GIGA-I^3 Center of Immunoendocrinology

The Physiology of The Thymus

From an 'accident of evolution' to the unique organ programming self-tolerance

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Head of clinics at University Hospital of Liege

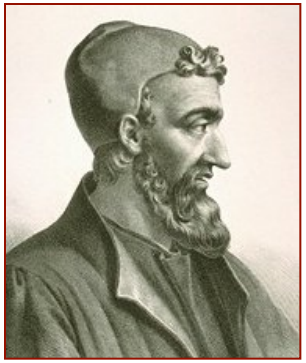
University of Maastricht − 13 November 2017

Paul EHRLICH (1854-1915)

Nobel Prize in physiology or medicine 1908 with Ilya METCHNIKOFF

« Horror autotoxicus »

The moving place of the thymus in the history of medicine

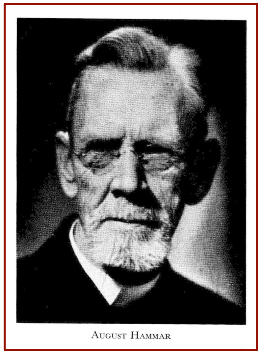


Claude Galen – 2nd father of Western medicine (129 – 230 AD)
Born in Pergame (Ionian Greece, now Turkey)

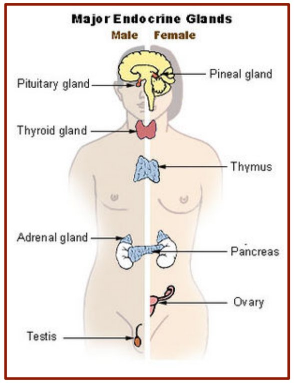
Thymos (Θymos) ← *Thymus cunula* (sarriette)

"A protection between the sternum and mediastinum vessels"

Temple of Peace, Roma



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

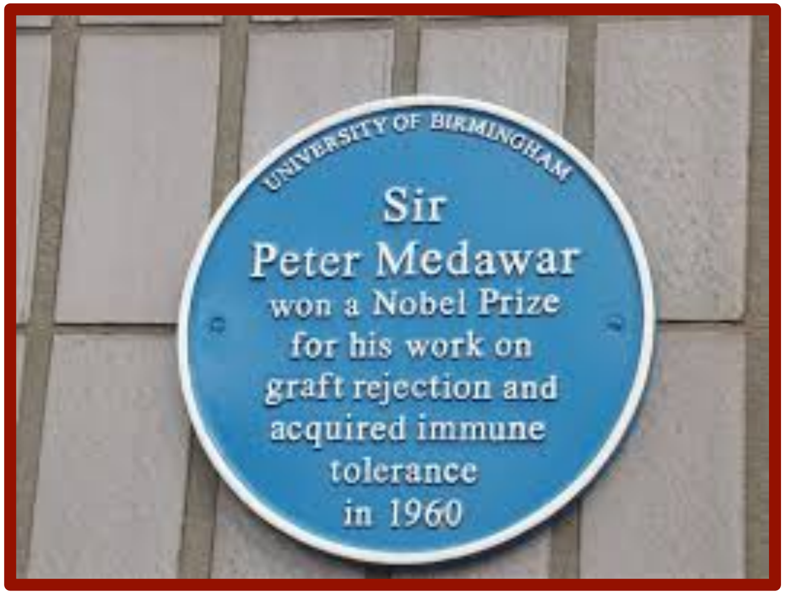


Jacques FAP Miller
Role of the thymus in murine leukemia. *Nature* (1959) 183:1069
Immunological function of the thymus. *Lancet* (1961) 2:748-9

Pollards Wood Research Station, UK

An ‘accident of evolution’

Peter Medawar and Frank MF Burnet



“We shall come to regard the presence of lymphocytes in the thymus as an evolutionary accident of no very great significance.”

Sir Peter MEDAWAR (1964)

The thymus is required for generation of diversity of the whole T-cell repertoire

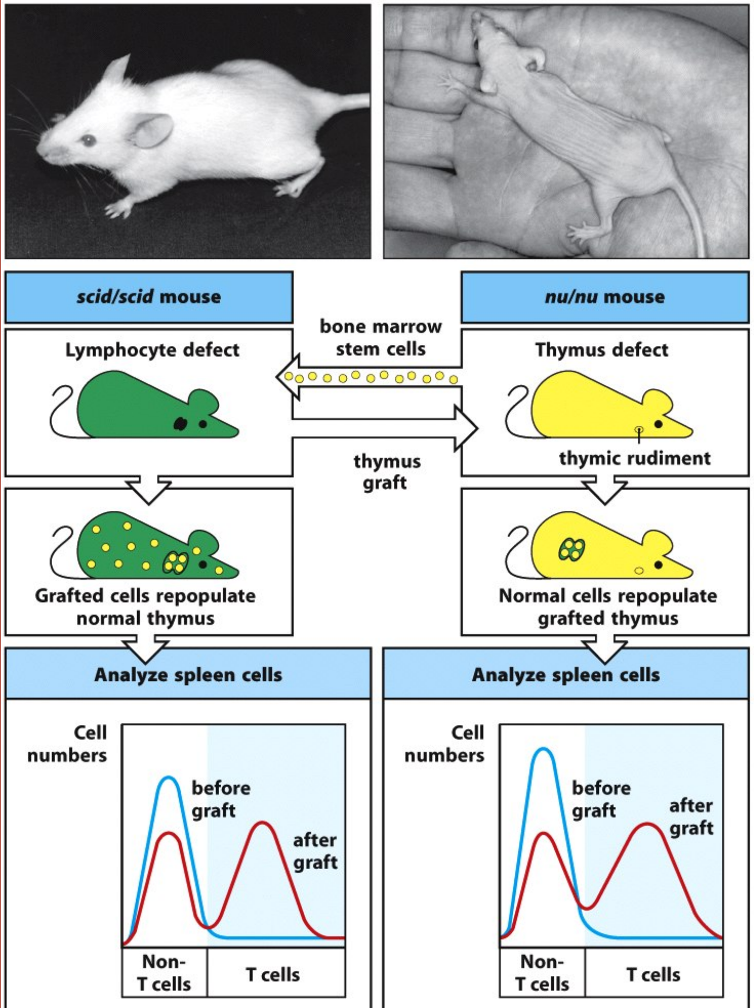
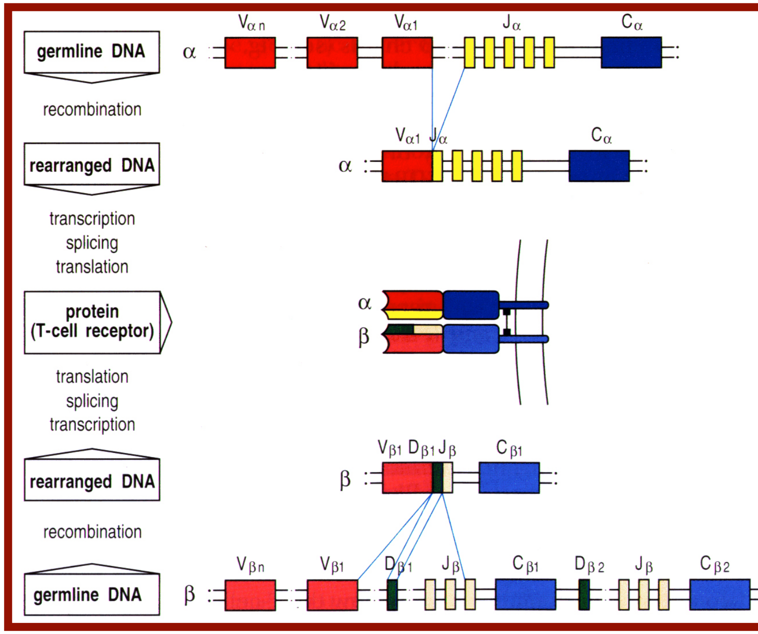


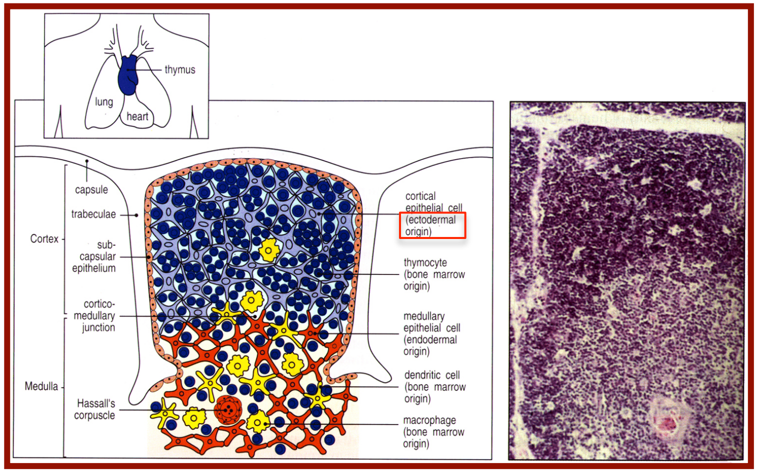
Figure 7-17 Immunobiology, 7ed. (© Garland Science 2008)



Quantification by **sjTREC** of the thymic output of new T cells

Quantification by **sj/Dβ TREC** of intrathymic T-cell proliferation

Cell populations in the thymus & Developmental biology



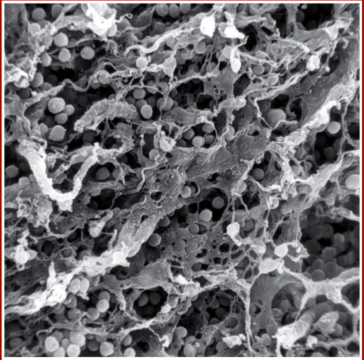
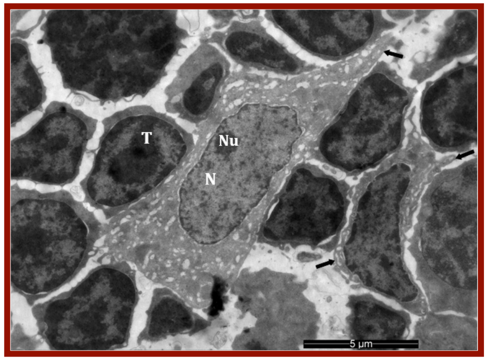


Figure 7-16 Immunobiology, 7ed. (© Garland Science 2008)

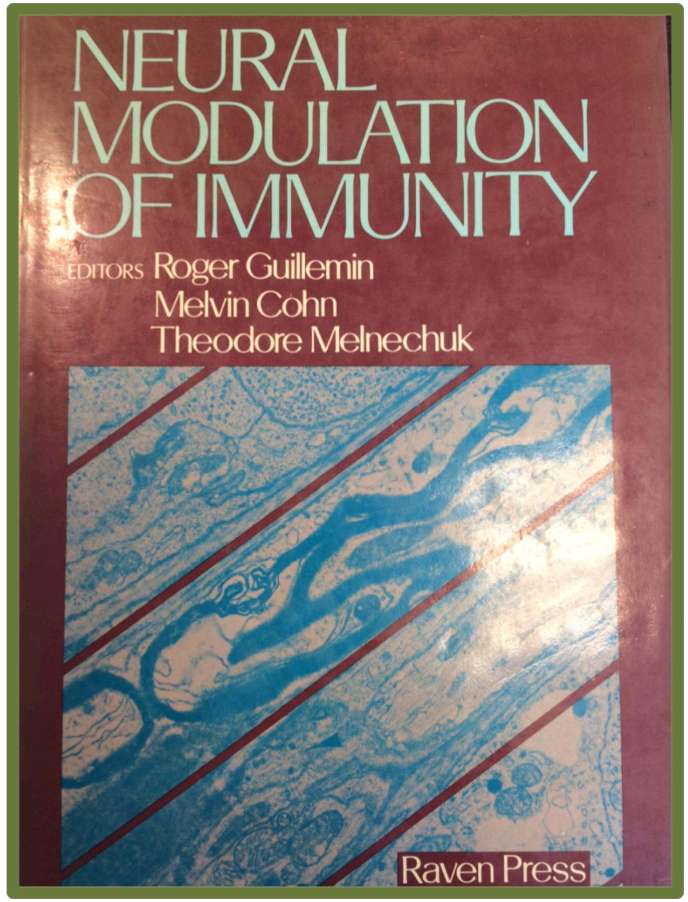


HOXA3 At ED10.5 expression in the 3rd pharyngeal pouch endoderm and neural crest mesenchyme. Absence of thymus and parathyroid hypoplasia in *Hoxa3*$^{-/-}$ mice.

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.

