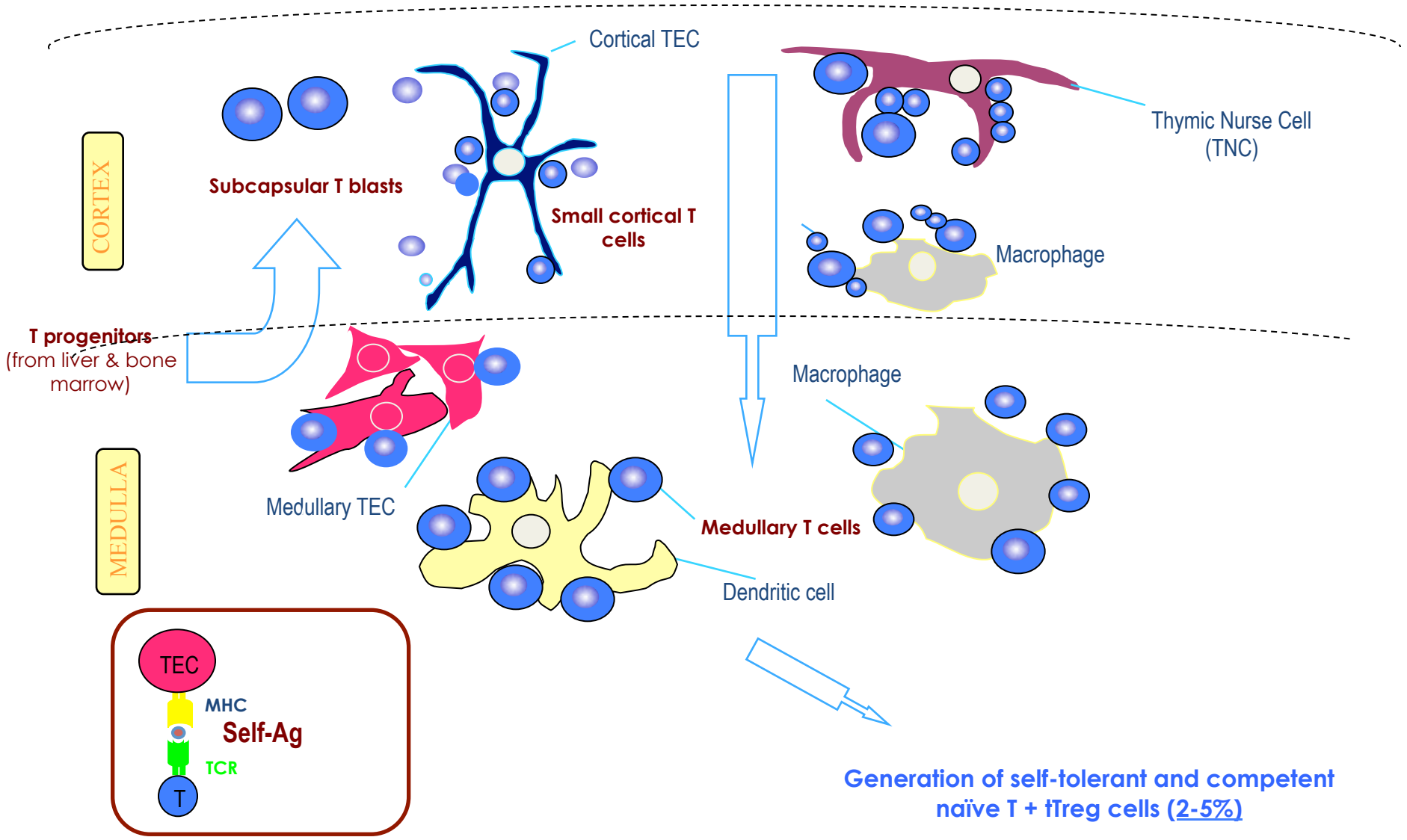
FOXN1 First expressed at ED12.5 in the 3rd pharyngeal pouch endoderm, then in thymic epithelial cells. *Foxn1* mutation results in the 'nude mouse' phenotype.

‘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



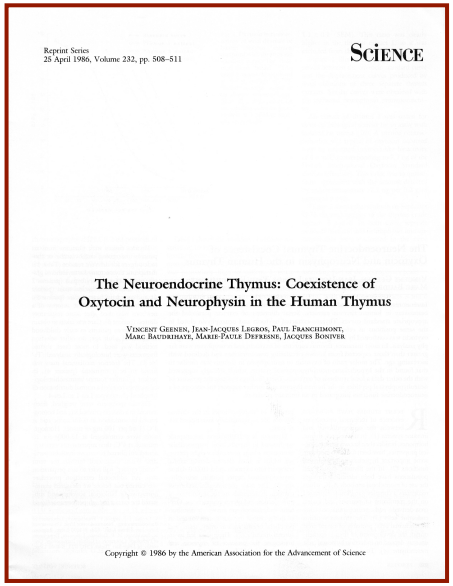
T-cell differentiation in the thymus



1. *Negative selection of self-reactive T cells during fetal life*
2. *Generation of self-specific tTreg cells early after birth*

Thymic neuropeptides: Organisation of the repertoire

The galactogogue action of the thymus and corpus luteum.
Ott I & Scott JC
Proc Soc Exp Biol Med (1910) 8:49-54



Reprint Series
28 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

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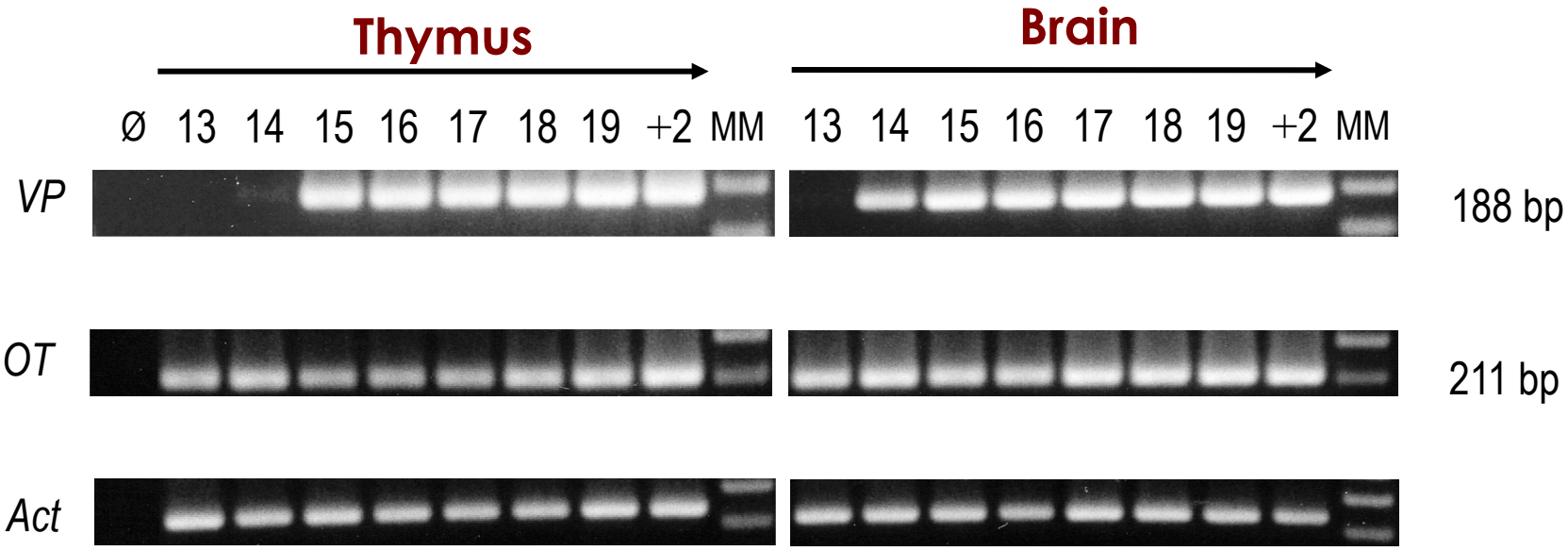
FAMILY	THYMIC NEUROPEPTIDES
Neurohypophysial family	Oxytocin / OT (>> Vasopressin / VP)
Neuromedins	Neurotensin / NT
Tachykinin family	Neurokinin A
Natriuretic peptide family	ANP
Somatostatin family	Cortistatin
Insulin family	IGF-2 (> IGF-1 > Insulin)

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

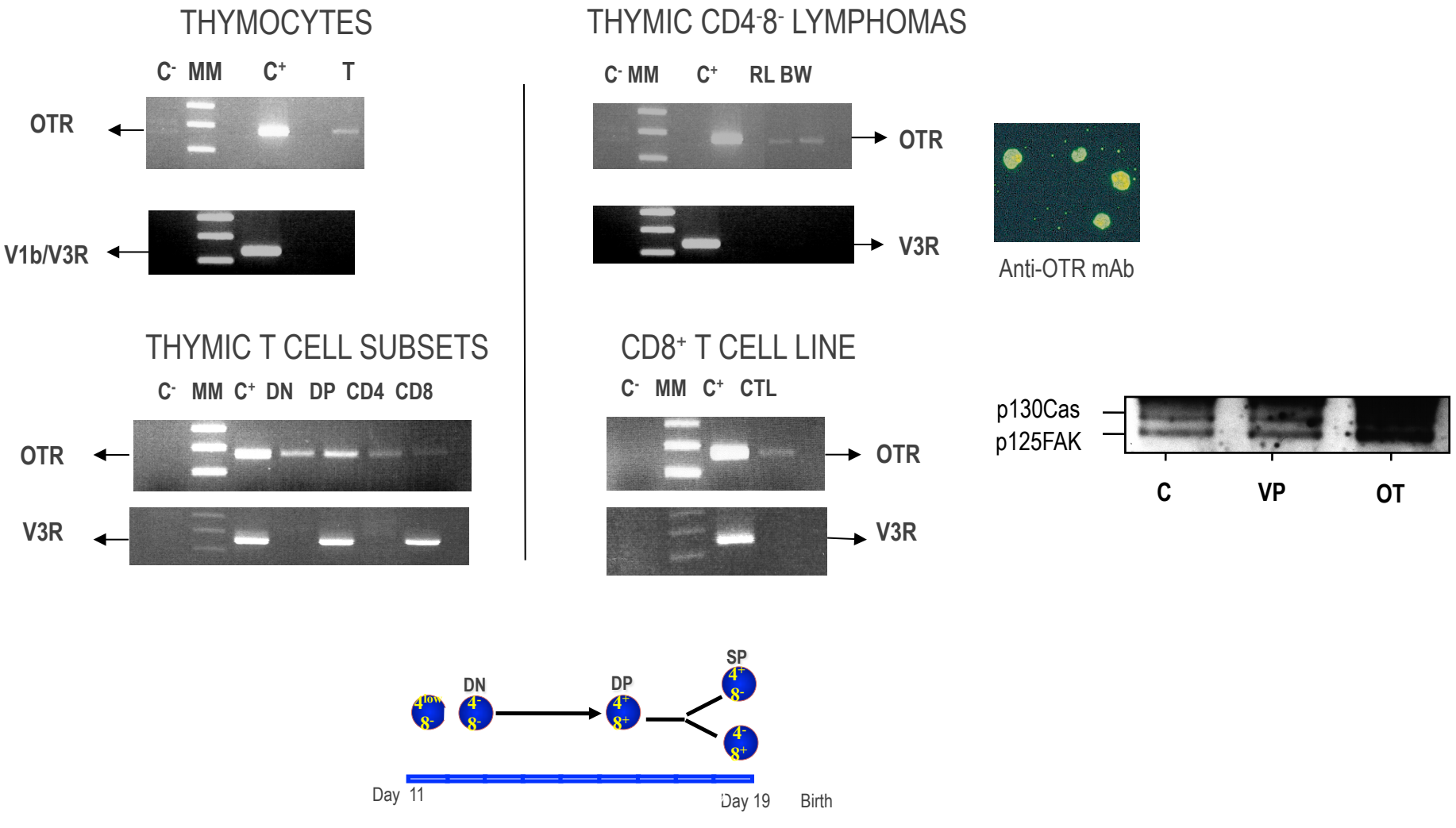
Geenen V et al. *Encyclopedia of Neuroscience 3rd Edition* (2003)

