Athens, April 28, 2017

Infections and Immune Dysregulation:

Two Facets of Primary Immune Deficiency Diseases

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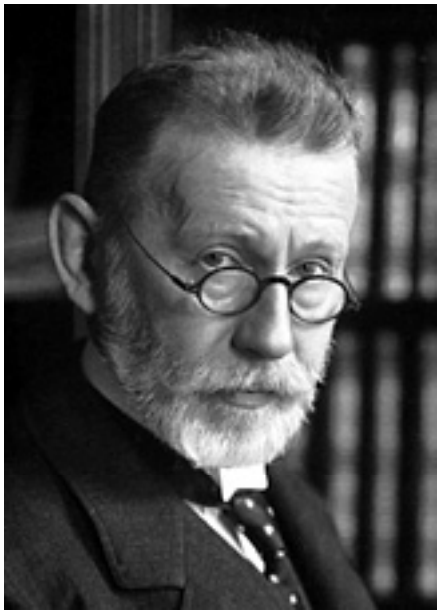
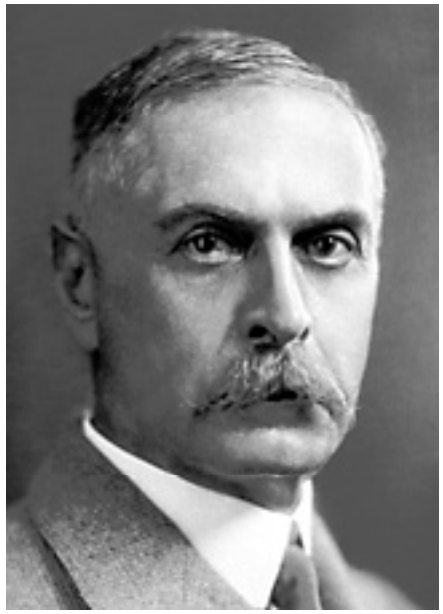
The quest to discover the bases of autoimmune diseases: The early days and many decades of fierce debate



Paul Ehrich

"It would be dysteleologic in the highest degree, if under these circumstances self-poisons of the parenchyma—autotoxins—were formed" -