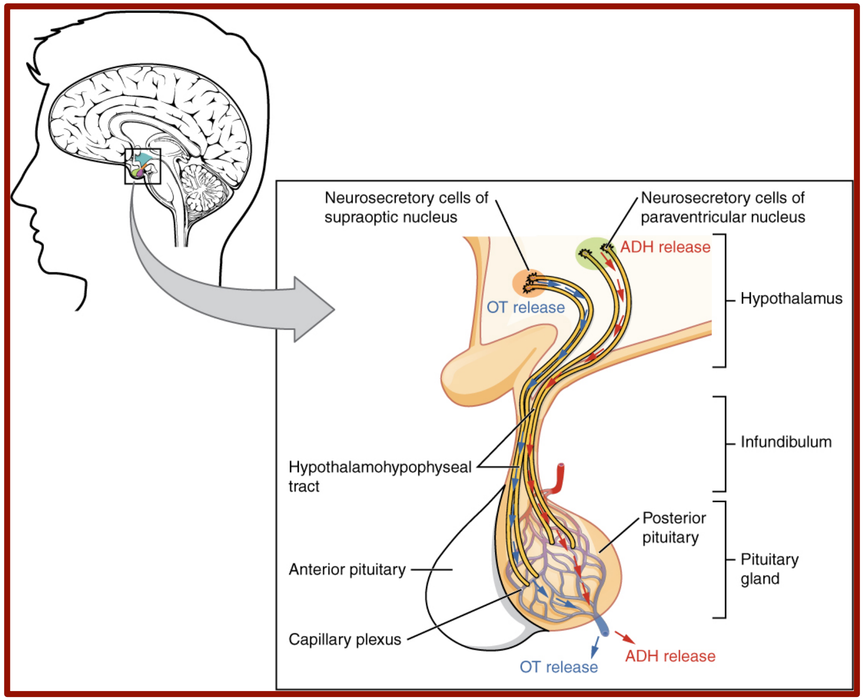
Symposium Princess Lilian Cardiology Foundation,

Brussels, 27-28 October 1983



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

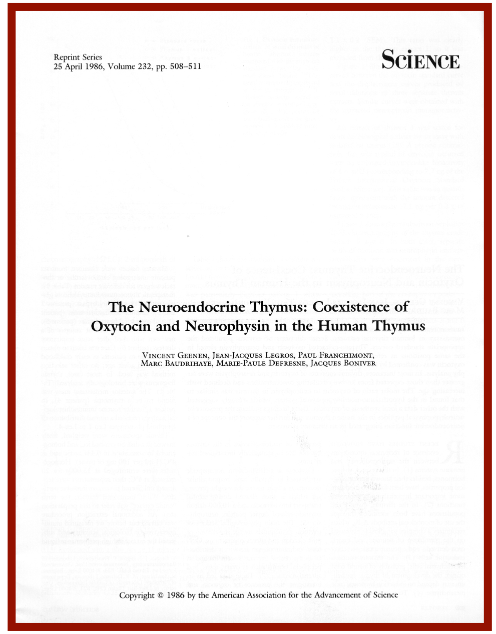
About the neurohormone/neuropeptide oxytocin (OT)



OT as a neurohormone

OT as a neuropeptide

The power of *metaphor*: application to thymic OT



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

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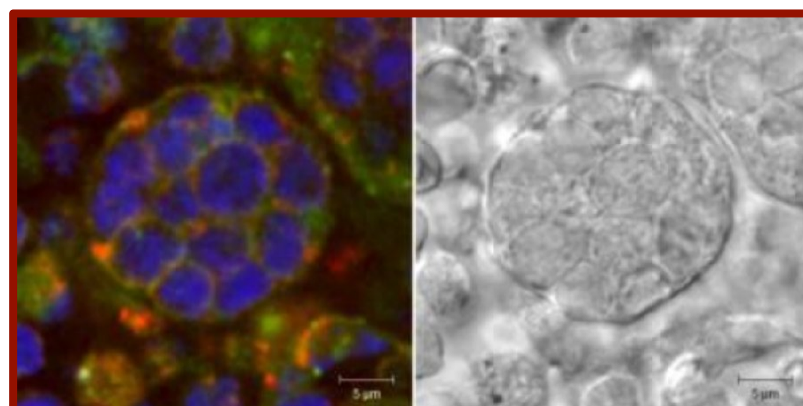
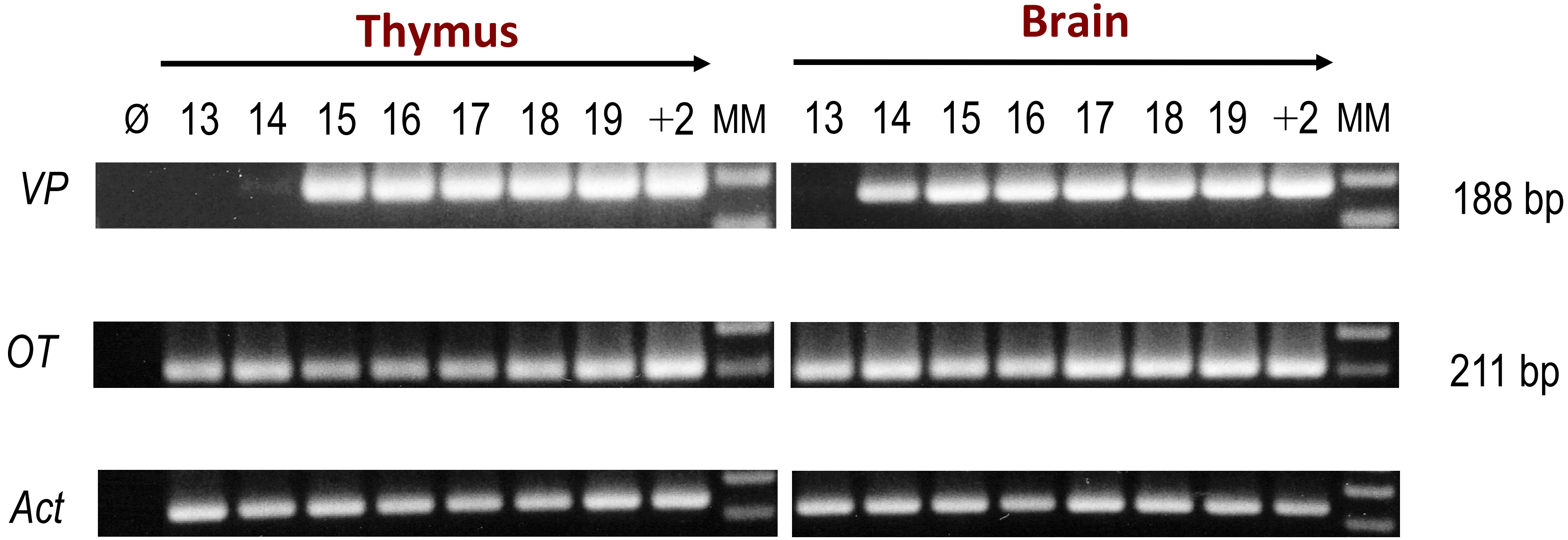


Thymic ‘nurse’ cells

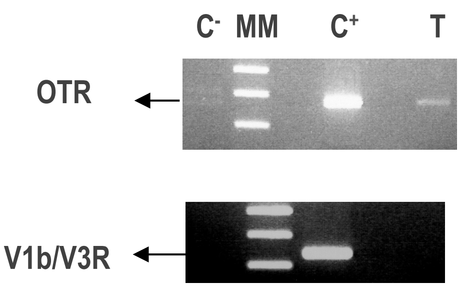
Ontogeny of *OT* and *VP* expression



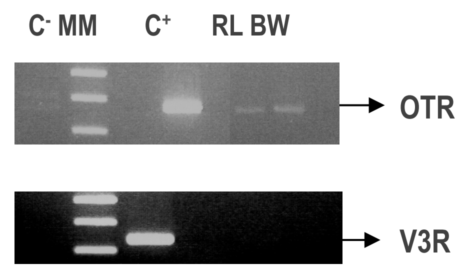
OT transcription coincides in brain and thymus, but precedes *VP* in both sites.
VP transcripts are not translated in the thymus!

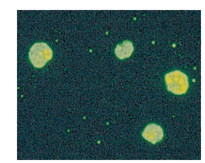
Neurohypophysial receptor expression by thymic T cells

THYMOCYTES



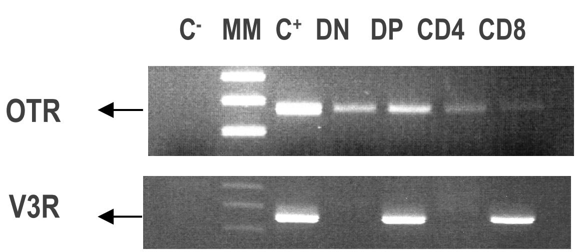
THYMIC CD4-8- LYMPHOMAS



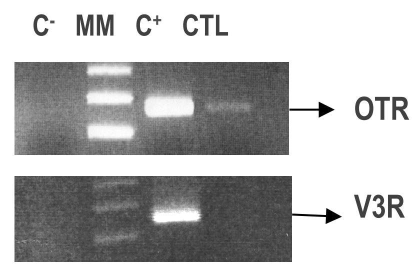


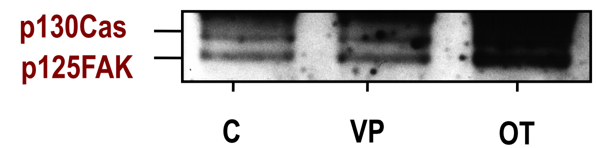
Anti-OTR mAb

THYMIC T CELL SUBSETS

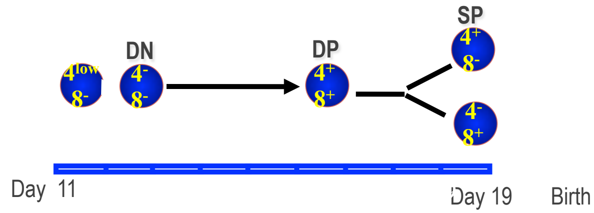


CD8+ T CELL LINE



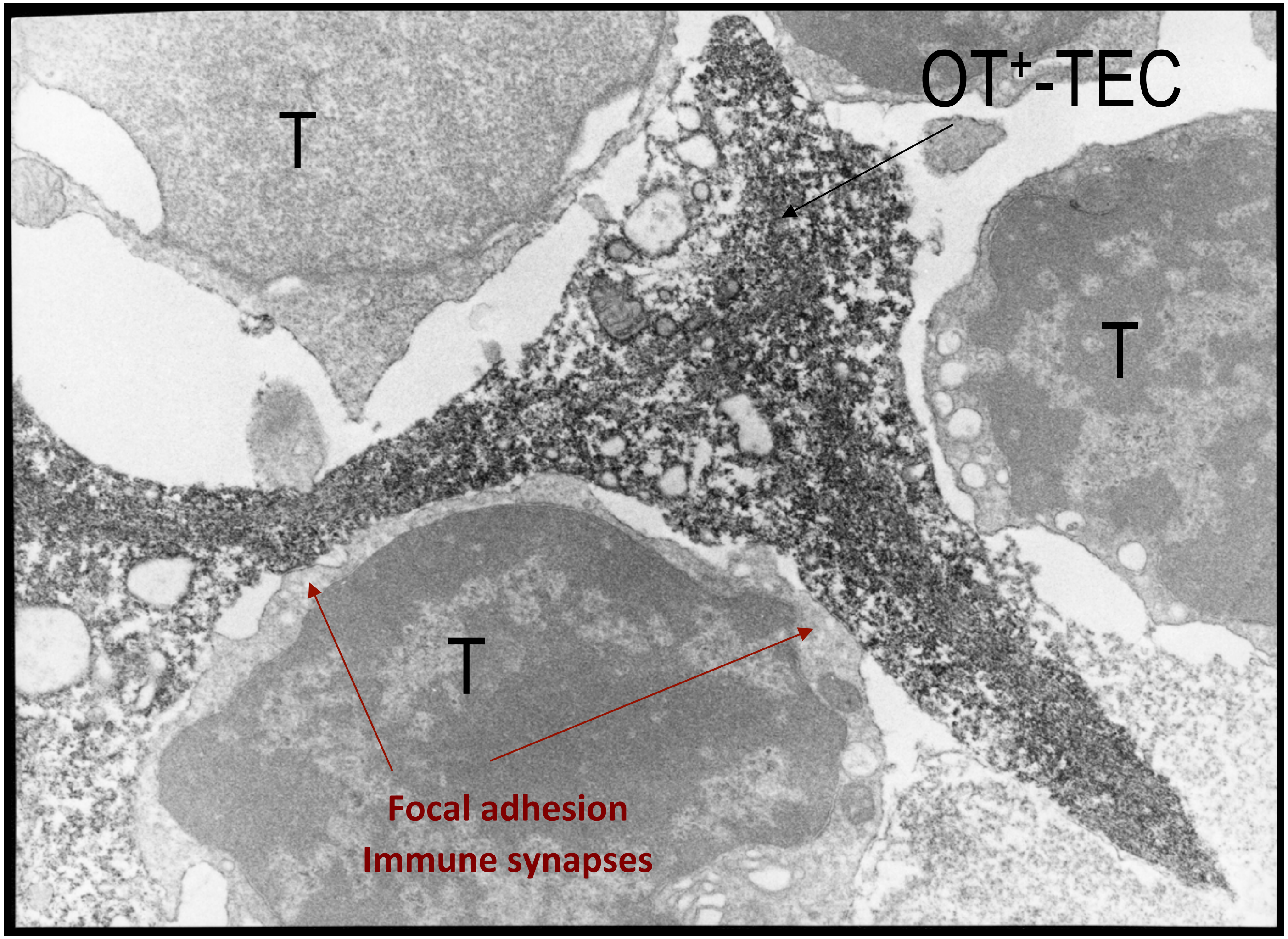


No expression of V1aR and V2R!