Ontogeny of *OT* and *VP* expression



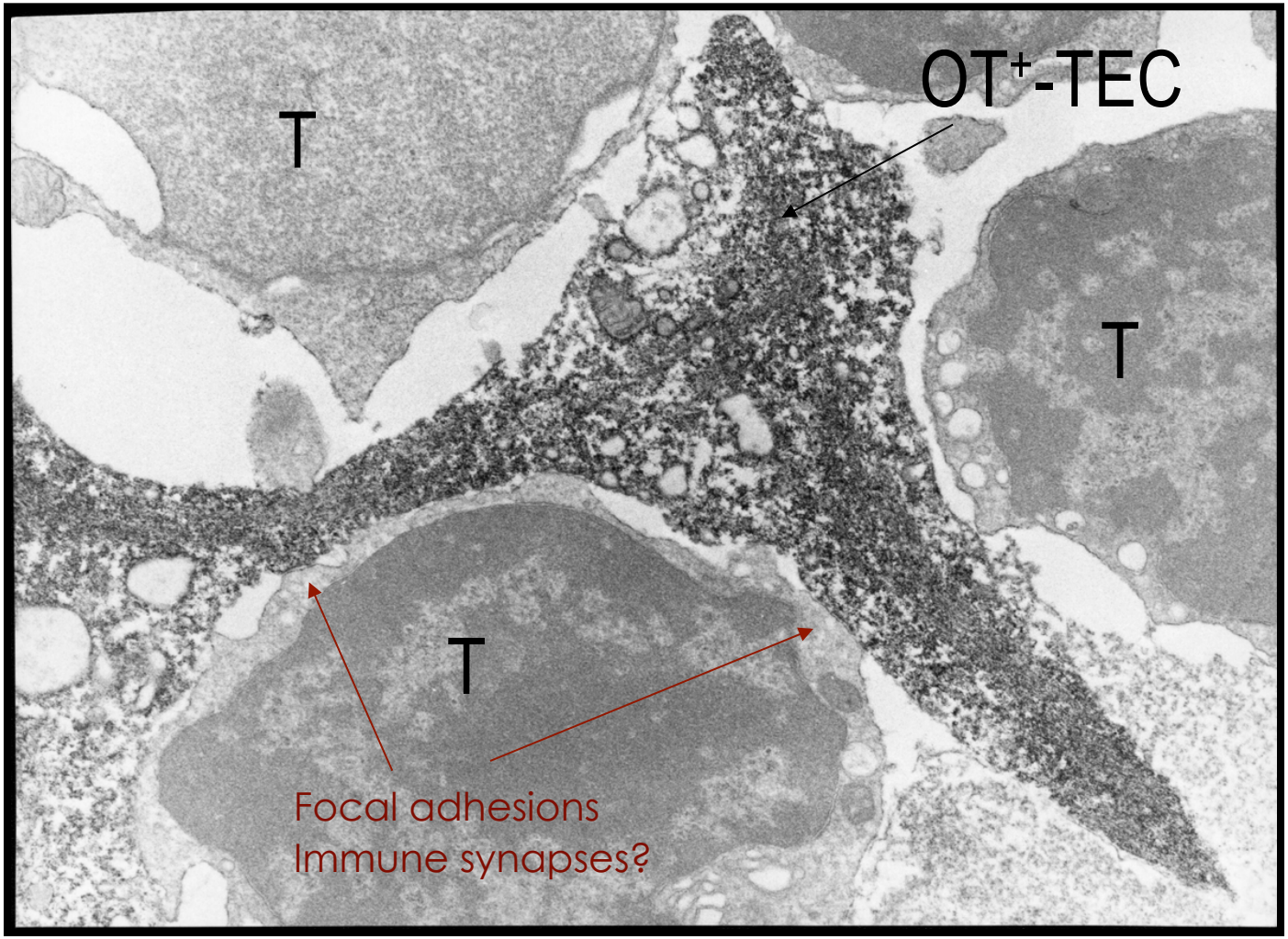
OT expression coincides in brain and thymus, but precedes onset of *VP* transcription in both sites

Neurohypophysial receptor expression by thymic T cells



Martens H et al. *Neuroendocrinology* (1998) 67:282-9
Hansenne I et al. *Clin Dev Immunol.* (2004) 11:45-51
Hansenne I et al. *J Neuroimmunol* (2005) 158:67-75

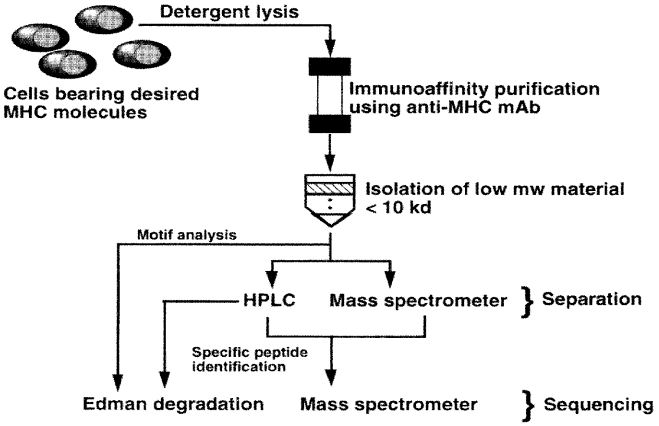
OT cryptocrine secretion in human TEC

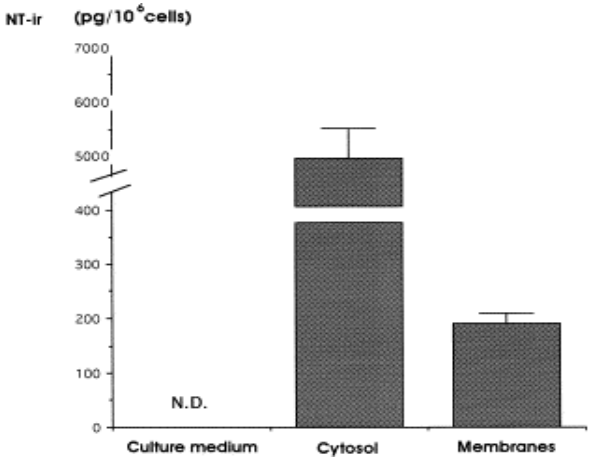


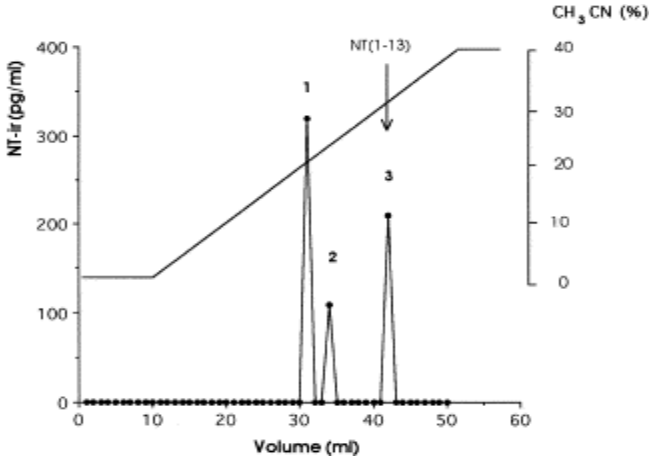
MHC-I presentation of neurotensin by human TEC

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

= ELYENKPRRPYIL





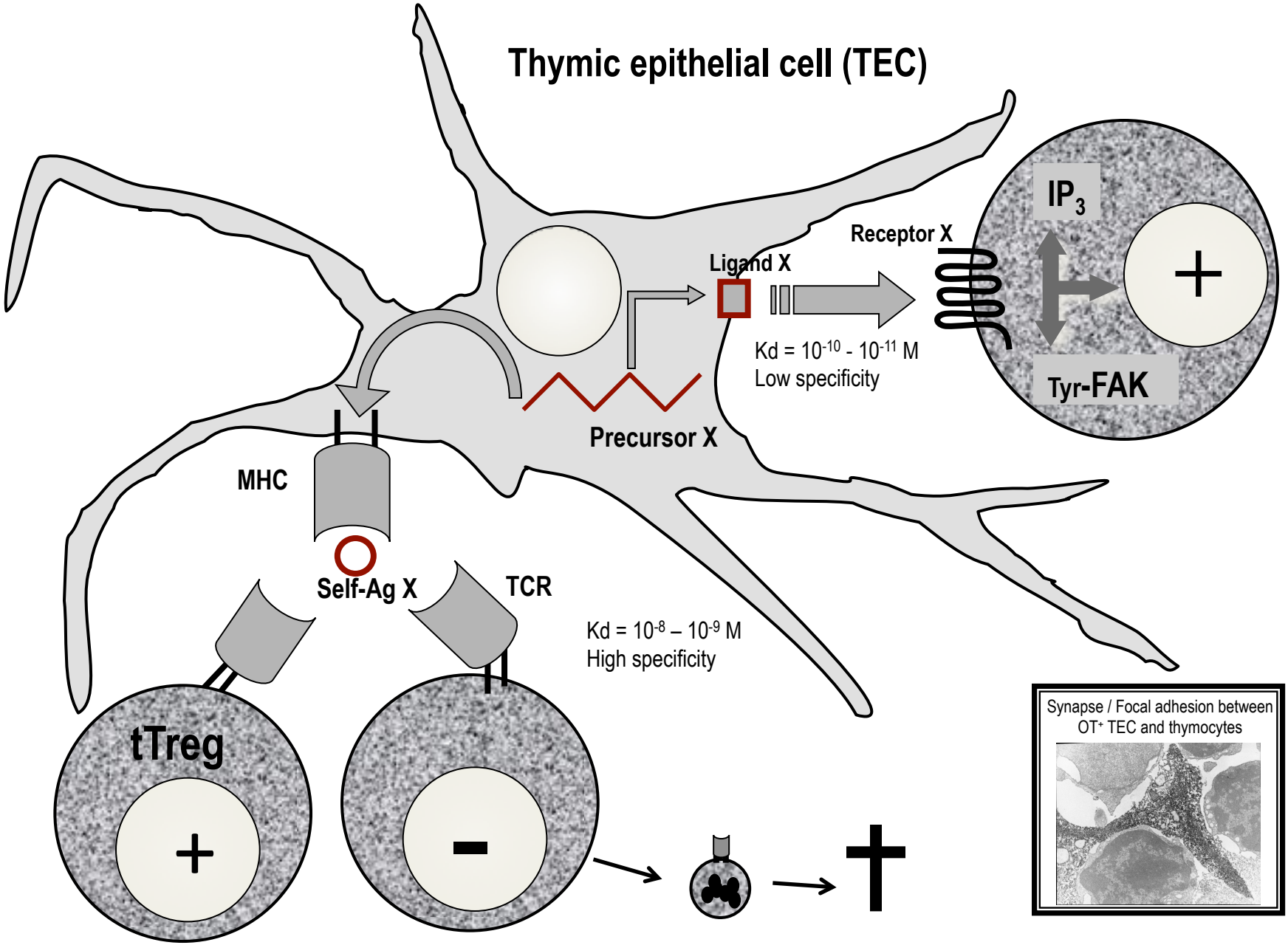


Vanneste Y et al. *J Neuroimmunol* (1997) 76:161-6

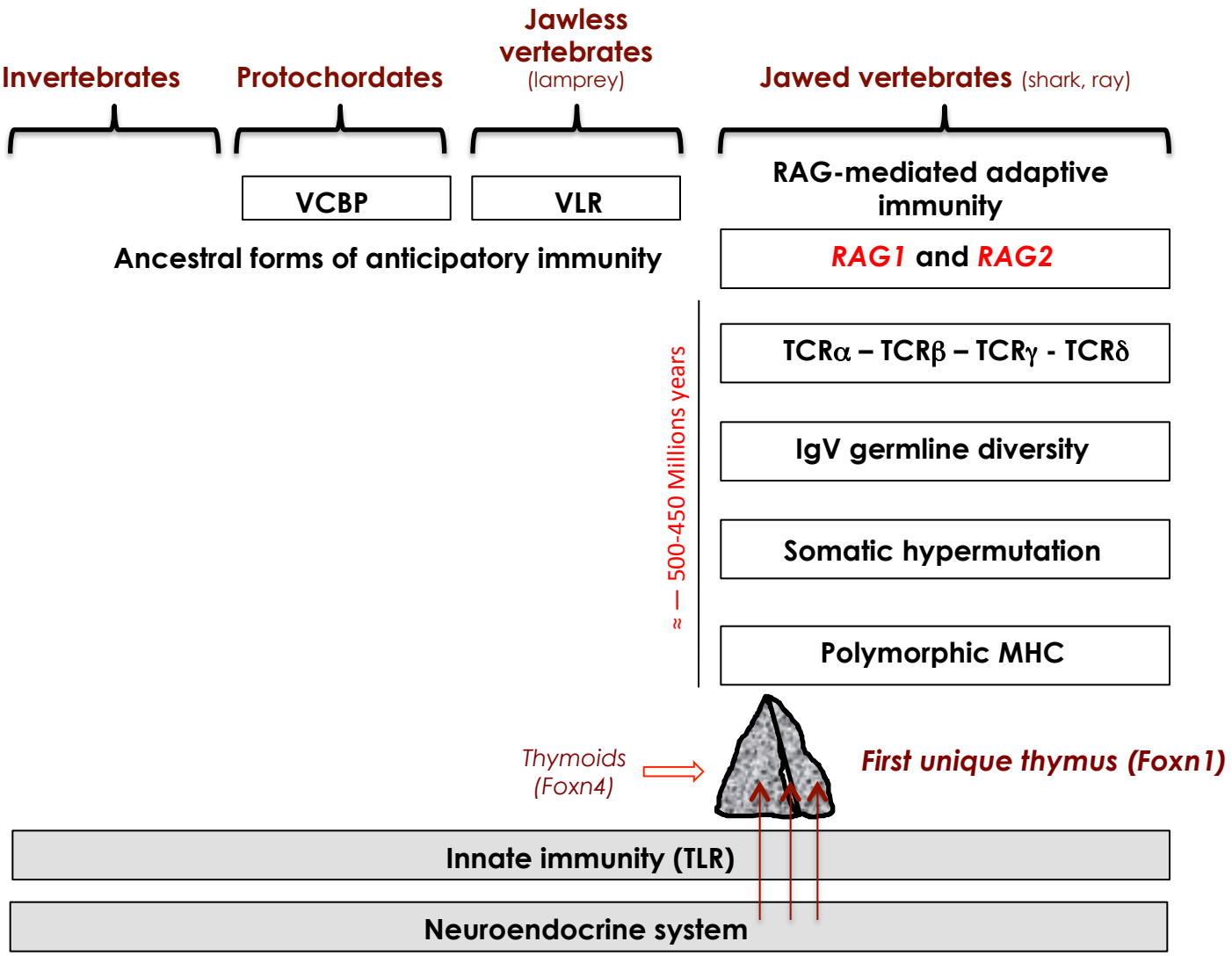
A paradigm shift: from thymic neuropeptides to '*neuroendocrine self-peptides*'

- 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).*
- Thymus-specific epigenetic regulation (*i.e. IGF2*).
- NO SECRETION but processing through MHC pathways for antigen presentation.

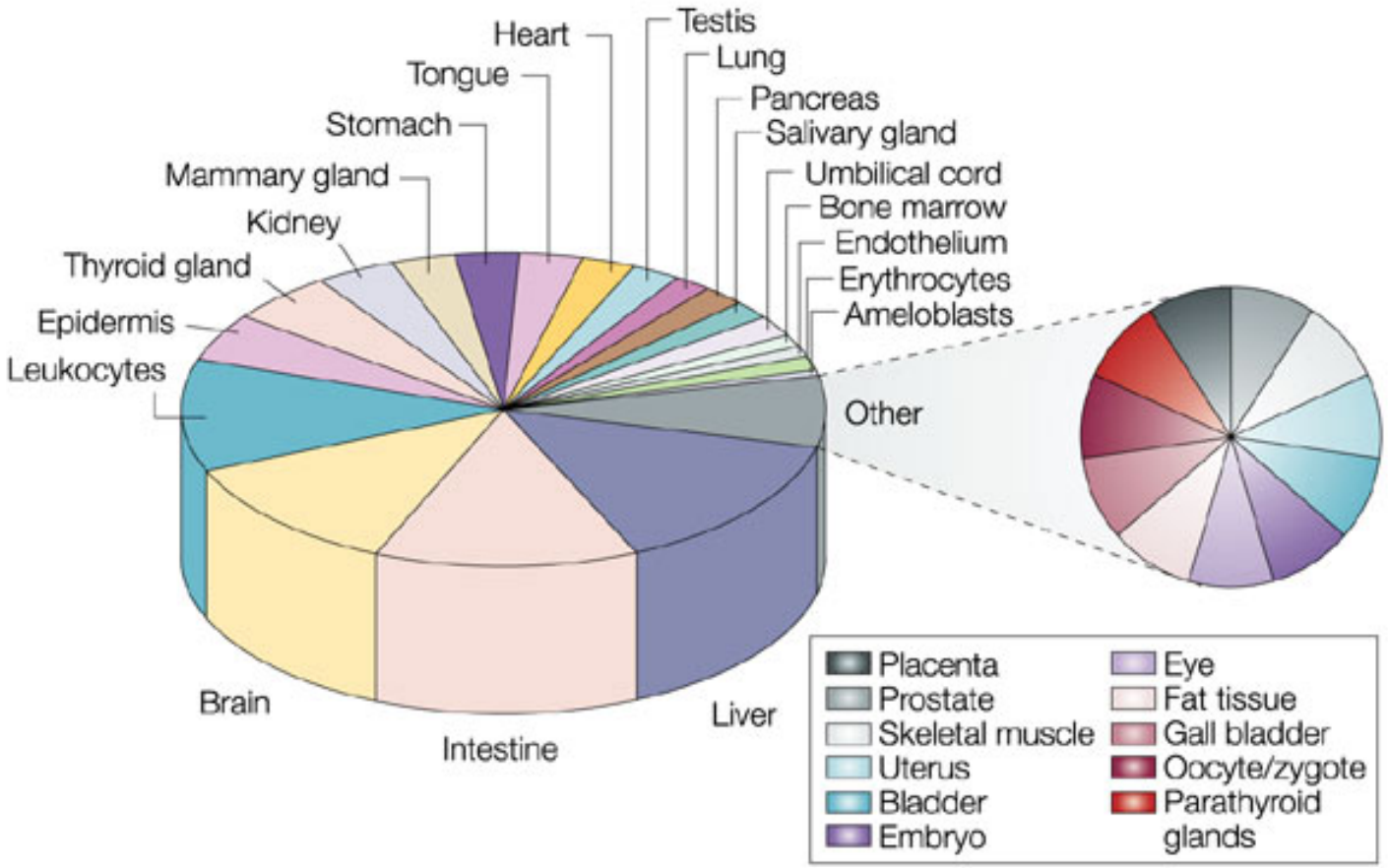
The dual role of neuroendocrine self-antigen precursors



Integrated coevolution of the immune and neuroendocrine systems



Intrathymic expression of tissue-restricted antigens



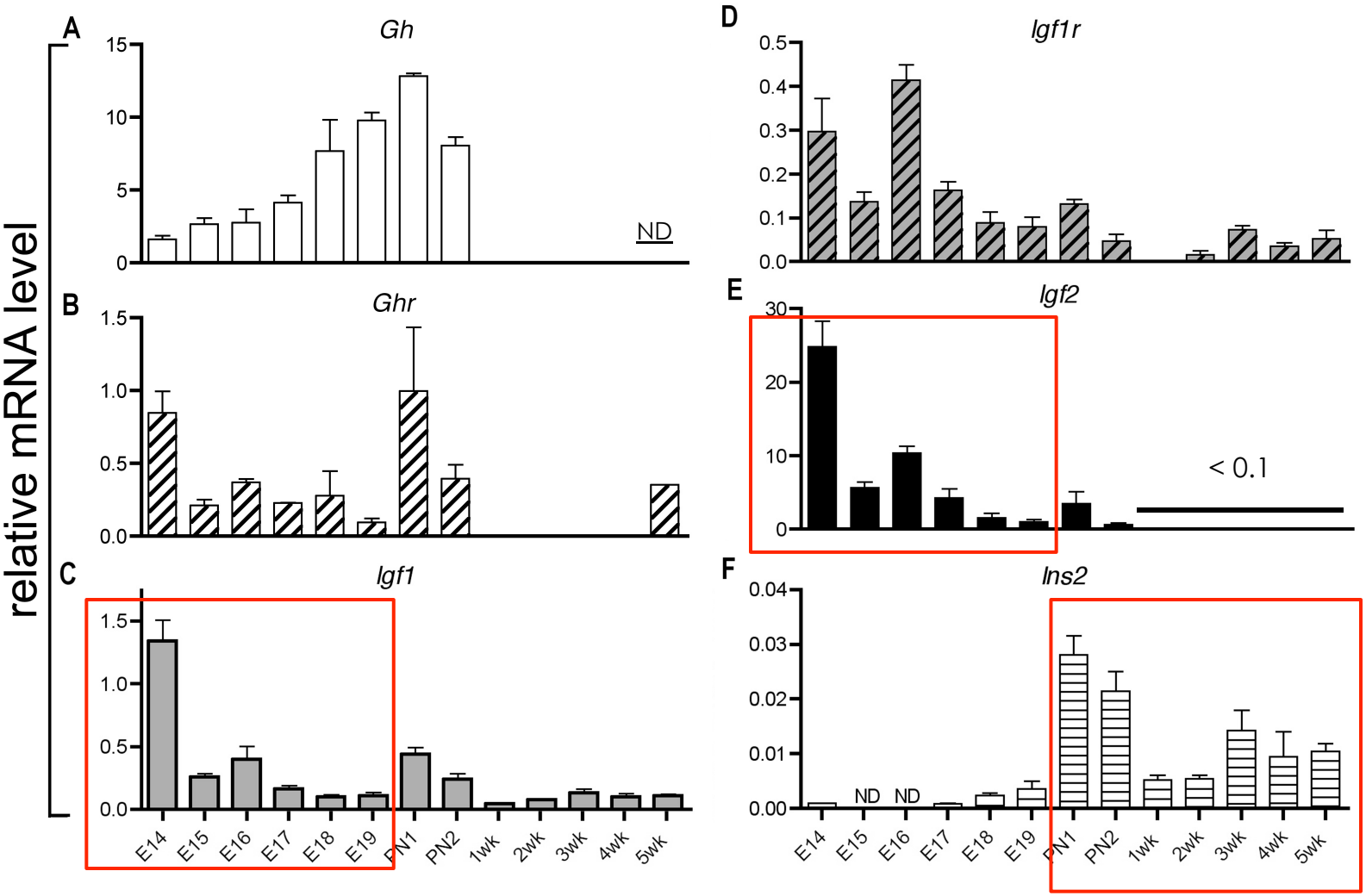
Nature Reviews | Immunology

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

Thymic expression of *INS*-related genes and tolerance to β cells

- Hierarchy and topography of intrathymic transcription:
  IGF2 (cTEC + mTEC) > *IGF1* (cTEC + mTEC) >> *INS* (rare subsets of mTEC)