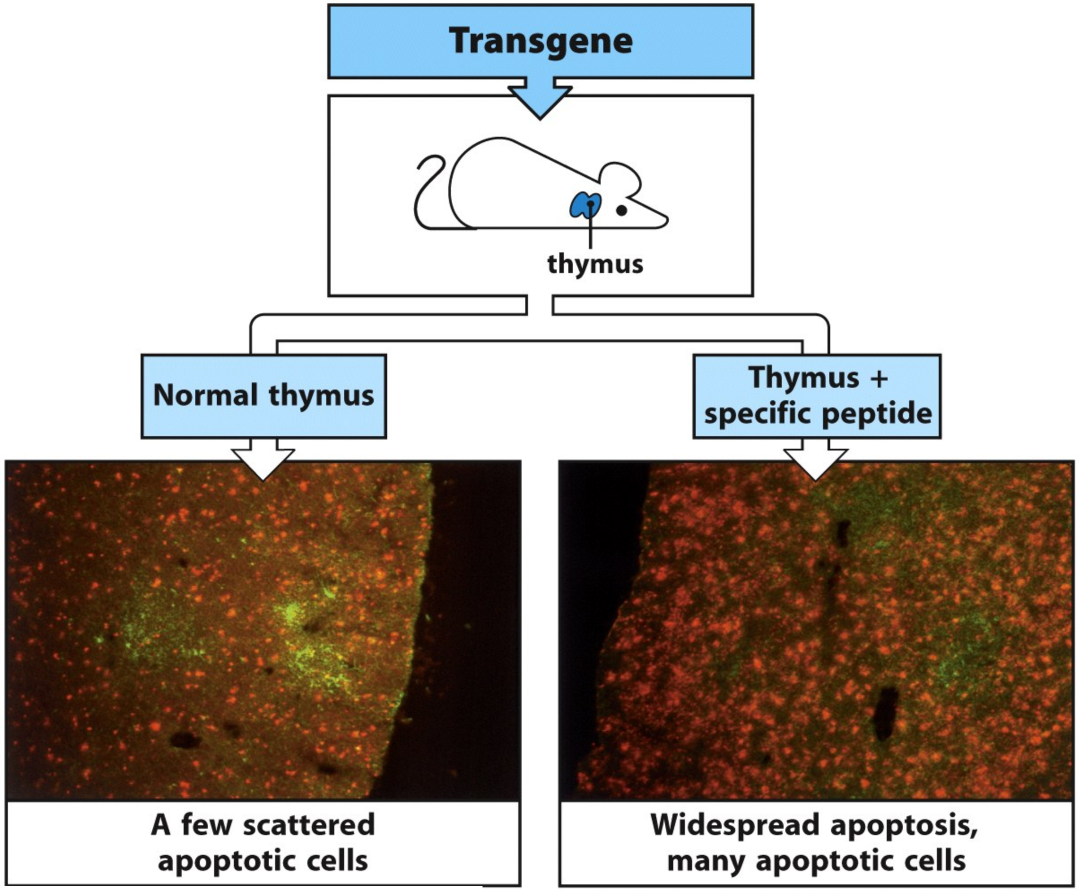
Martens H et al. *Neuroendocrinology* (1998) 67:282-9
Hansenne I et al. *Clin Dev Immunol.* (2004) 11:45-51
Hansenne I et al. *J Neuroimmunol* (2005) 158:67-75

The thymus microenvironment

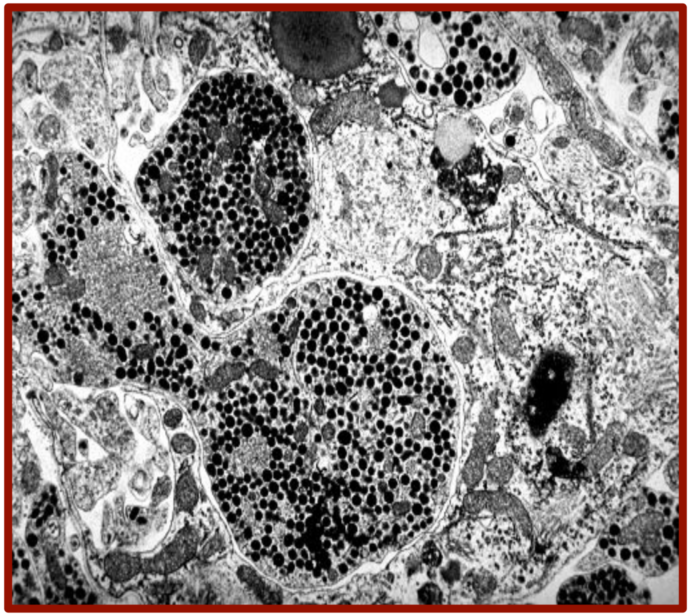


Central self-tolerance induction in the thymus

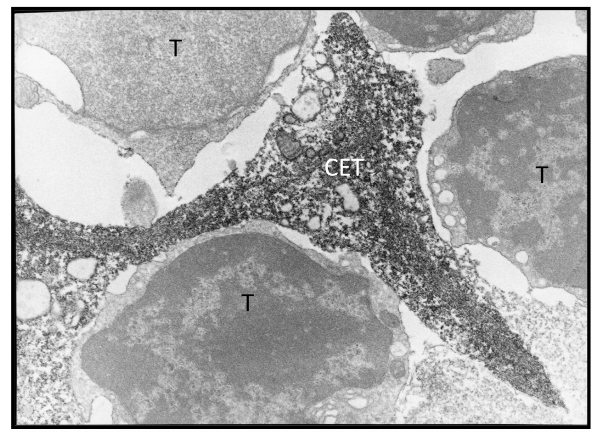
Ohki H, Martin C, Corbel C, Coltey M & Le Douarin NM *Science* 1987
Kappler JW, Roehm N & Marrack P *Cell* 1987
Kisielow P, Bluethmann H, Staerz UD, Steinmetz M & von Boehmer H *Nature* 1988



A paradigm shift for thymic oxytocin:
From a neurohormone/neuropeptide to a *self-antigen*



Neurohypophysis
Neurosecretory granules and
neuroendocrine communication

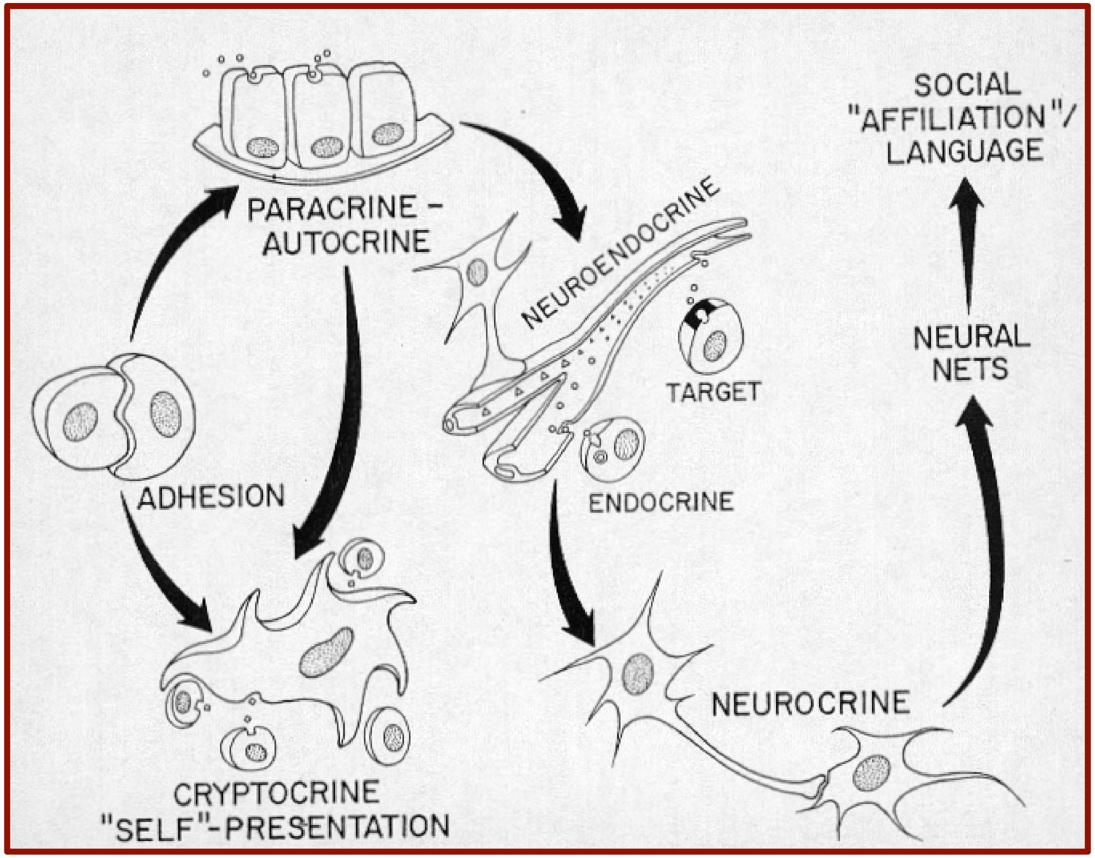


Thymic epithelial cell
Importance of the unseen
Cryptocrine communication
Self-presentation!

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

« Ontogeny recapitulates phylogeny. »

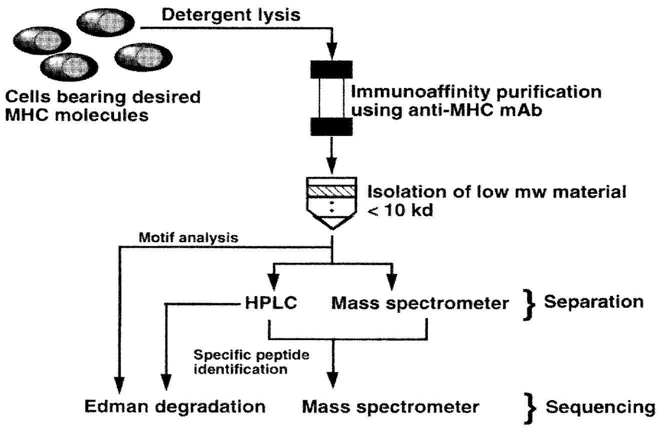
Ernst HAECKEL (1834-1919)

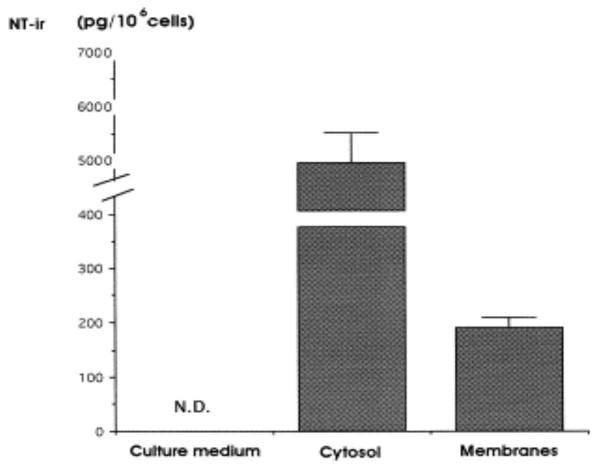


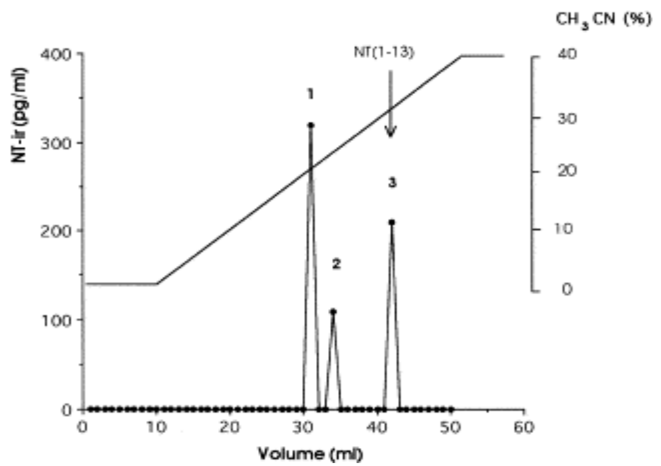
MHC-I presentation of Neurotensin by human TEC