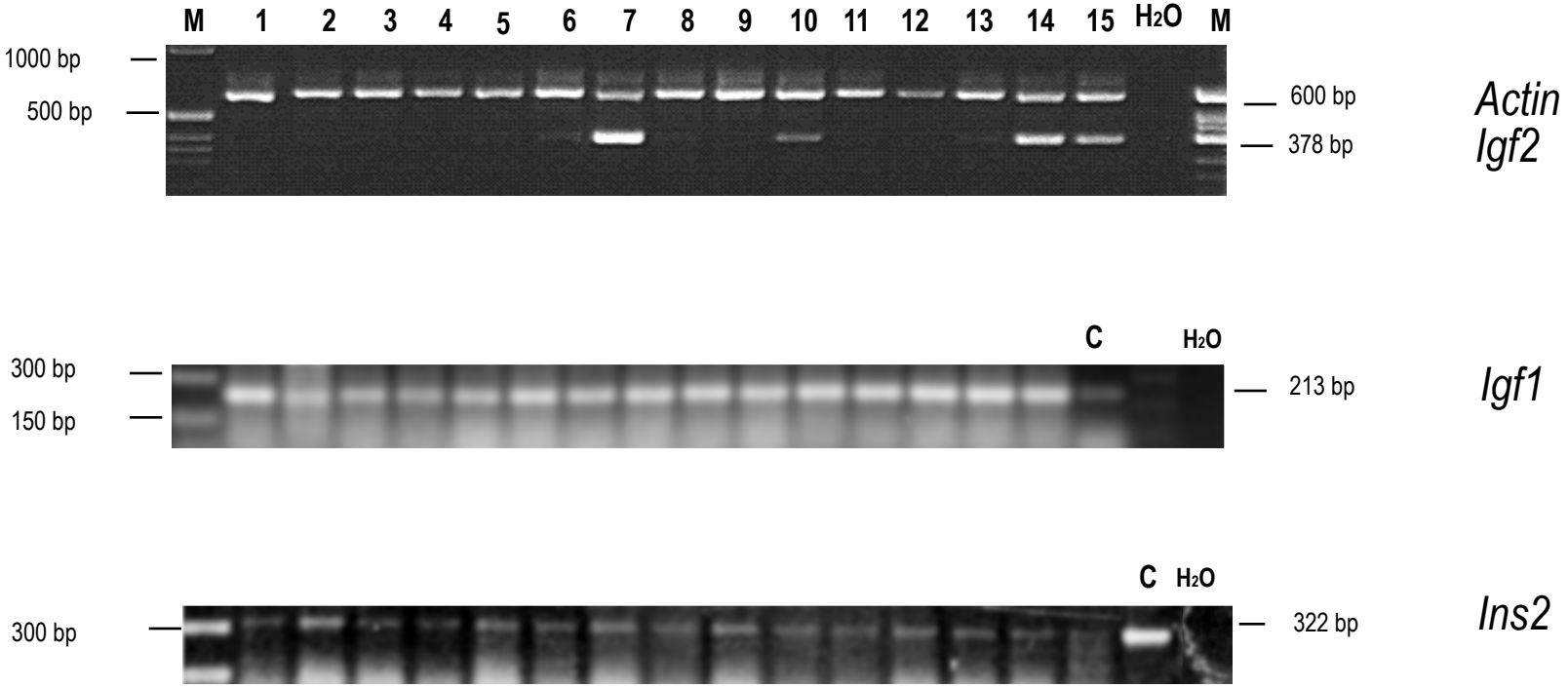
- Tolerance primarily concerns dominant epitopes
  - Tolerance to IGF-2 > IGF-1 >> Insulin
  - Active immunization (experimental breakdown of tolerance) :
    Frequency and titers of Abs to IGF-2 < IGF-1 << Insulin

- Oral Insulin and residual β cell function
  DPT (insulin administration to children at high risk)
  Anaphylaxis in NOD mice vaccinated with Insulin B9-23

  No tolerance !

- Immunogenicity of Insulin and prevalence of IAA (± 5% of whole population): related to the very low level of *INS* transcription in the thymus?

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



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

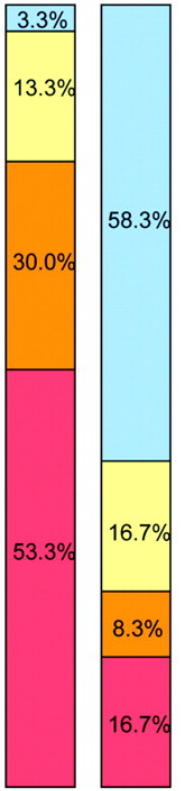
APS-I or APECED syndrome

- 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

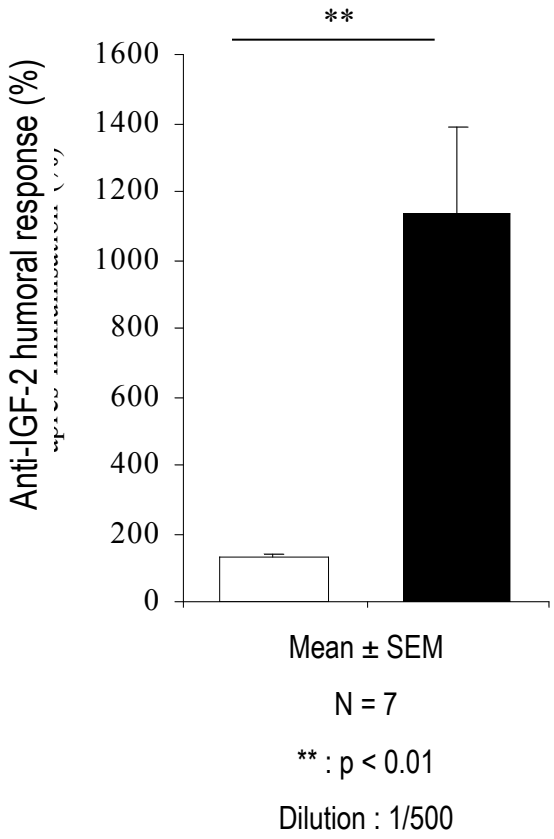


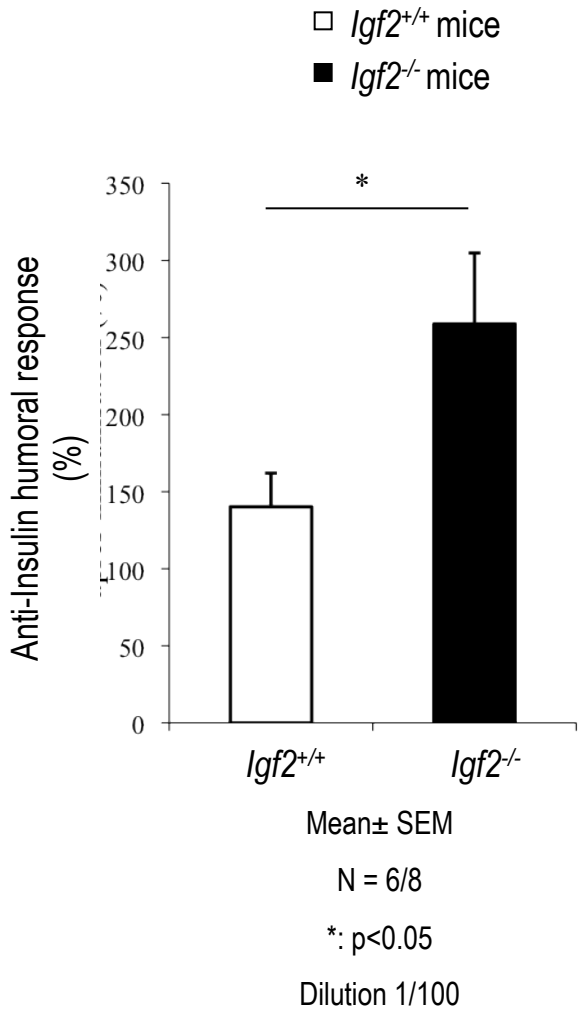
Anderson MS et al., *Science* (2002) 298:1395-1401

Transgenic mice models in autoimmune diabetes

T1D auto-antigens	Characteristics	Transgenic NOD	Influence on diabetes
GAD	Catalyzes GABA synthesis. Two isoforms : GAD65 and GAD67 GAD67 >> GAD65 in mTEC	*GAD65-/-* NOD *GAD67-/-* mouse GAD65/GAD67 antisense transgene (insulin promoter)	Insulitis/diabetes Death Suppression of diabetes
IA-2	Protein tyrosin phosphatase-like molecule. Two isoforms: IA-2 and IA-2b.	*IA-2-/-* NOD *IA-2b-/-* NOD	Insulitis/diabetes Insulitis/diabetes
ICA69	Neuroendocrine protein. Unknown function	*ICA69-/-* NOD	Insulitis/diabetes
Insulin	Two genes present in the mouse genome: *Ins1* and *Ins2* *Ins1* predominates in islet β cells *Ins2* predominates in mTEC	*Ins1-/-* NOD *Ins2-/-* NOD *Ins1-/-* x *Ins2-/-* NOD *Ins1-/-* x *Ins2* -/-Thy	Reduced insulitis/diabetes and T1D auto-antibodies Increased insulitis/diabetes and T1D auto-antibodies No insulitis/diabetes No T1D auto-antibodies **T1D diabetes in 3 weeks**

Contribution of *Igf2* expression to immunological tolerance toward INS

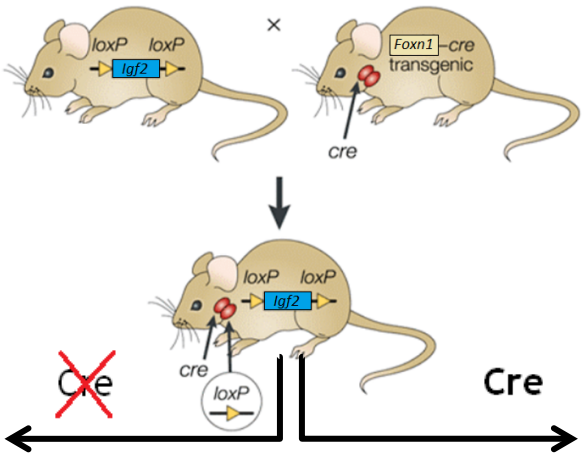




Hansenne I et al. *J Immunol* (2006) 176:4651-7

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

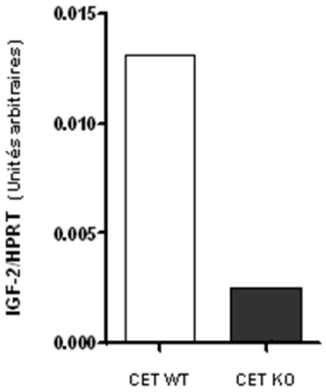
(Pr M. Constancia, University of Cambridge)



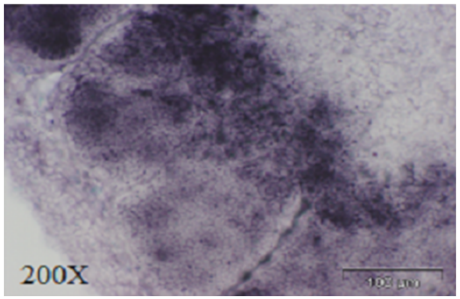
(Pr G. Holländer, University of Basel)

Igf2 $^{fl/fl}$ Control

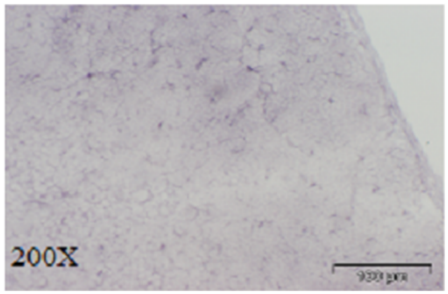
Igf2 $^{\Delta Thy}$



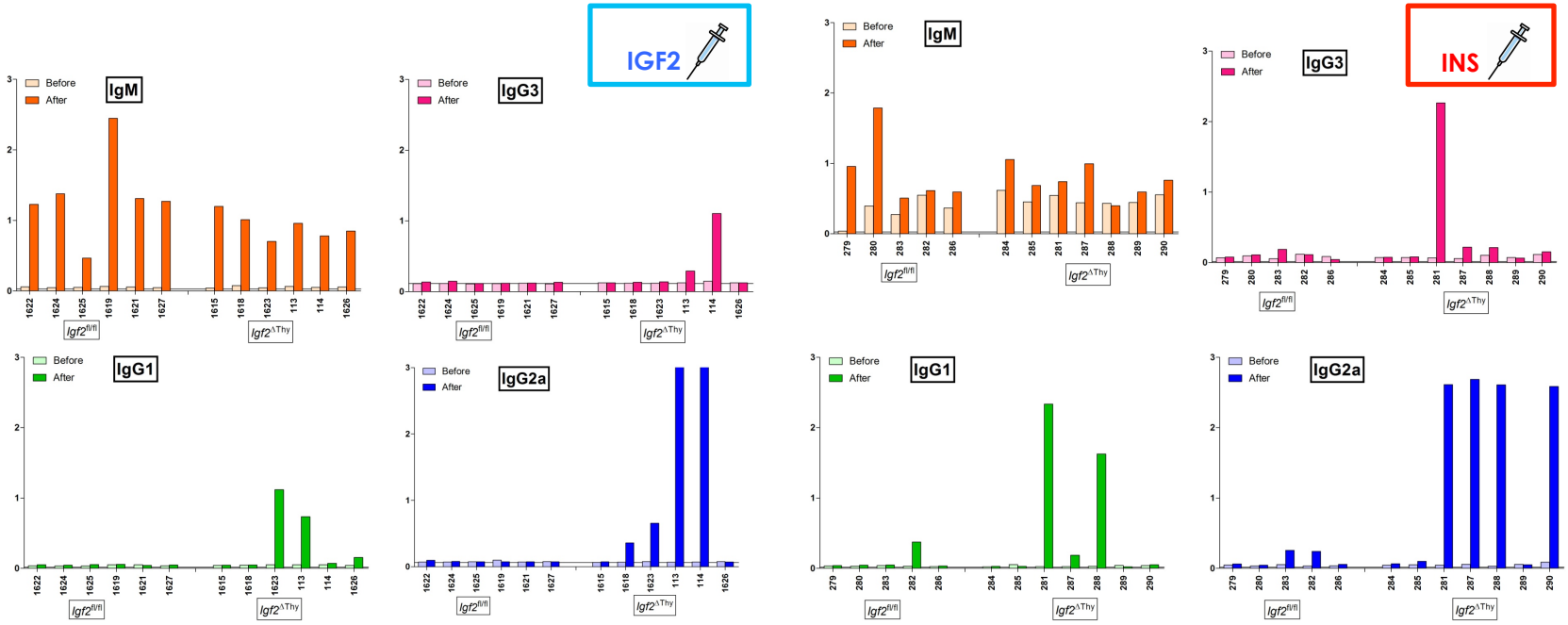
Thymus *Igf2* $^{fl/fl}$ 20 µm



Thymus *Igf2* $^{\Delta Thy}$ 20 µm



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



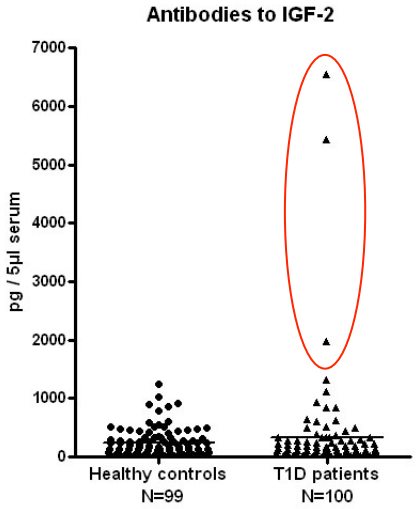
- Despite its ubiquitous expression, *Igf2* deletion in the sole thymus leads to loss of tolerance toward IGF2.
- *Igf2* deletion in the sole thymus also lowers the level of immunological tolerance toward INS (central cross-tolerance between IGF2 and INS).