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

= E**L**YENKPRR**P**Y**IL**







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

Economical organization of the thymic repertoire of *neuroendocrine self-antigens*

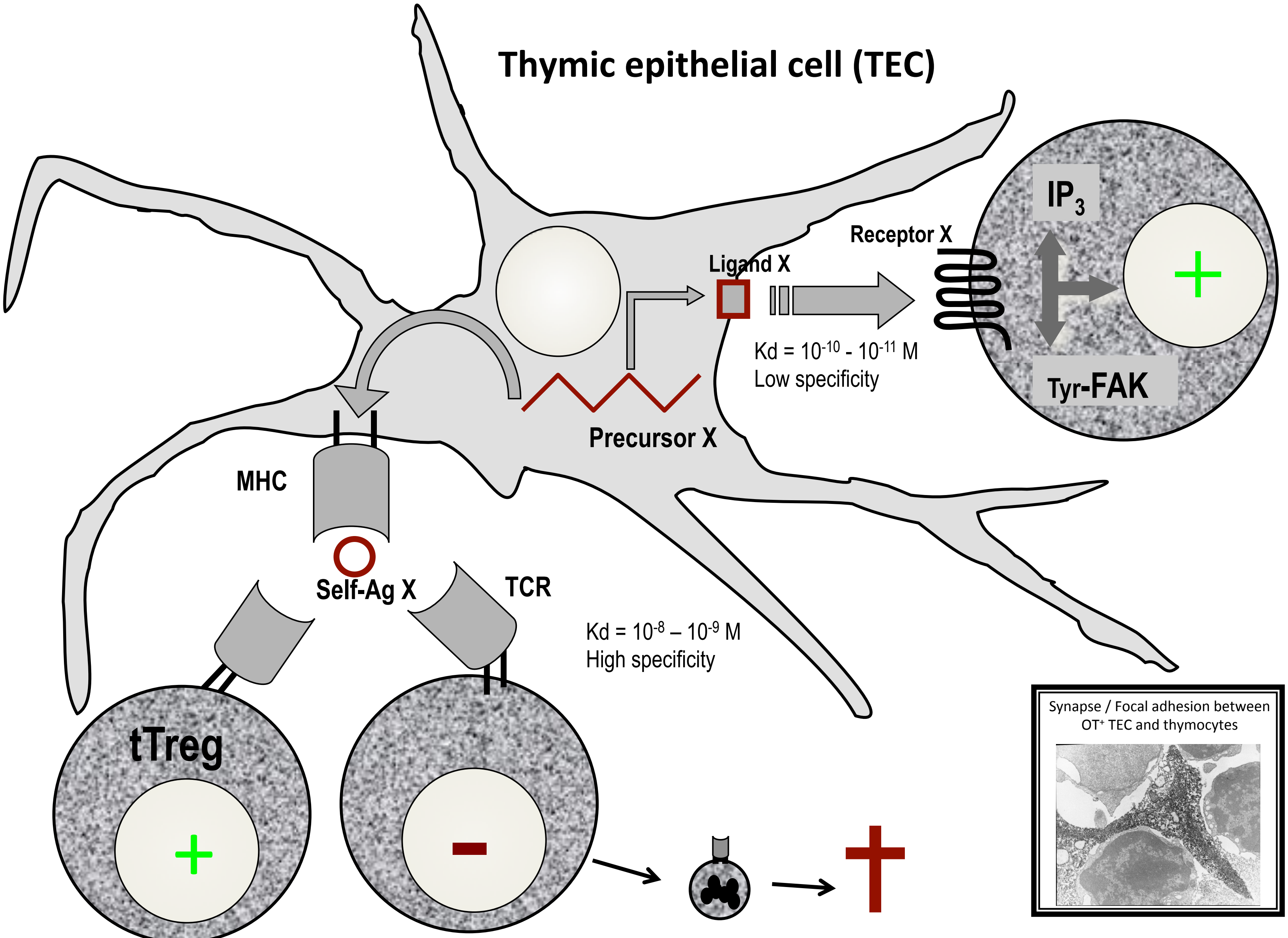
Family	Thymic neuropeptides
Neurohypophysial family	Oxytocin (OT) (>> Vasopressin VP)
Neuromedins	Neurotensin
Tachykinins	Neurokinin A
Atrial natriuretic peptides	ANP
Somatostatins	Cortistatin
Insulin family	IGF-2 (> IGF-1 > Insulin)

Geenen V et al. In *Immunoendocrinology in Health and Disease*.
(Geenen V & Chrousos G, eds.) New York, Marcel Dekker (2004).

The biochemical nature of neuroendocrine *self*

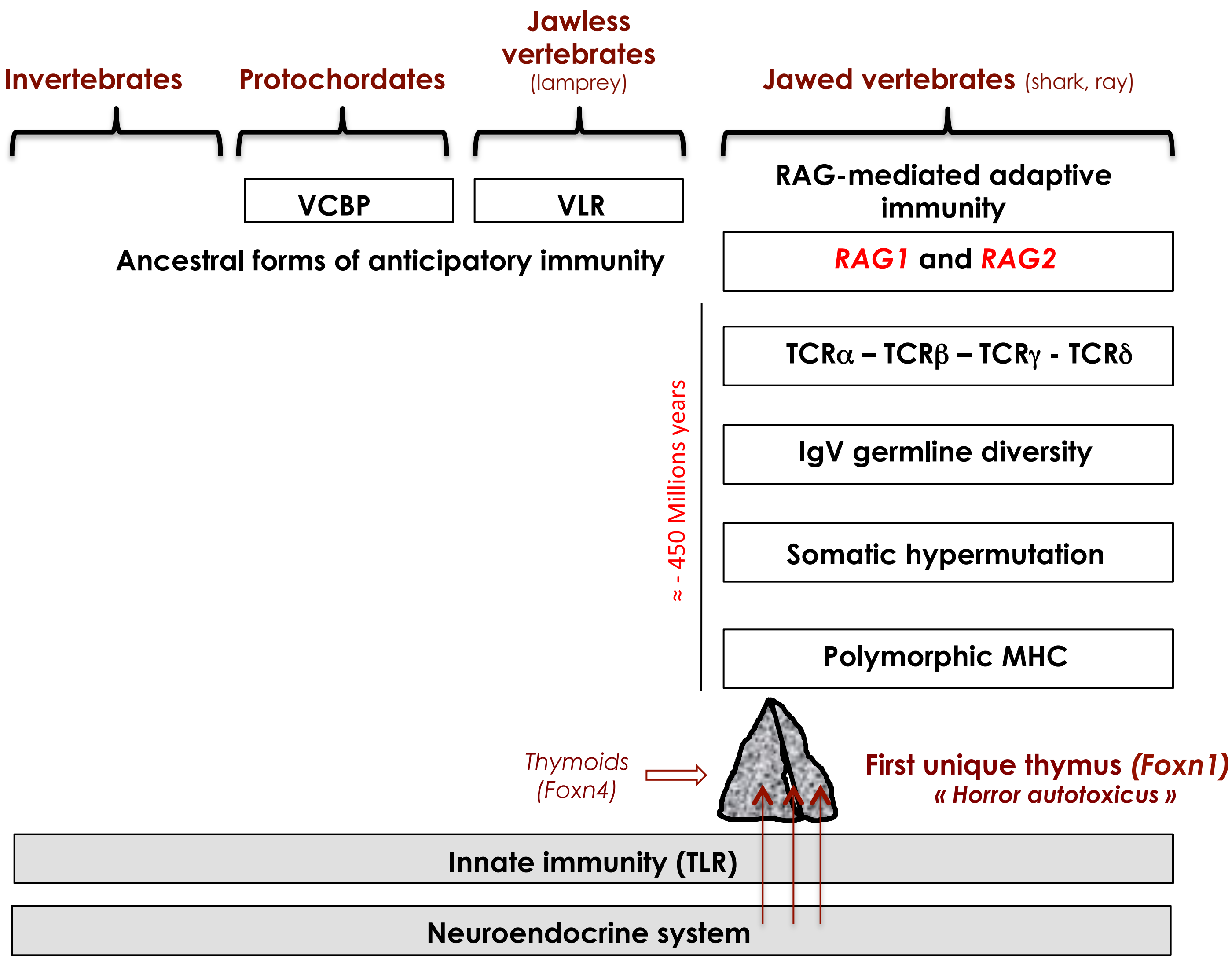
- Dominant member of a neuroendocrine gene family expressed in the thymus.
- Highly conserved sequences throughout evolution of a family.
- Intrathymic transcription before expression in orthotopic tissues *(i.e. OT).*
- Importance for species preservation *(OT > VP).*
- Thymus-specific epigenetic regulation (*i.e. Igf2*).
- <u>NO SECRETION</u> but processing through MHC pathways for antigen presentation.

The triple role of neuroendocrine self-antigen precursors

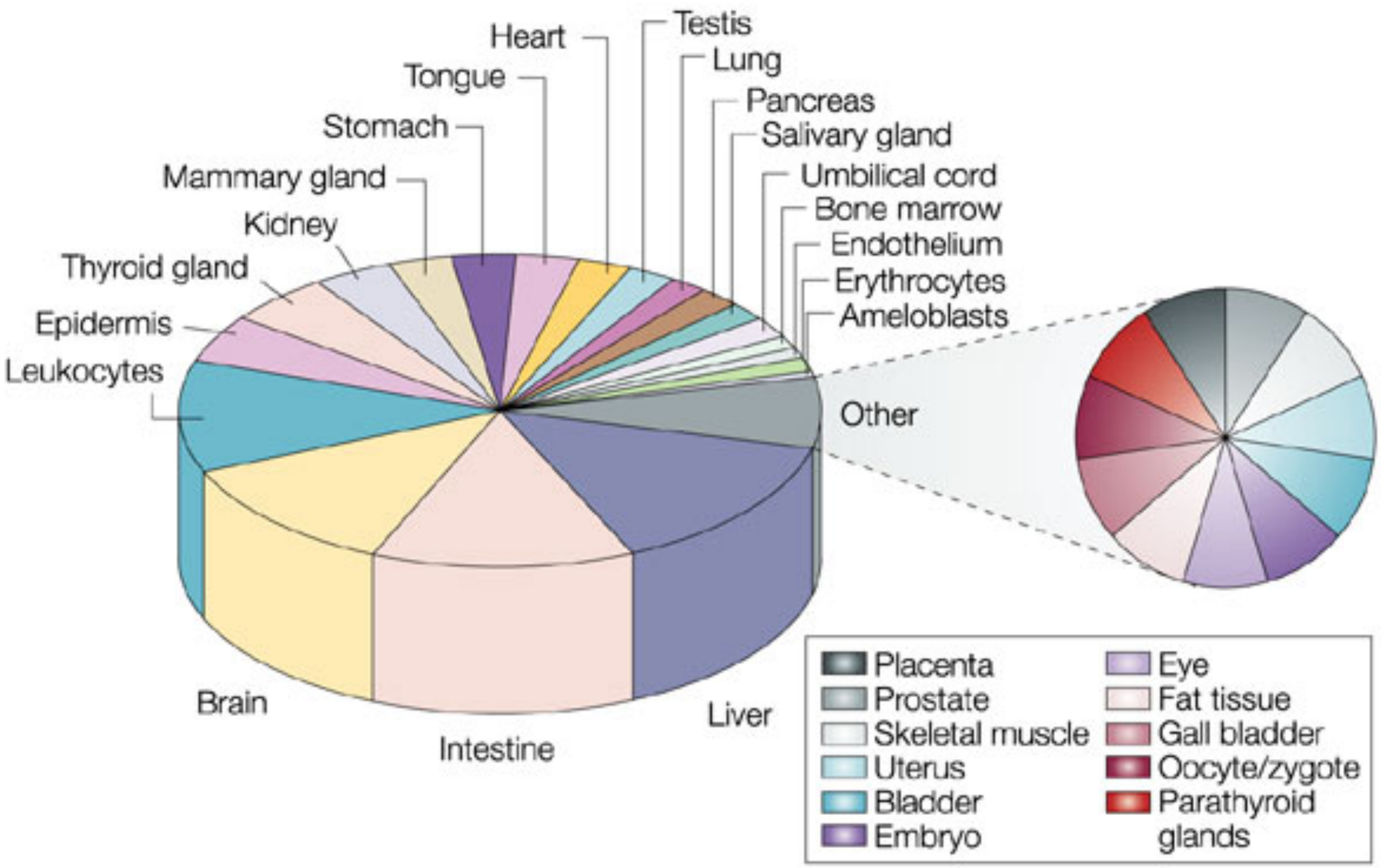


Geenen V. *Annals of the New York Academy of Sciences* (2012)