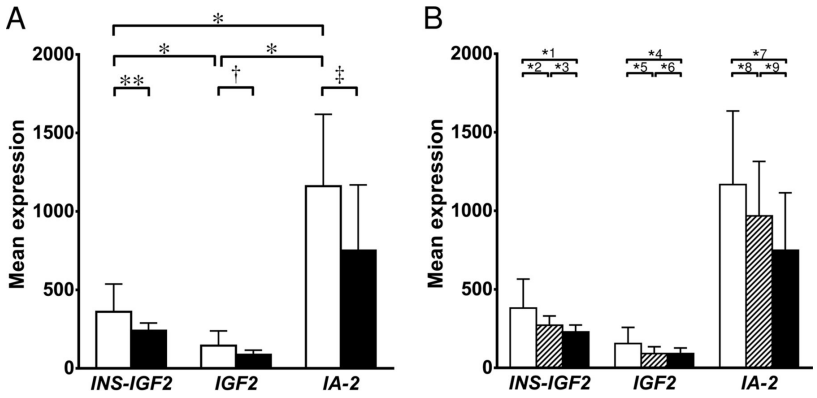
Bodart G et al. *Submitted*

Humoral response to IGF-2 in T1D patients

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

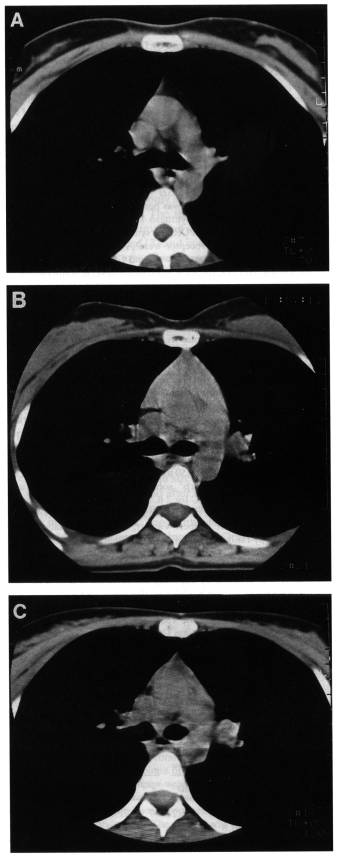
Quantification
Standard curve of a monoclonal antibody anti- human IGF-2 (CBL82)





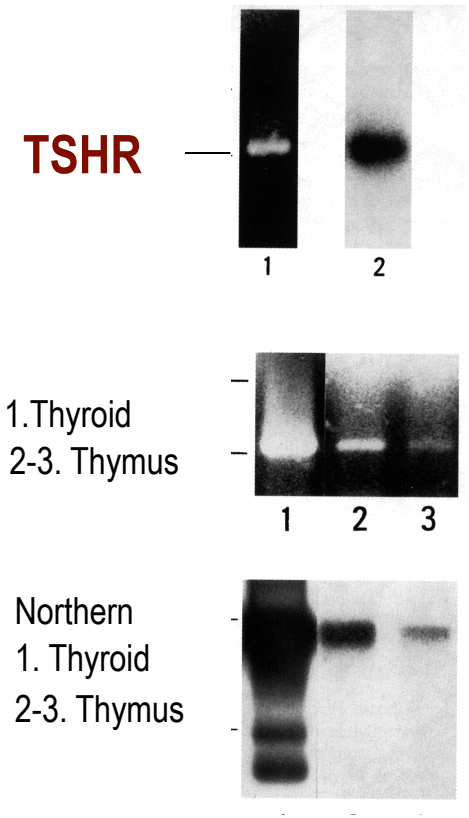
Kanatsuna N et al. *J Biol Chem* (2013) 288:29013-23

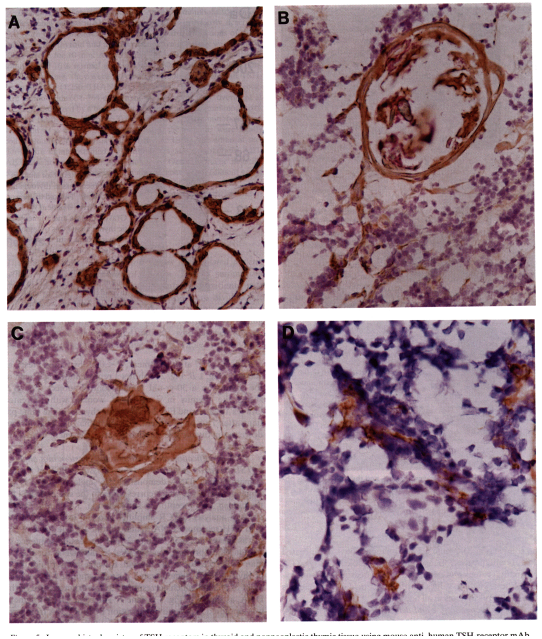
Thymus and Graves' disease (Type 3 AI thyroiditis)



CT-scan

A. Control
B. Before treatment
C. After treatment



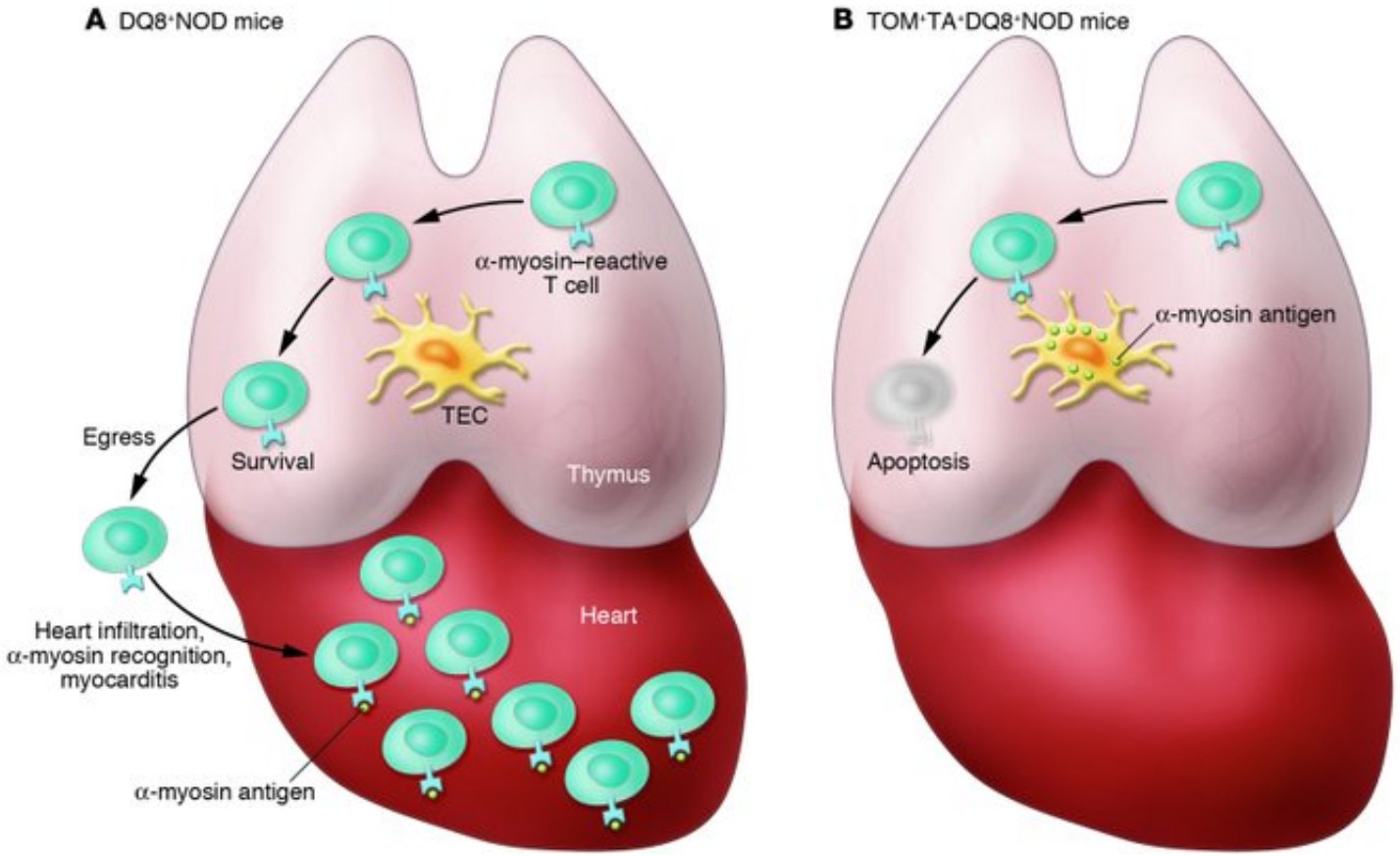


ICC for TSHR

A. Thyroid
B-D. Thymus

Paschke R & Geenen V *J Mol Med* (1995) 73:577-80
Murakami M et al. *J Clin Invest* (1996) 98:2228-34
Colobran R et al. *Hum Mol Genet* (2011) 20:3415-23

Defect in thymic tolerance to α-myosin promotes autoimmune myocarditis



Lv H et al. *J Clin Invest* (2011) 121:1561-73

A thymus defect in autoimmune neuroendocrine diseases

Thymus physiology

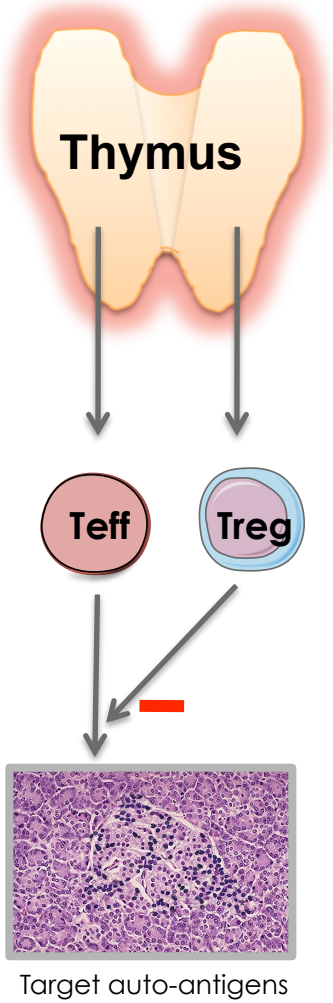
- AIRE-regulated transcription of neuroendocrine self-peptides in thymus epithelium.
- Deletion of T cells with high affinity for MHC/neuroendocrine self-peptide complexes.
- Selection of CD4+ CD25+ Foxp3+ tTreg, specific of neuroendocrine self-peptides.