Coevolution of the immune and neuroendocrine systems



Intrathymic expression of tissue-restricted antigens (TRA)

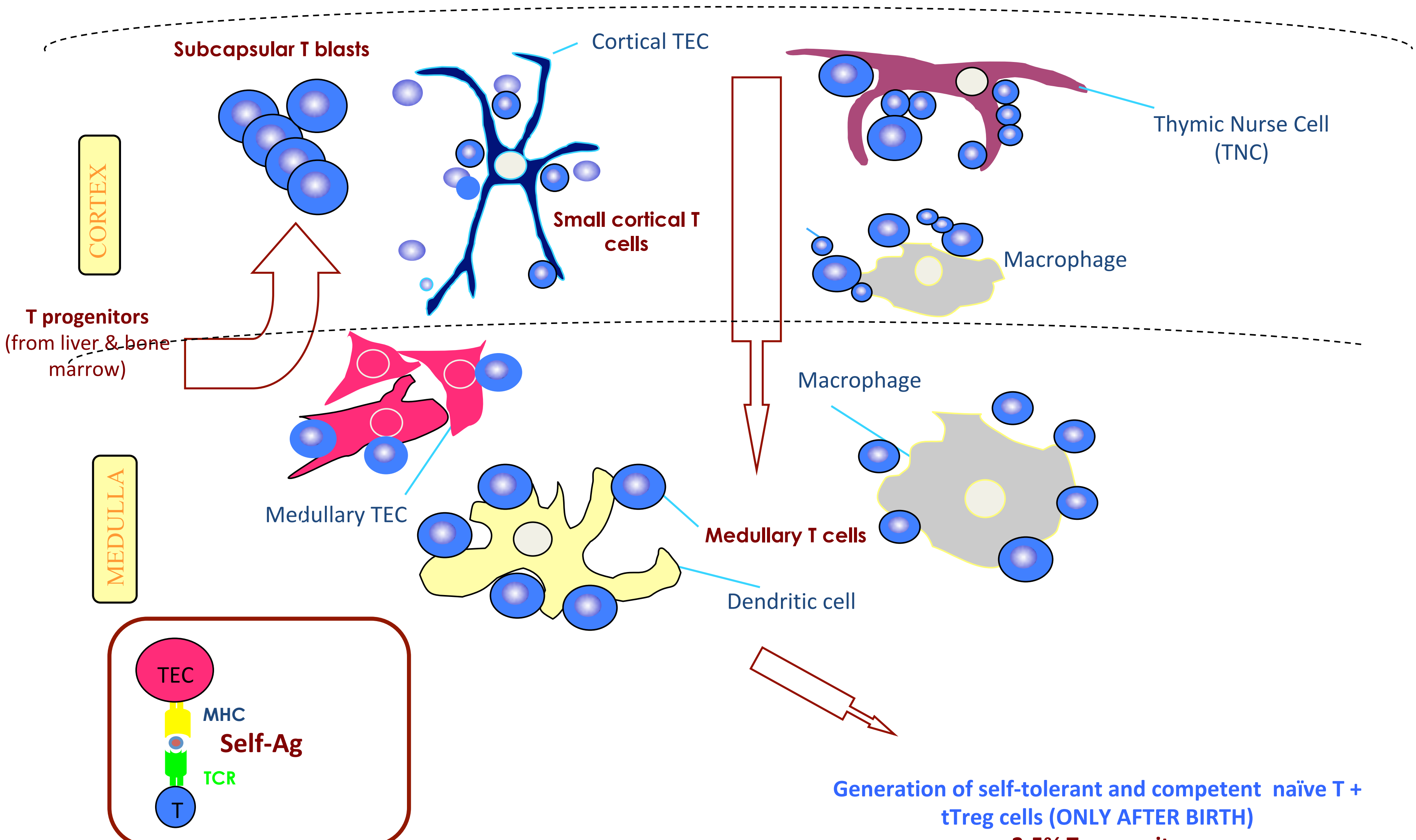


Nature Reviews | Immunology

Kyewski B et al. (2004)

Kyewski B & Klein L. *Annu Rev Immunol* (2006) 24:571-606.

T-cell differentiation in the thymus: an overview



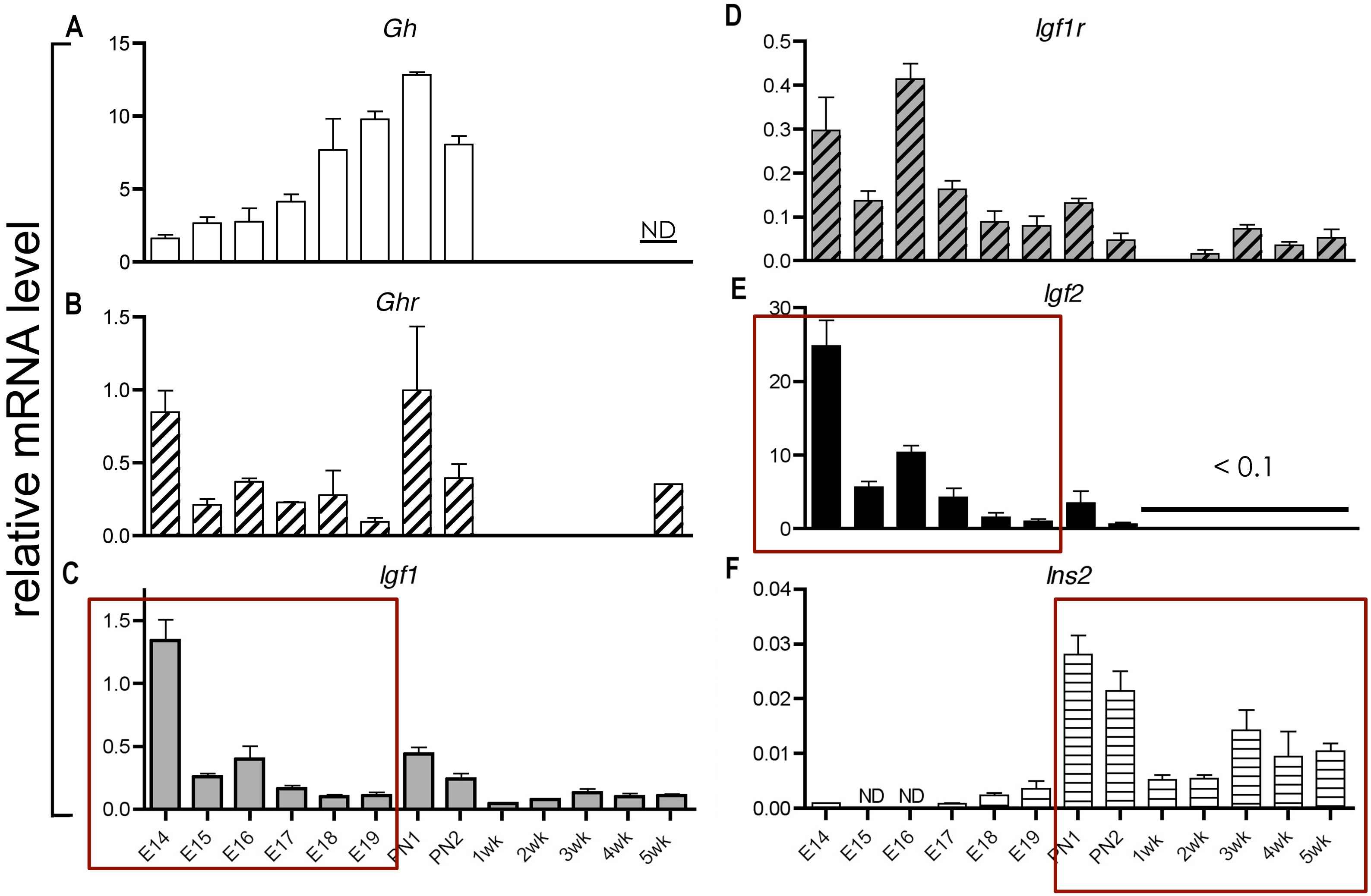
Generation of self-tolerant and competent naïve T + tTreg cells (ONLY AFTER BIRTH)
= 2-5% T progenitors

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

The origin of organ/cell-specific autoimmunity:

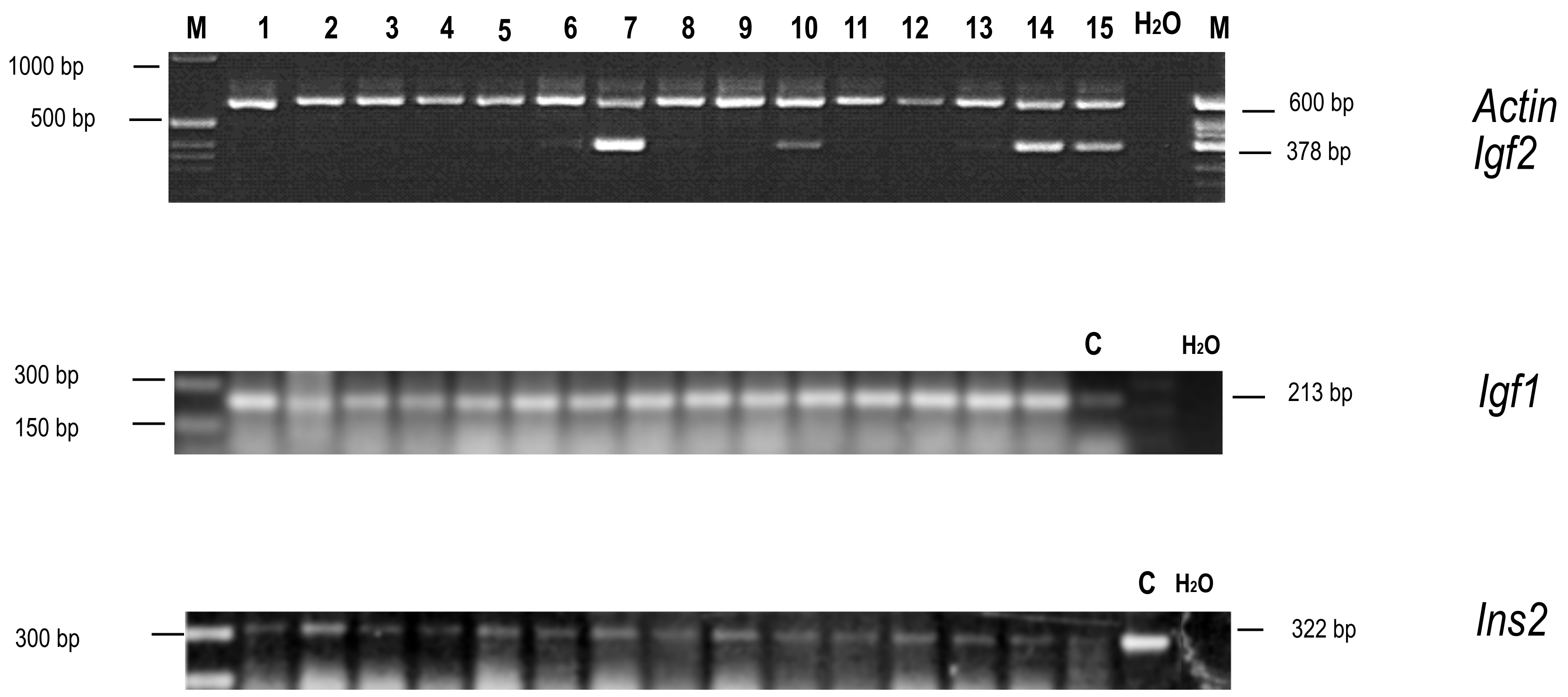
A thymus defect in programming self-tolerance?

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)

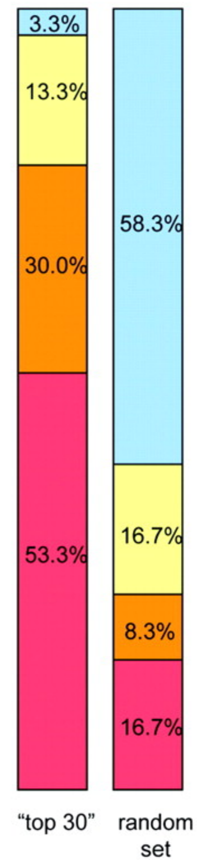
APS-I or APECED syndrome

- 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	prepronenuropeptide 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	-

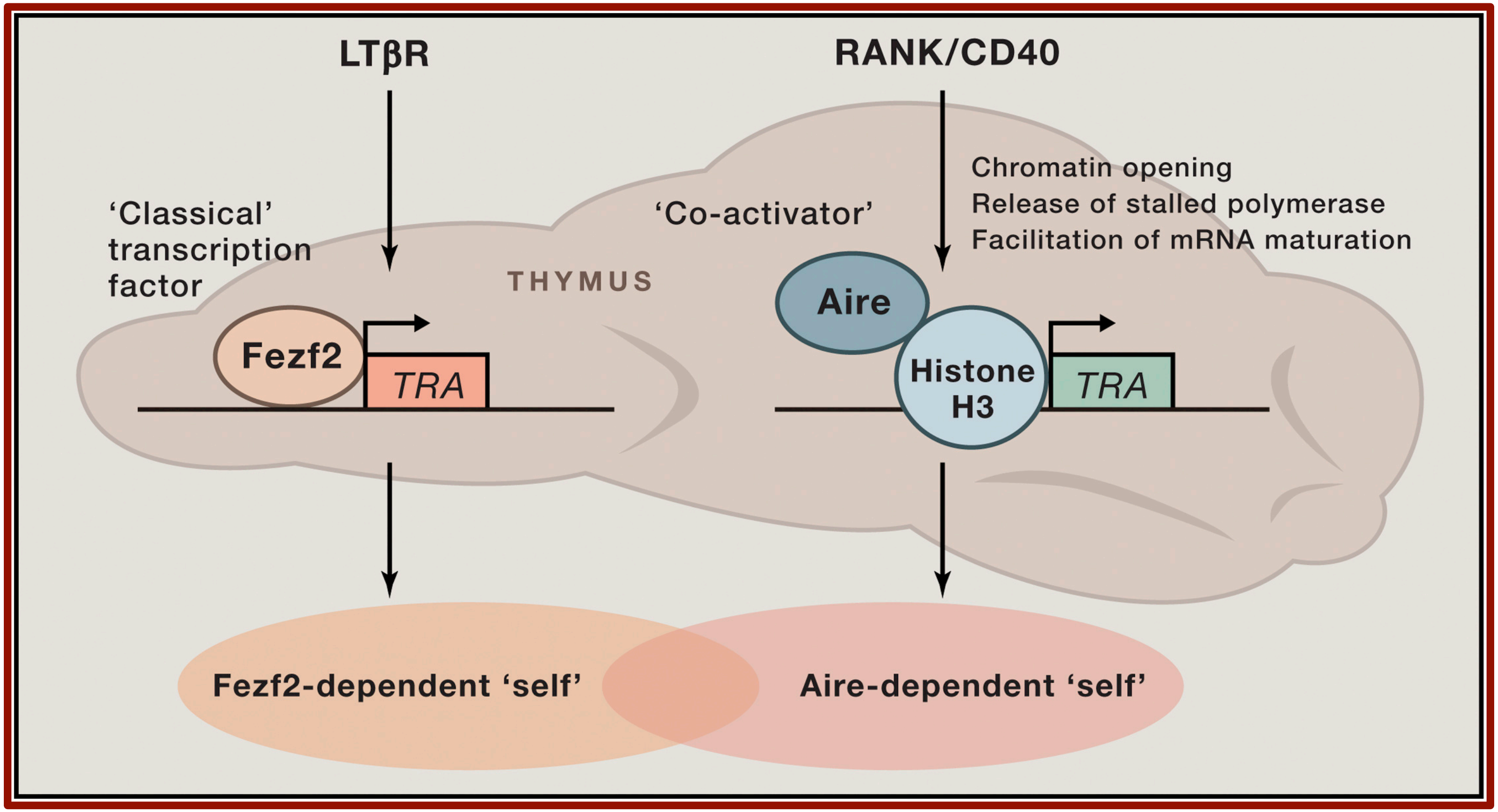
B



one specific tissue
several specific tissues
hematopoietic cells
housekeeping

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

Fezf2 and *Aire* control intrathymic transcription of tissue-restricted antigens (TRA)

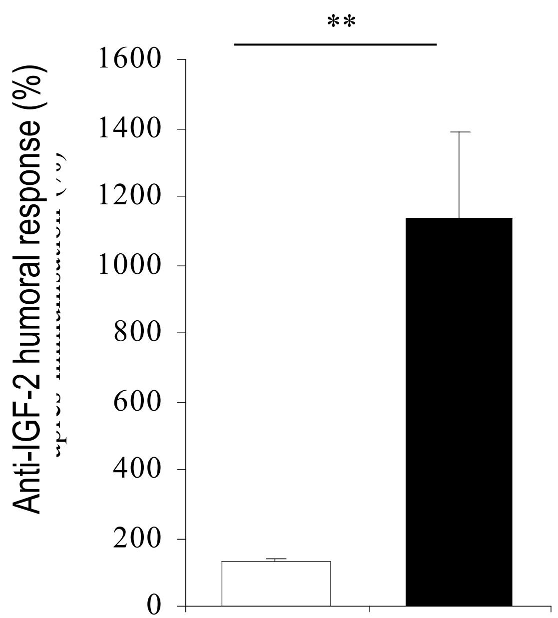


Takaba H et al. *Cell* (2015) 163:975-87

Fezf2 promotes neuronal differentiation through localised activation of Wnt/β-catenin signalling during forebrain development.
Zhang S, Li J, Lea R, Vleminckx K, Amaya E.
Development (2014) 2):4794-805.

Gonadal steroids regulate the level of *Aire* transcription in thymic epithelial cells (Berrih-Aknin S and colleagues).

Igf2 expression controls the level of tolerance to Insulin

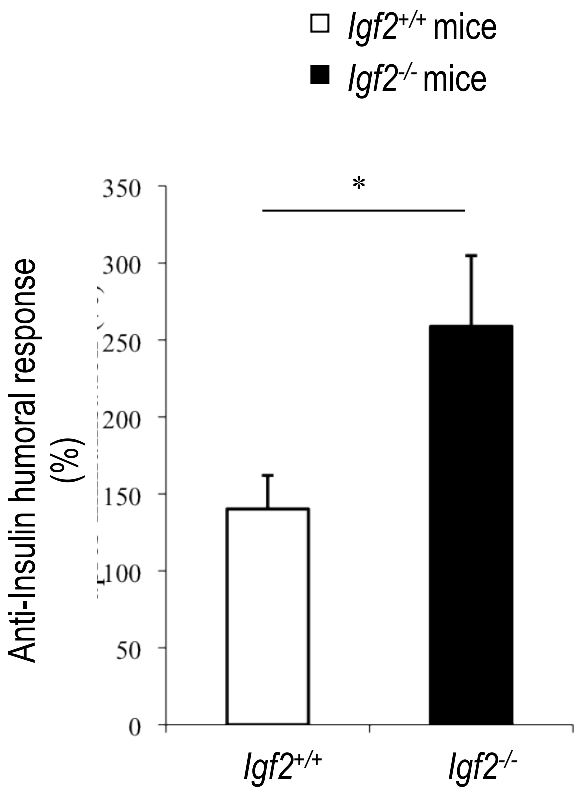


Mean ± SEM

N = 7

** : $p < 0.01$

Dilution : 1/500



Mean± SEM

N = 6/8

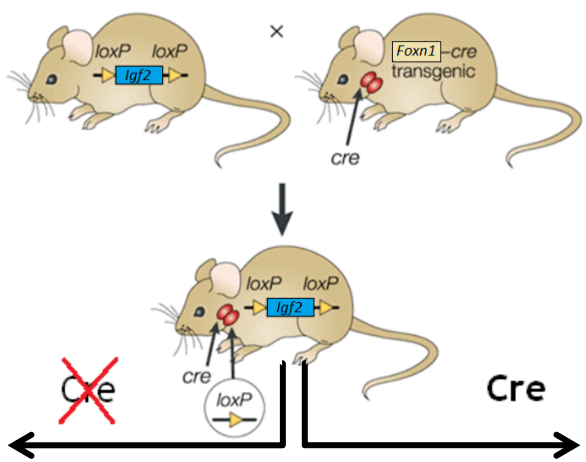
*: $p<0.05$

Dilution 1/100

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

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

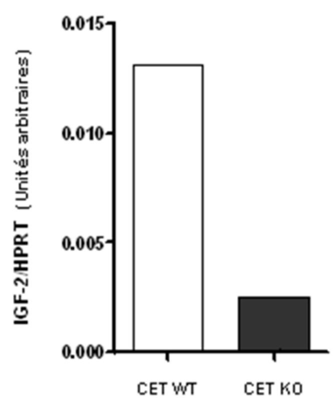
(Pr M. Constancia, University of Cambridge)



(Pr G. Holländer, University of Basel)

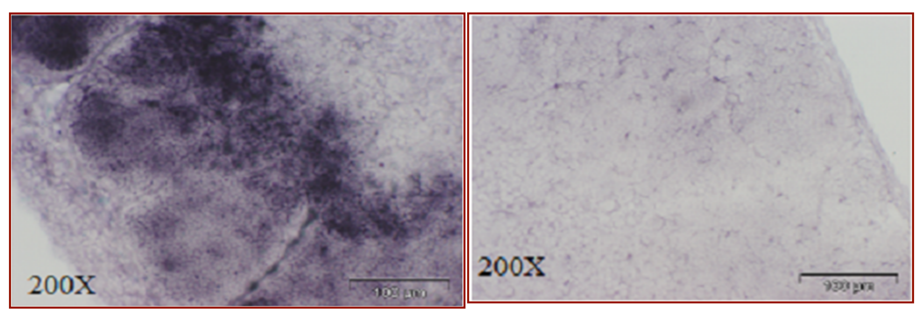
Igf2$^{fl/fl}$
Control

Igf2$^{\Delta Thy}$

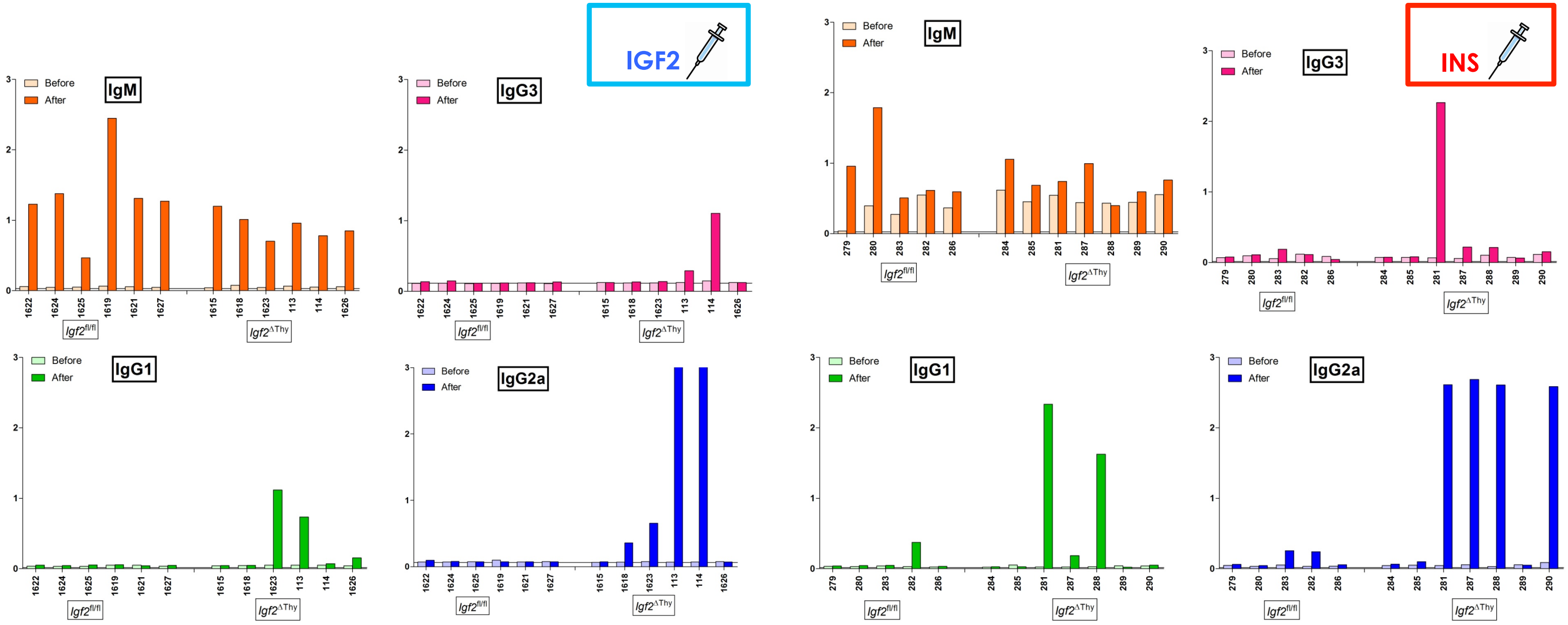


Thymus *Igf2*$^{fl/fl}$ 20 µm

Thymus *Igf2*$^{\Delta Thy}$ 20 µm



Titres and isotypes of Ig 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).