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

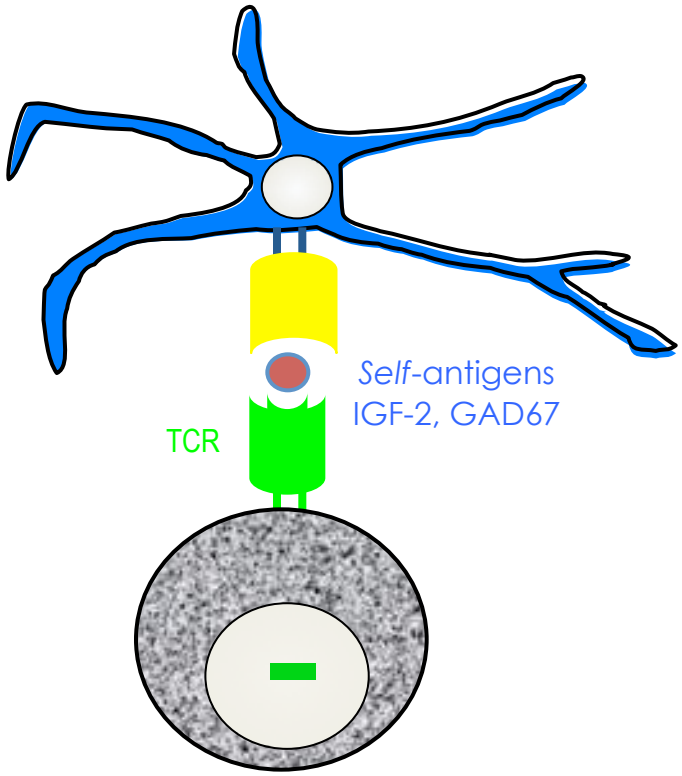
- Role of environmental factors (**viruses**, diet, vitamin D deficiency, stress…)



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

The concept of « negative *self*-vaccination »

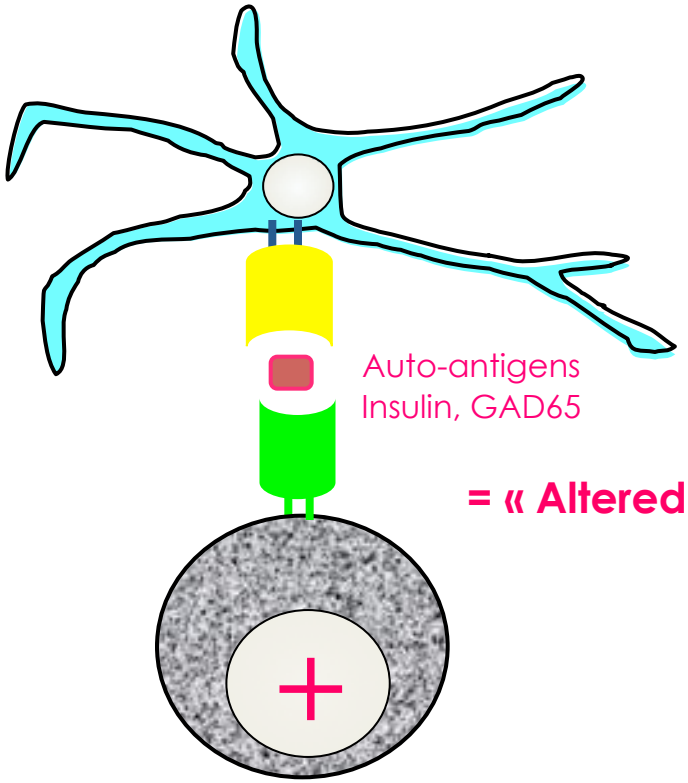
Thymus antigen-presenting cell



SELF-TOLERANCE TO β CELLS

Clonal deletion of self-reactive T cells
Generation of specific yTreg

Islet antigen-presenting cell



= « Altered » *self*

AUTOIMMUNITY TO β CELLS

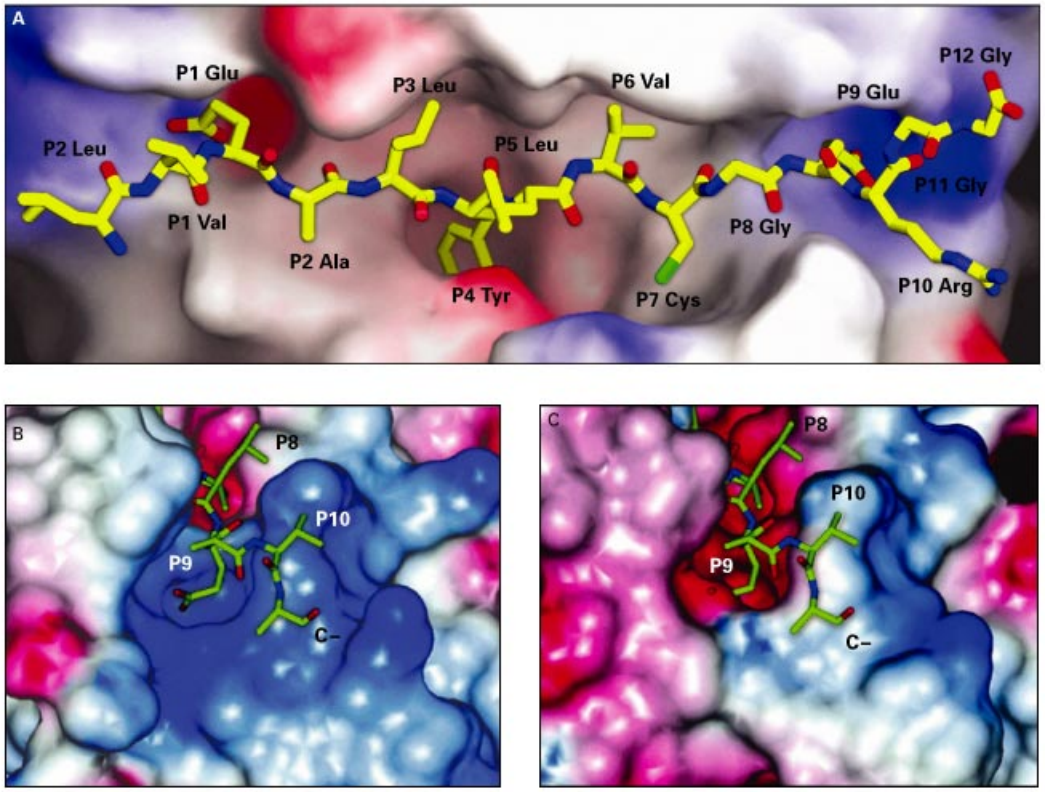
Activation of self-reactive T cells
Induction of memory T cells

Geenen V et al. *Curr Opin Pharmacol* (2011) 10:461-72

DQ8 presentation of Insulin $B_{9\text{-}23}$

In memoriam

Don Craig WILEY
(1944-2001)



Lee W, Wucherpfennig K & Wiley D, *Nat Immunol* (2001)

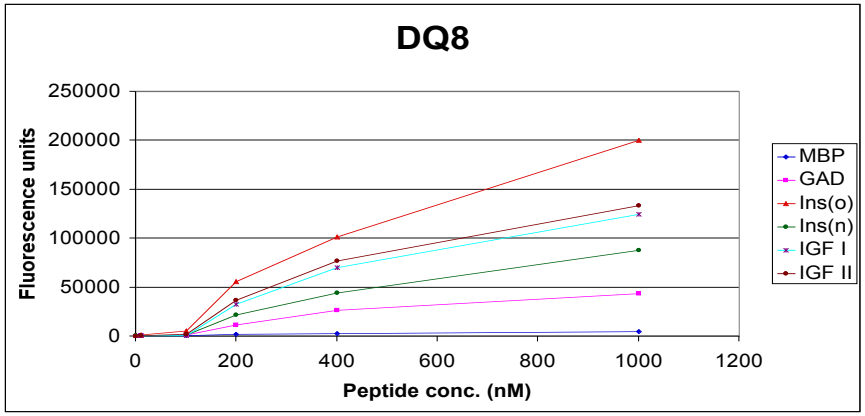
HLA-DQ8 = combination chain α (HLA-DQA1*0301) + ß (HLA-DQB1*0302)

HLA-DQ2 = combination chain α (HLA-DQA1*0501) + ß (HLA-DQB1*0201)

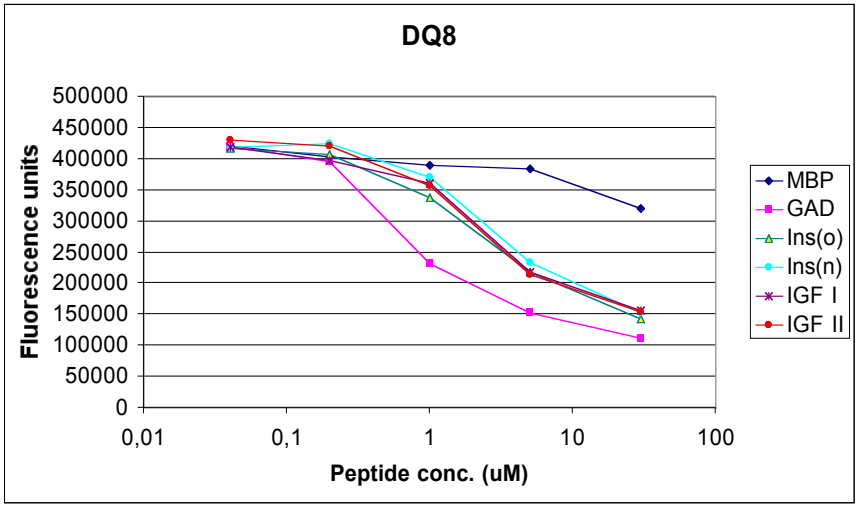
Both alleles confer major susceptibility to T1D