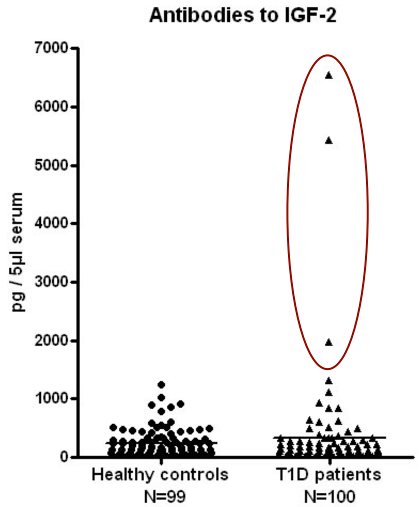
Bodart G et al., *in preparation*

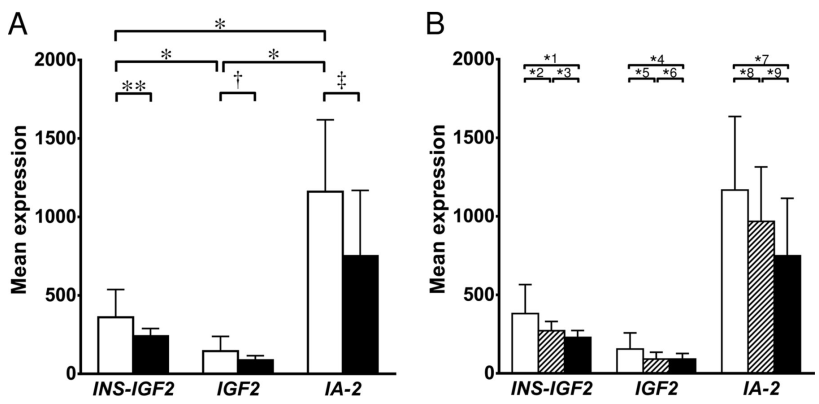
Humoral response to IGF-2 in T1D patients

(in collaboration with the Belgian Diabetes Registry – VUBrussels)

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

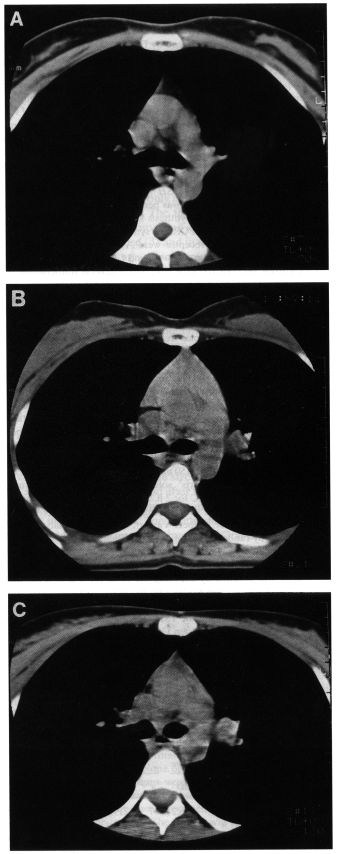
Quantification
Standard curve of mAb to human IGF-2 (CBL82)





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

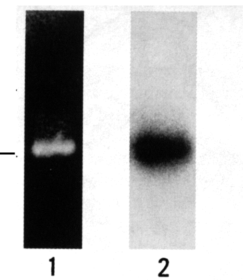
Thymus and Graves' disease (Type 3 AI thyroiditis)



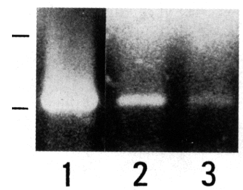
CT-scan

A. Control
B. Before treatment
C. After treatment

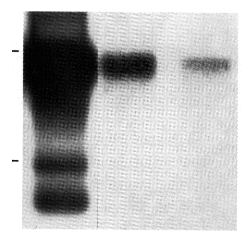
TSHR

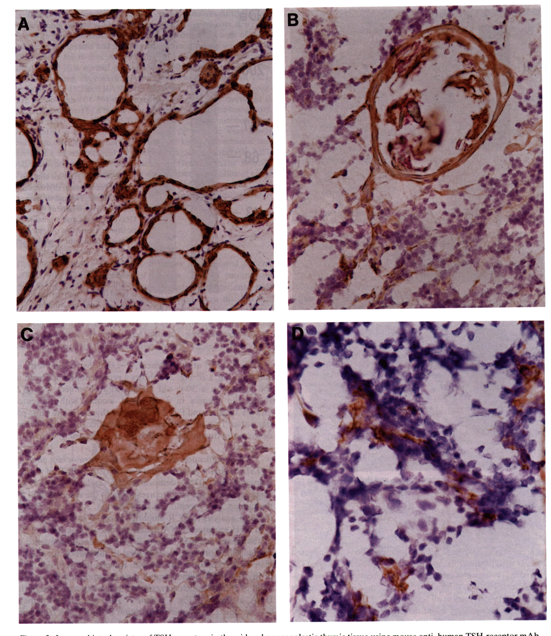


1.Thyroid
2-3. Thymus



Northern
1. Thyroid
2-3. Thymus





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

A thymus defect in autoimmune neuroendocrine diseases

Thymus physiology

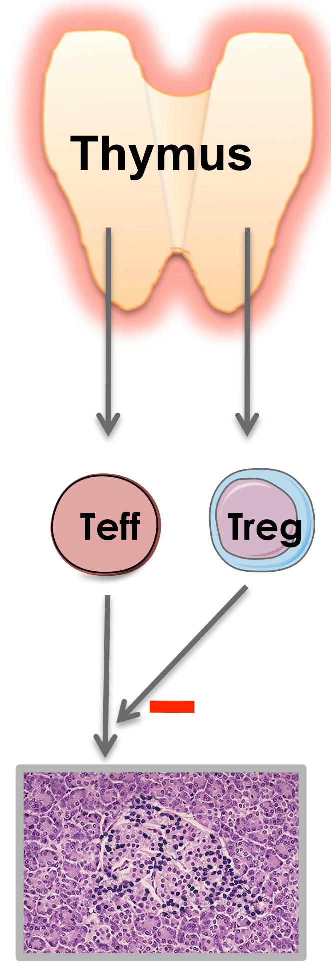
- *Aire* and *Fezf2*-regulated transcription of genes encoding self-peptides in thymus epithelium.
- Deletion of T cells with high affinity for MHC/neuroendocrine self-peptide complexes.
- Selection of CD4+ CD25+ Foxp3+ tTreg, specific of neuroendocrine self-peptides.

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…)

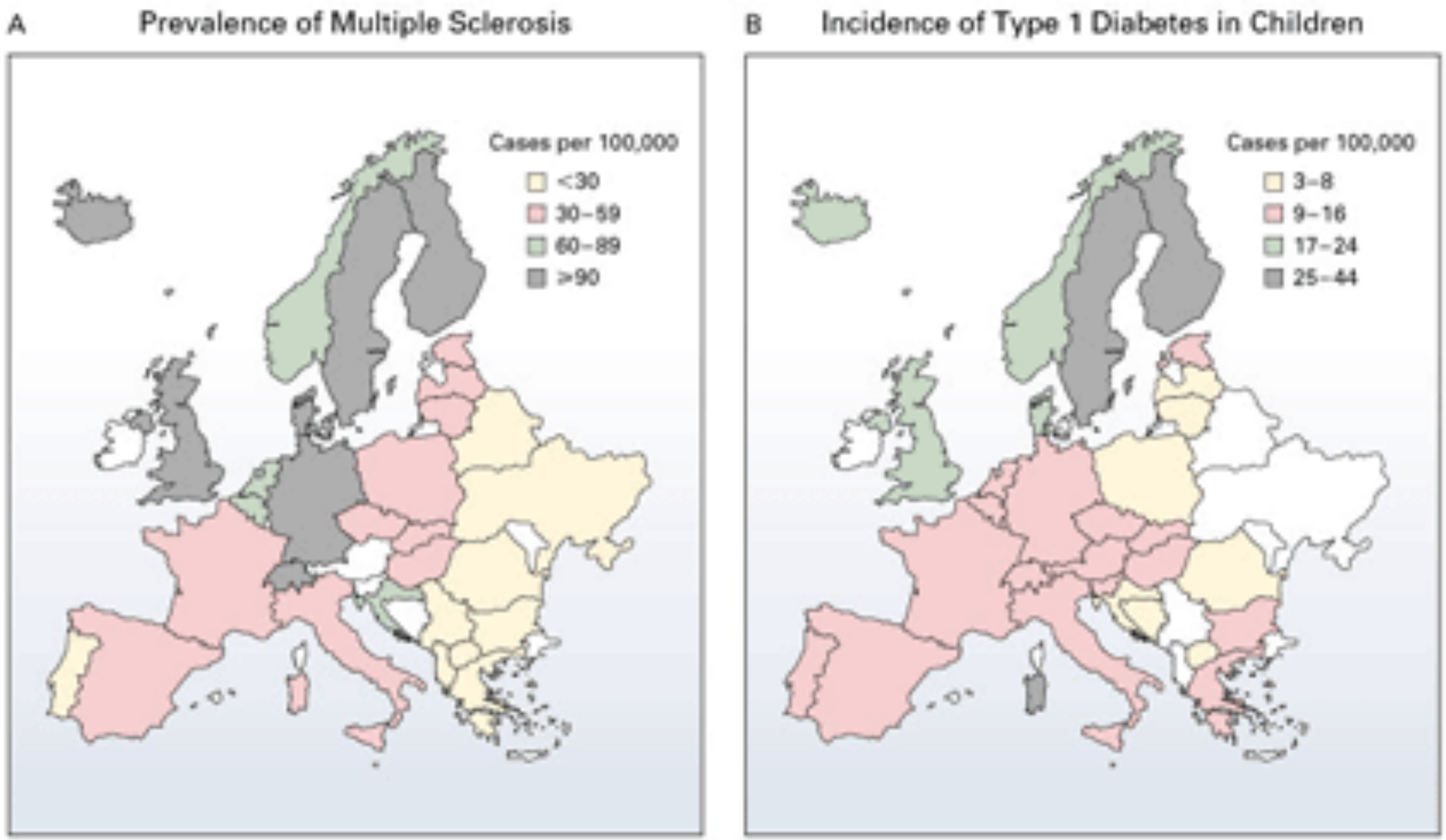


Target auto-antigens

Geenen V et al. *Front Neuroscience* (2013) 7:1-12
Geenen V *Médecine/Sciences* (2017) 33:653-663

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 CV-B4, thymus and T1D pathogenesis

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

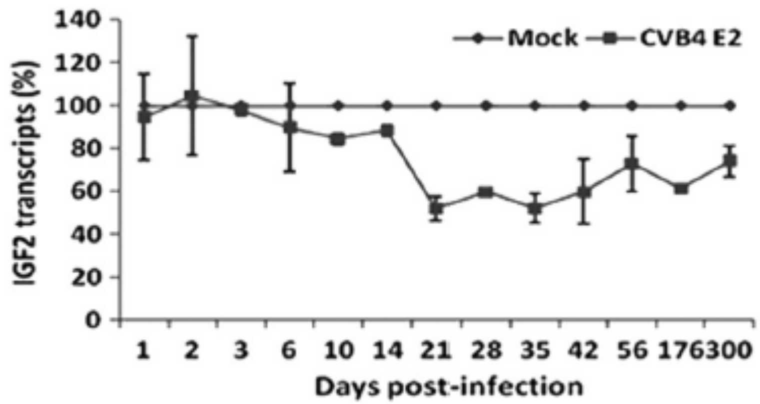
- Persistent infection of human thymic epithelial cells by Coxsackievirus B4.
  F. Brilot *et al*. *J Virol* (2002) 76:5260-5265.

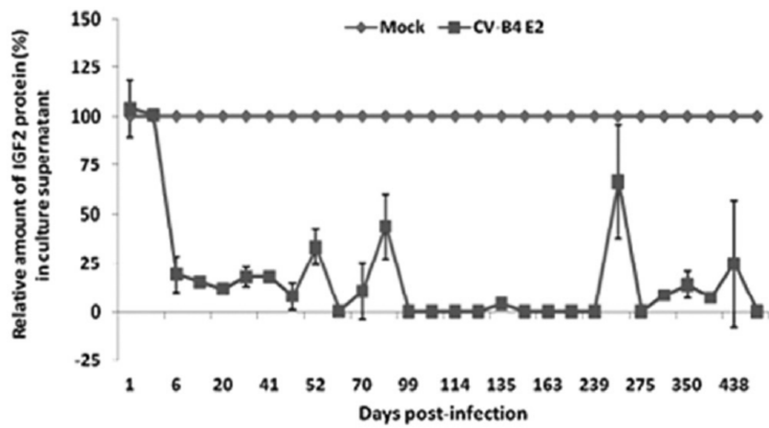
- Coxsackievirus B4 infection of human fetal thymus cells.
  F. Brilot, V. Geenen, D. Hober & C. Stoddart, *J Virol* (2004) 78:9854-9861.

- 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 CV-B4 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

Take-home messages

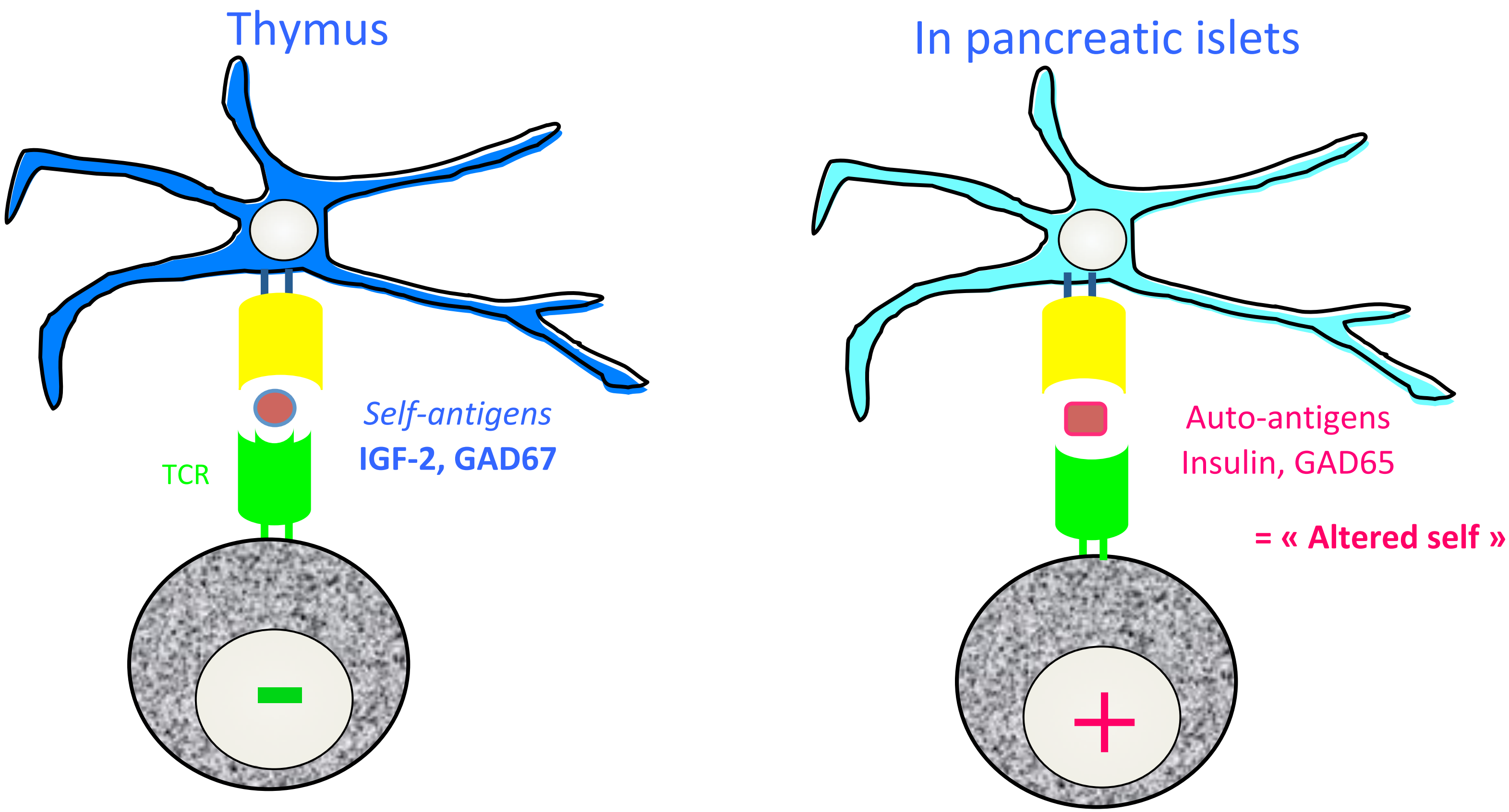
- Presentation of ***neuroendocrine self-peptides*** in the thymus programs central tolerance to neuroendocrine functions, which ensured an integrated and harmonious coevolution between the neuroendocrine and adaptive immune systems.

- A genetic or acquired 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; environmental influences also intervene.

From a ‘creative’ metaphor to innovation in therapeutics

Concept of « negative » self-vaccination



Tolerance to islet β cells

Deletion of 'forbidden' T cells

Generation of self-specific tTreg cells

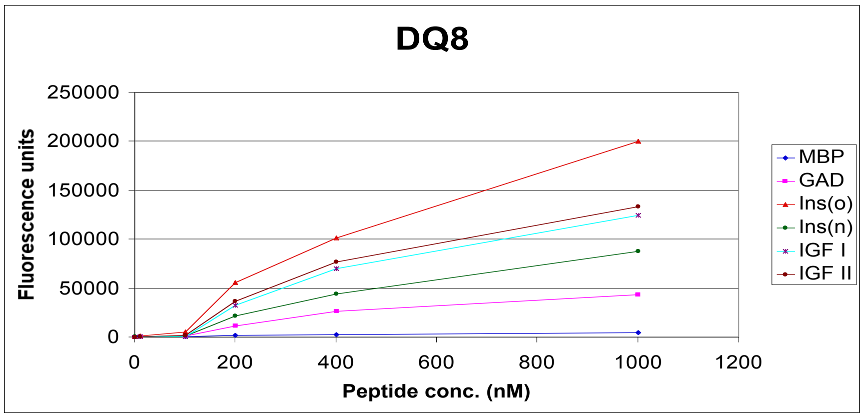
Autoimmunity to β cells

Activation of 'forbidden' T cells

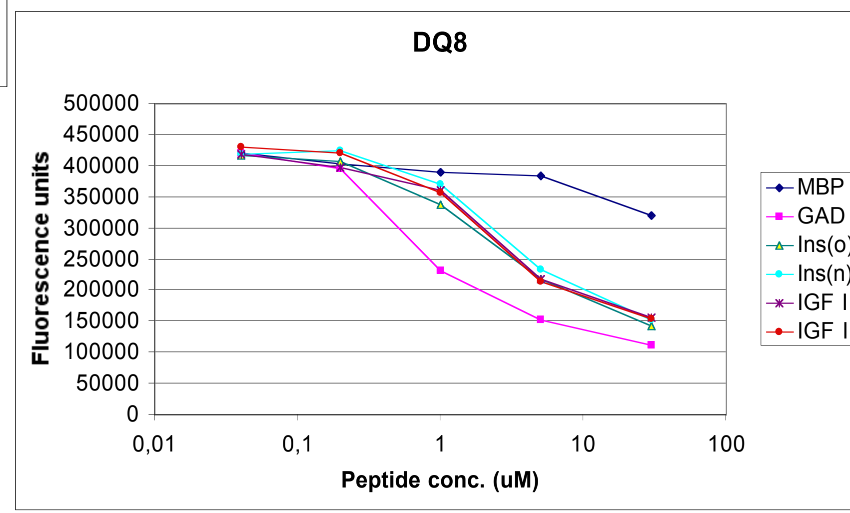
Induction of mermory T cells

Binding to DQ8 of INS and IGF-2 homologous sequences

Direct binding



Competition

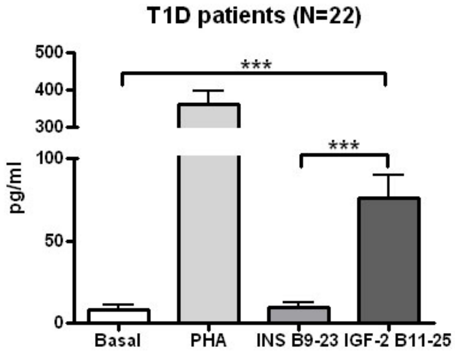


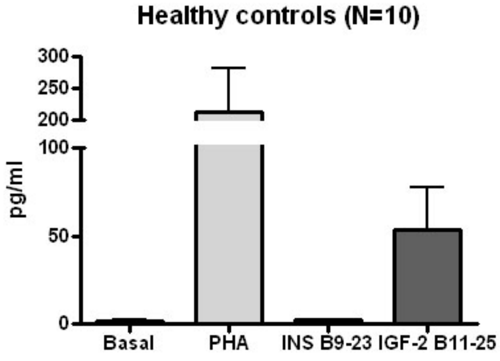
Ins B9-23 = SHLVEALYLVCGERG

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

Immune cellular response to IGF-2

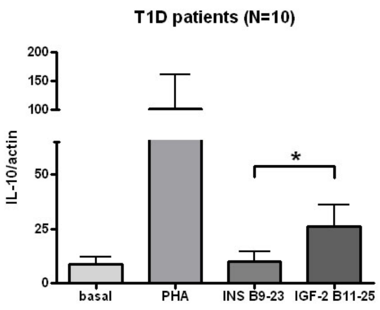
IL-10

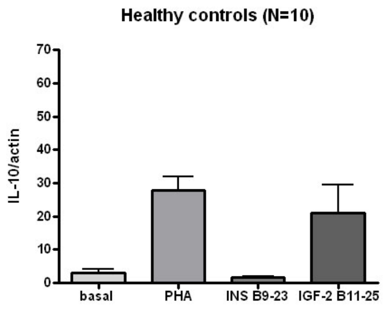




M ± SEM
* p < 0.05
*** p < 0.001 *t*-test

IL10 transcripts





+ IGF-2 stimulates Treg and Breg cell functions (shown by two other laboratories)

Acknowledgments

GIGA-I³ Immunoendocrinology

Henri Martens, PI
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Roberto Salvatori, MD, PhD

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Waleo 3 - Tolediab

JDRF Juvenile Diabetes Research Foundation International

EFSD

ARC « Somasthym » 2013-2017 FWB

Thank you for your kind invitation and attention!

Current research in GIGA-I^3
Immunoendocrinology

- Mechanisms of *Igf2* inhibition in thymic epithelium infected by CV-B4
  Hélène MICHAUX, BSc

- *In utero* vertical infection of fetal thymus by CV-B4 and interference with central tolerogenic mechanisms
  Aymen HALOUANI, BSc and Hela JAÏDANE, PhD
  Faculty of Sciences El Manar, Tunis and University of Monastir (Tunisia)

Hélène Michaux

Aymen Halouani & Hela Jaïdane

- Michaux H et al. How does thymus infection by coxsackievirus contribute to the pathogenesis of type 1 diabetes?
  Front Immunol (2015) 6: art. 338.
- Jaïdane H et al. *In utero* coxsackievirus B4 infection of the mouse thymus.
  Clin Exp Immunol (2017) 187: 399-407.

- Reassessment of the impact of the somatotrope GHRH/GH/IGF-1 in developmental on developmental and functional immunology.
Gwennaëlle BODART, BSc

- A surprising and dramatic neuroendocrine-immune phenotype of GHRH-deficient mice.
Khalil FARHAT, BSc (Granted by Lebanon government)

Gwennaëlle BODART

Khalil FARHAT

Roberto SALVATORI, MD, PhD
Johns Hopkins Hospital Baltimore
Ghrh$^{-/-}$ mice

Henri MARTENS, PI

Farhat K*, Bodart G* et al. Severe deficiency of the somatotrope GHRH/GH/IGF-1 axis induces a dramatic susceptibility to *S. pneumoniae* infection. *Best communication at the 50th Congress of the French Society of Immunology (Paris, November 2016) – « Immunity and infections »*

The balance between Treg cells and IL-17 producing cells at the materno-fetal interface: γδ T cells are the major producers of IL-17a

Barbara POLESE, BSc

Barbara POLESE

Pr Sophie PERRIER d'Hauterive, PI

Virginie GRIDELET, PhD

Polese B et al. γδ T cells are the main producers of IL-17a in the pregnant uterus. *Submitted for publication*.

TRANSLATIONAL RESEARCH

Investigation of the ocytocinergic system in Prader-Willi Syndrome

Pr Maïthé TAUBER, Hôpital des Enfants, Toulouse
Chantal RENARD, Laboratory Assistant
Henri MARTENS, PI

Pr Maïthé TAUBER

Chantal RENARD

Henri MARTENS, PI

Tauber M et al. The use of oxytocin to improve feeding and social skills in infants with Prader-Willi syndrome. *Pediatrics* (2017) DOI: 10.1542/peds.2016-2976.

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* 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**