Binding to DQ8 of INS and IGF-2 homologous sequences

Direct binding



Competition

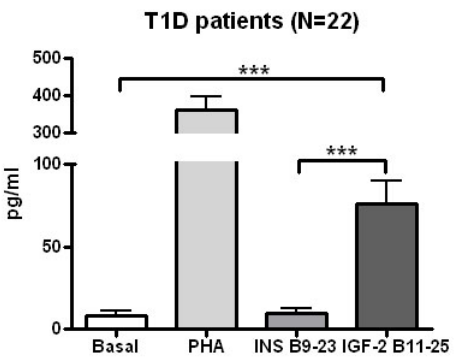


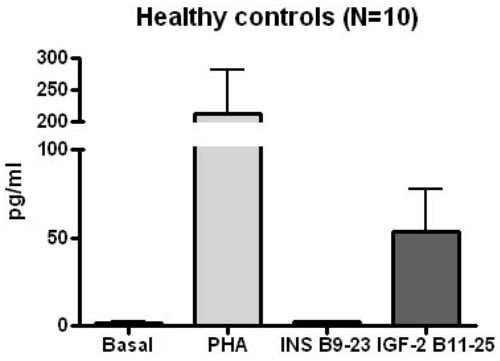
Ins B9-23 = SHLVEALYLVCGERG

IGF-2 B11-25 = GELVDTLQFVCGDRG 53% (8/15) homology

Immune cellular response to IGF-2

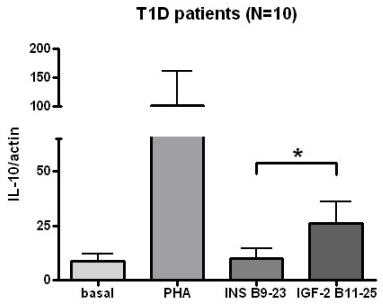
IL-10

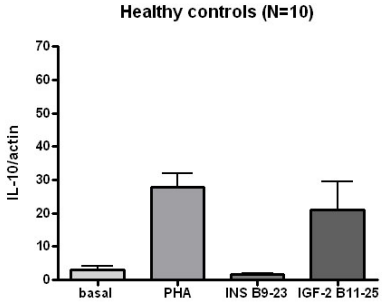




M ± SEM
* p < 0.05
*** p < 0.001 *t*-test

IL10 transcripts

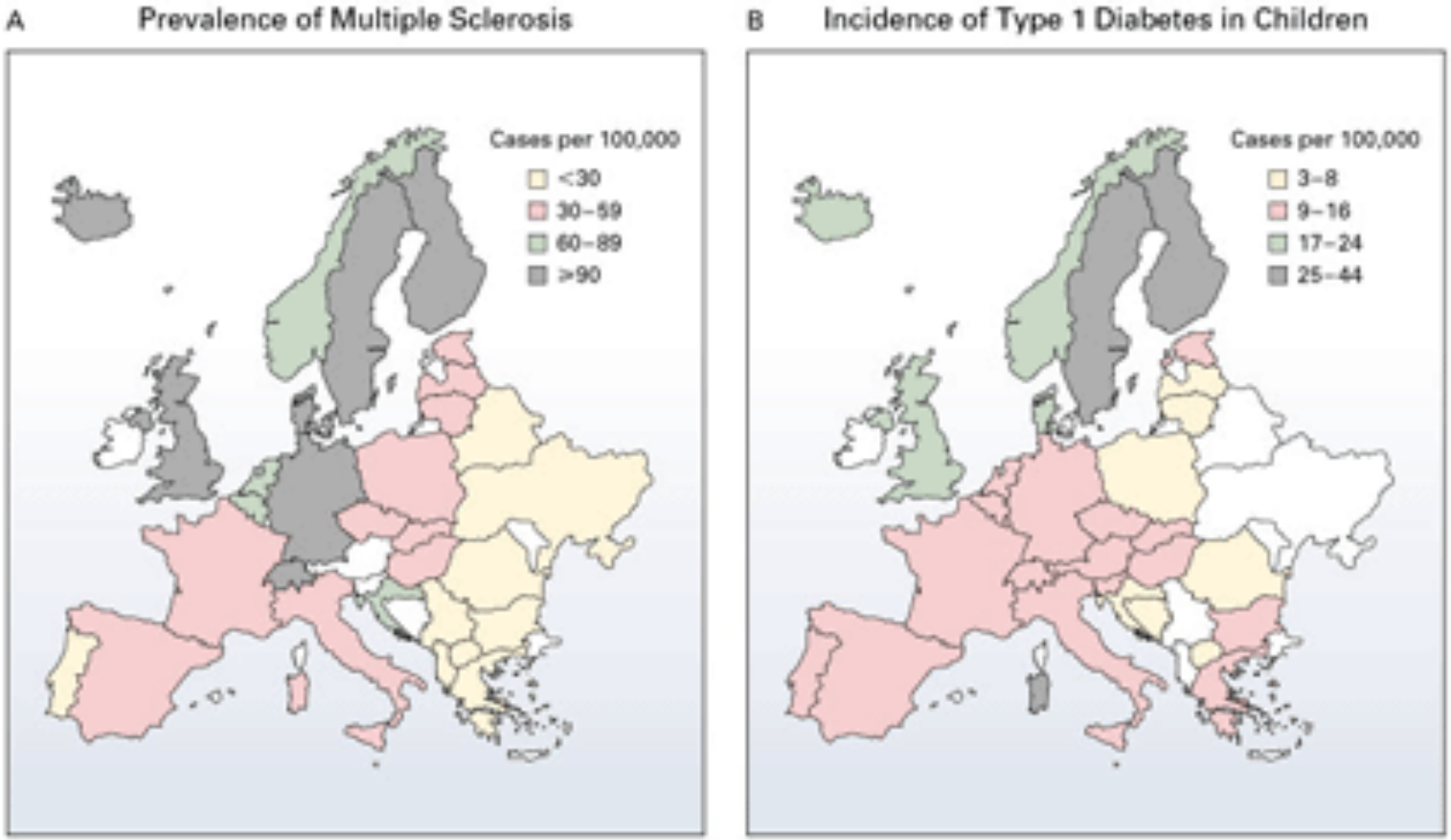




Geenen V et al. *Ann NY Acad Sci* (2006) 1037:59-64

The Role of Environment in T1D Pathogenesis

N-S gradient in MS and T1D incidence



Concordance of T1D in monozygotic twins: ± 40%

Coxsackie B (CVB)
- Epidemiological studies (serology, PCR)
- Virus isolated in pancreas of T1D dead child, able to induce autoimmune diabetes after injection to *susceptible* mice

BUT LACK OF ANY DIRECT EVIDENCE

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.

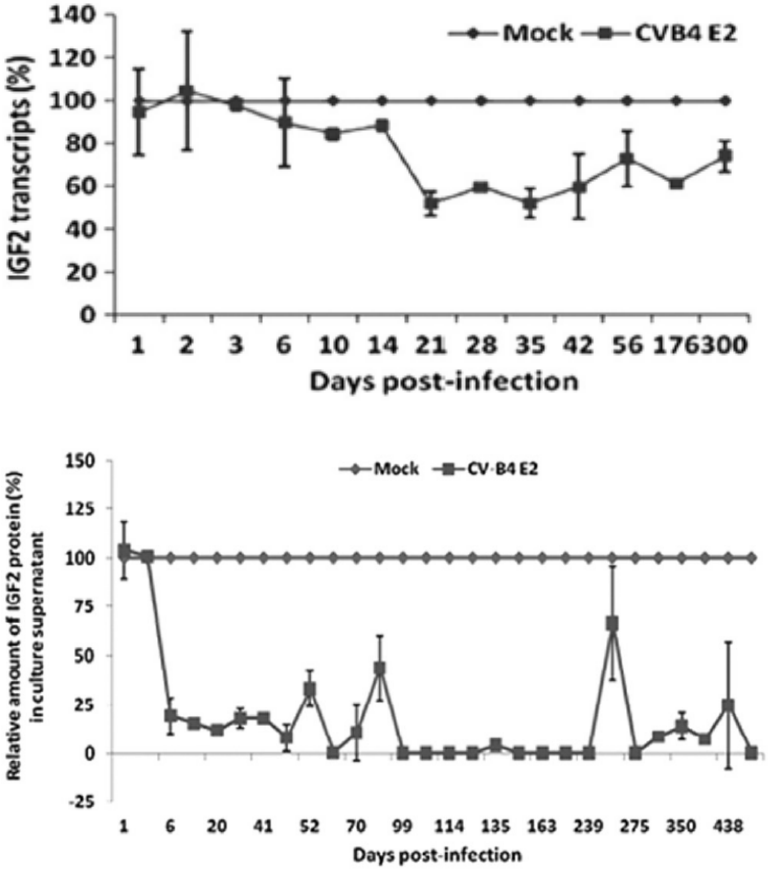
- Persistent infection of human thymic epithelial cells by Coxsackievirus B4.
  F. Brilot *et al*. *J Virol* (2002) 76:5260-5265.

- 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

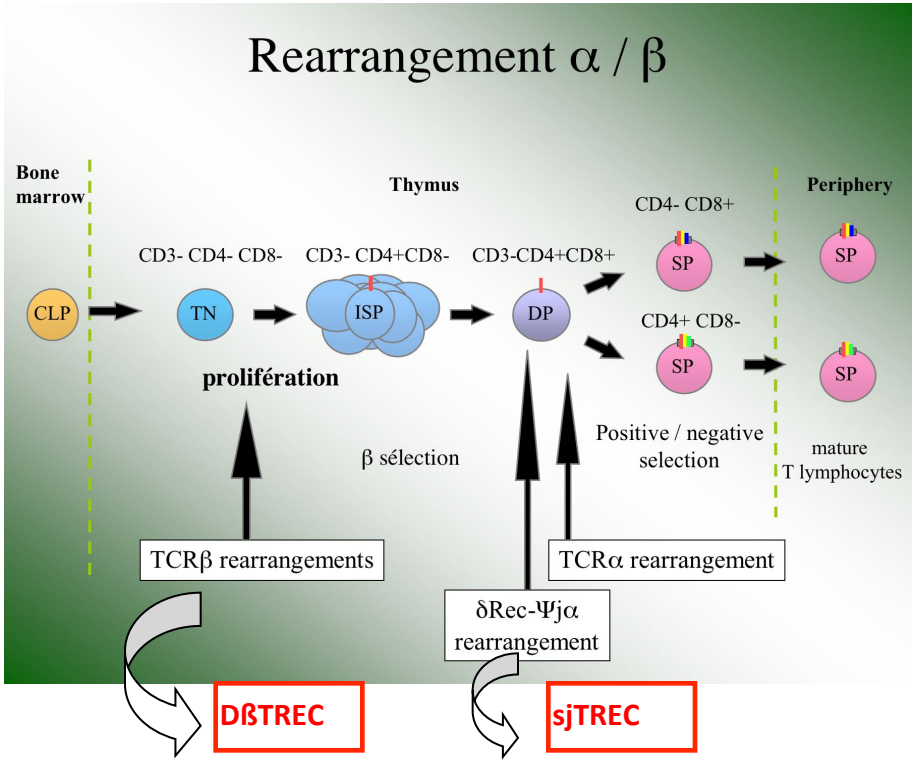
- The presentation of ***neuroendocrine self-peptides*** in the thymus ensured an integrated and harmonious evolution between the neuroendocrine and adaptive immune systems.

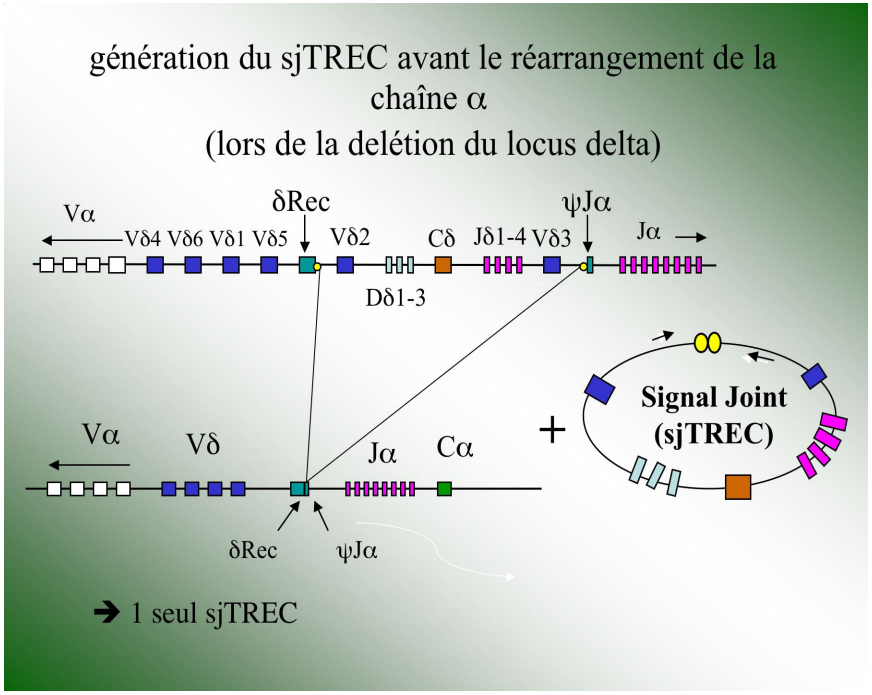
- A thymus dysfunction in programming central self-tolerance plays a primary role in the development of a specific autoimmune response directed against neuroendocrine organ/cell-restricted antigens.

- 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.

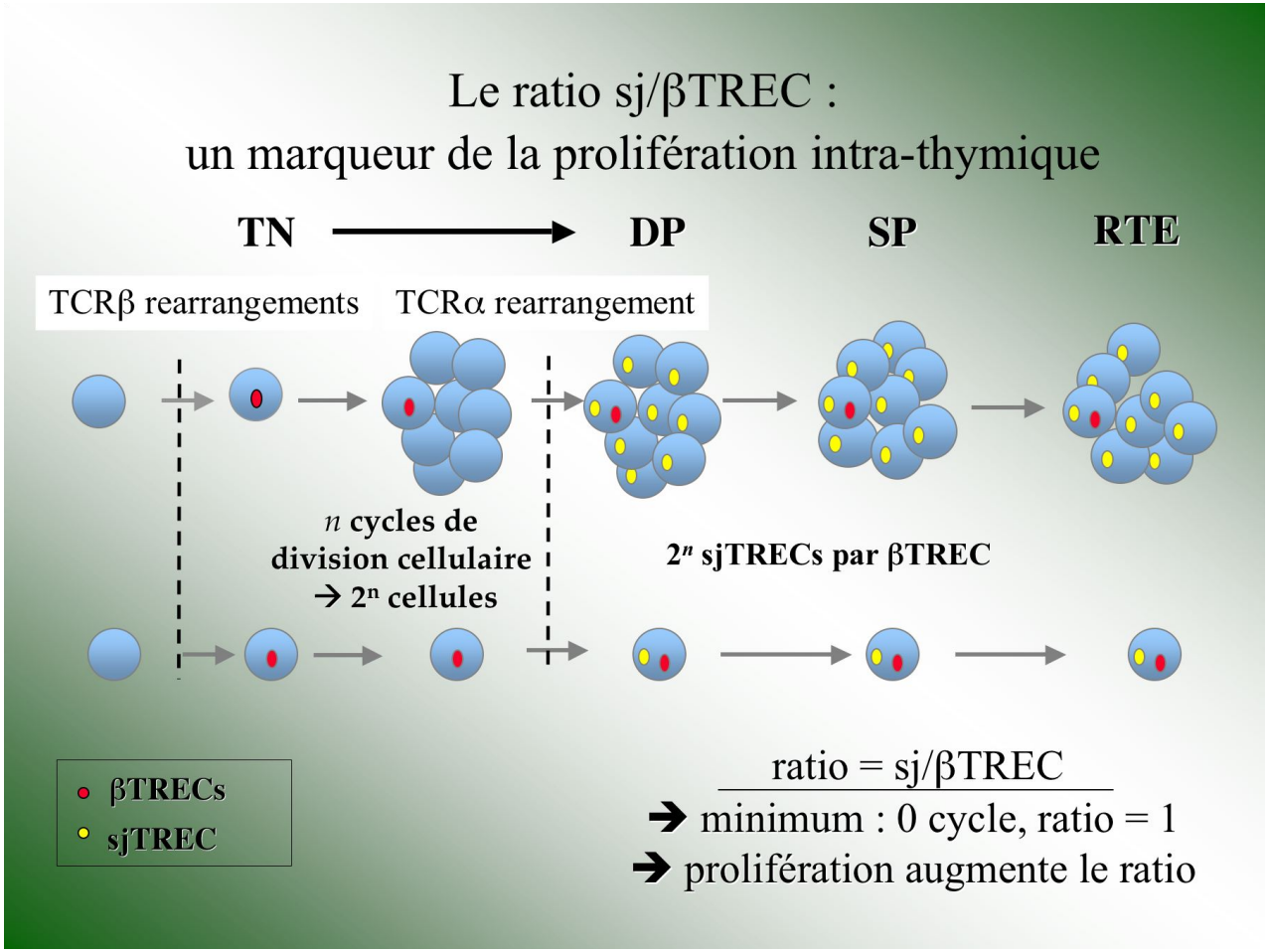
Clinical Investigation of Thymus Function

TCR excision circles: DβTREC and sjTREC

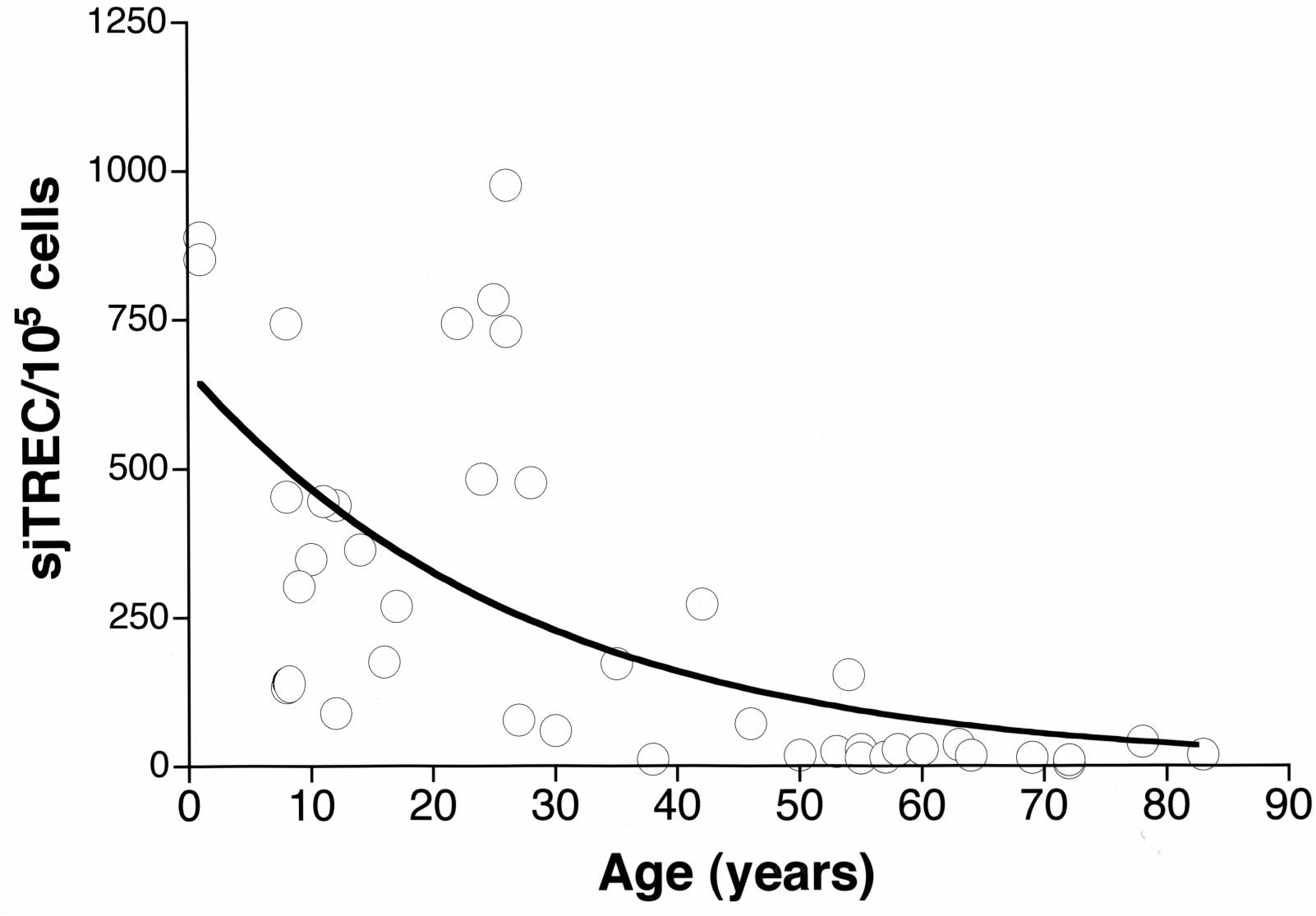




Sj/DβTREC ratio: a marker of intrathymic pre-T cell proliferation



SjTREC frequency during aging



Geenen V et al. *J Endocrinol* 176: 305-11, 2003

The somatotrope GH/IGF-1 axis regulates the maintenance of adult thymopoiesis

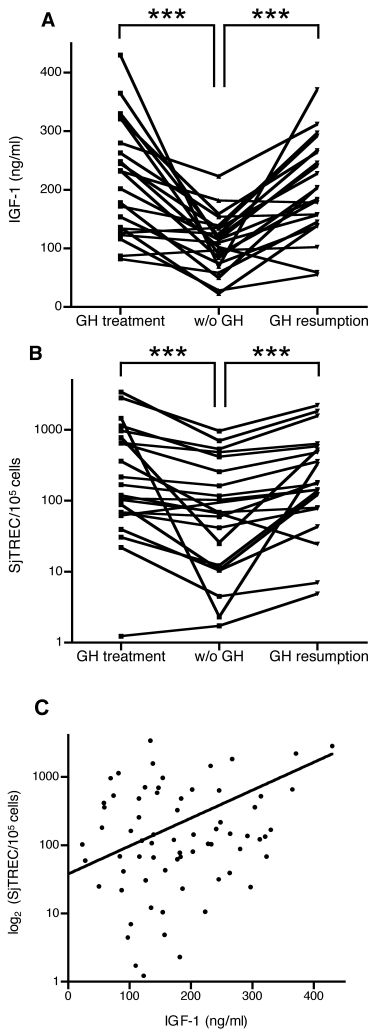


Figure 1

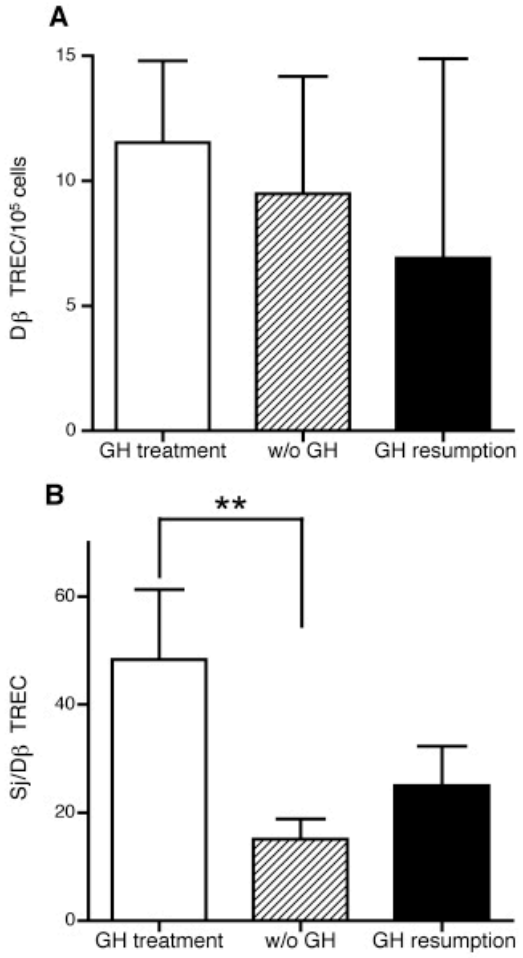


Figure 2

Morrhaye G et al. *PLoS ONE* 4: e5668, 2009

Acknowledgments

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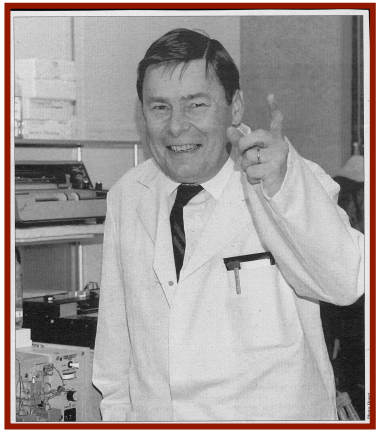

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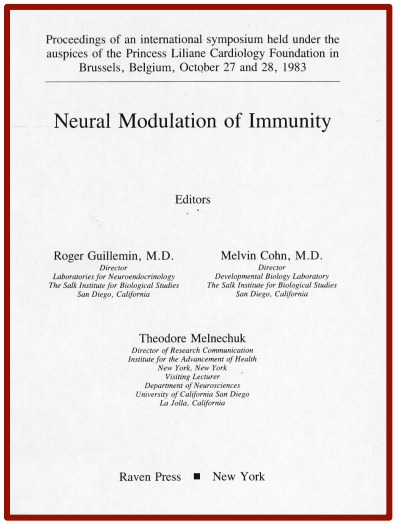

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Neural Modulation of Immunity

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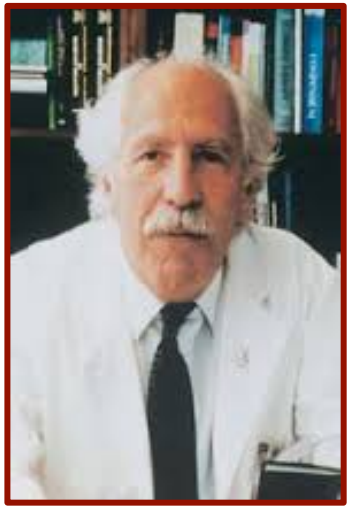

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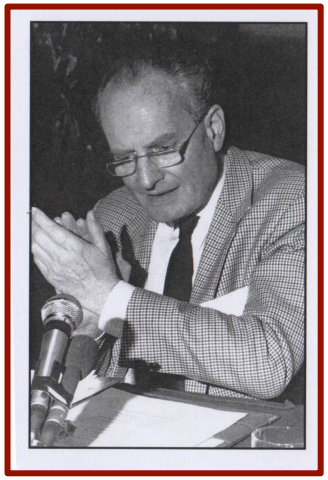

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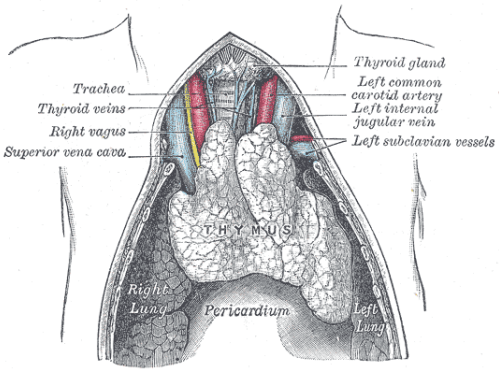

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