Julius Donath



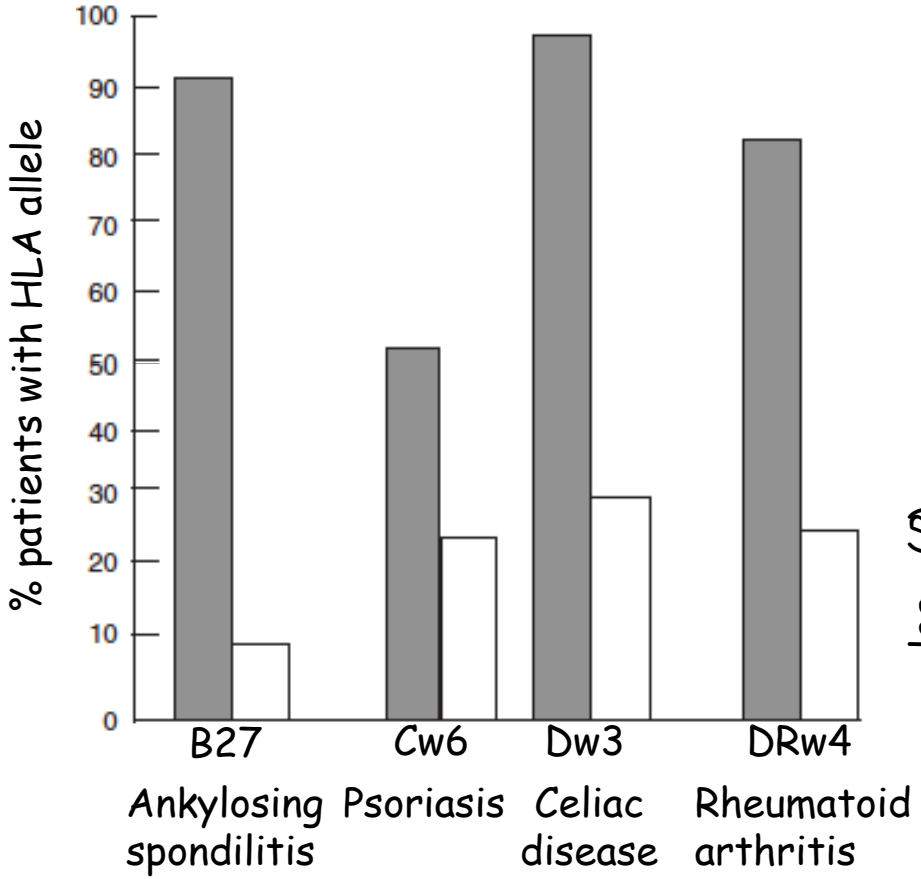
Karl Landsteiner

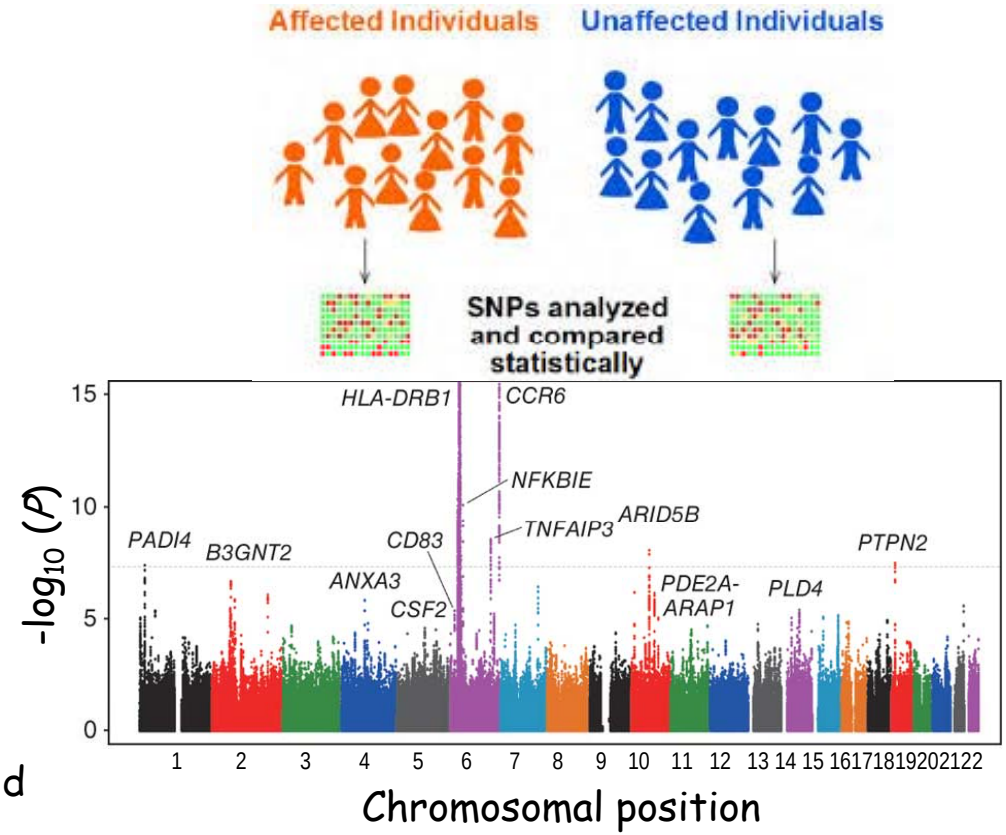
"The development of autotoxic substances, which are bound to the organism's own cells, can be related to the process of antibody formation, a possibility which, as far as we know, has not been previously discussed"

The quest to discover the bases of autoimmune diseases: From 'quantitative inheritance' to GWAS studies

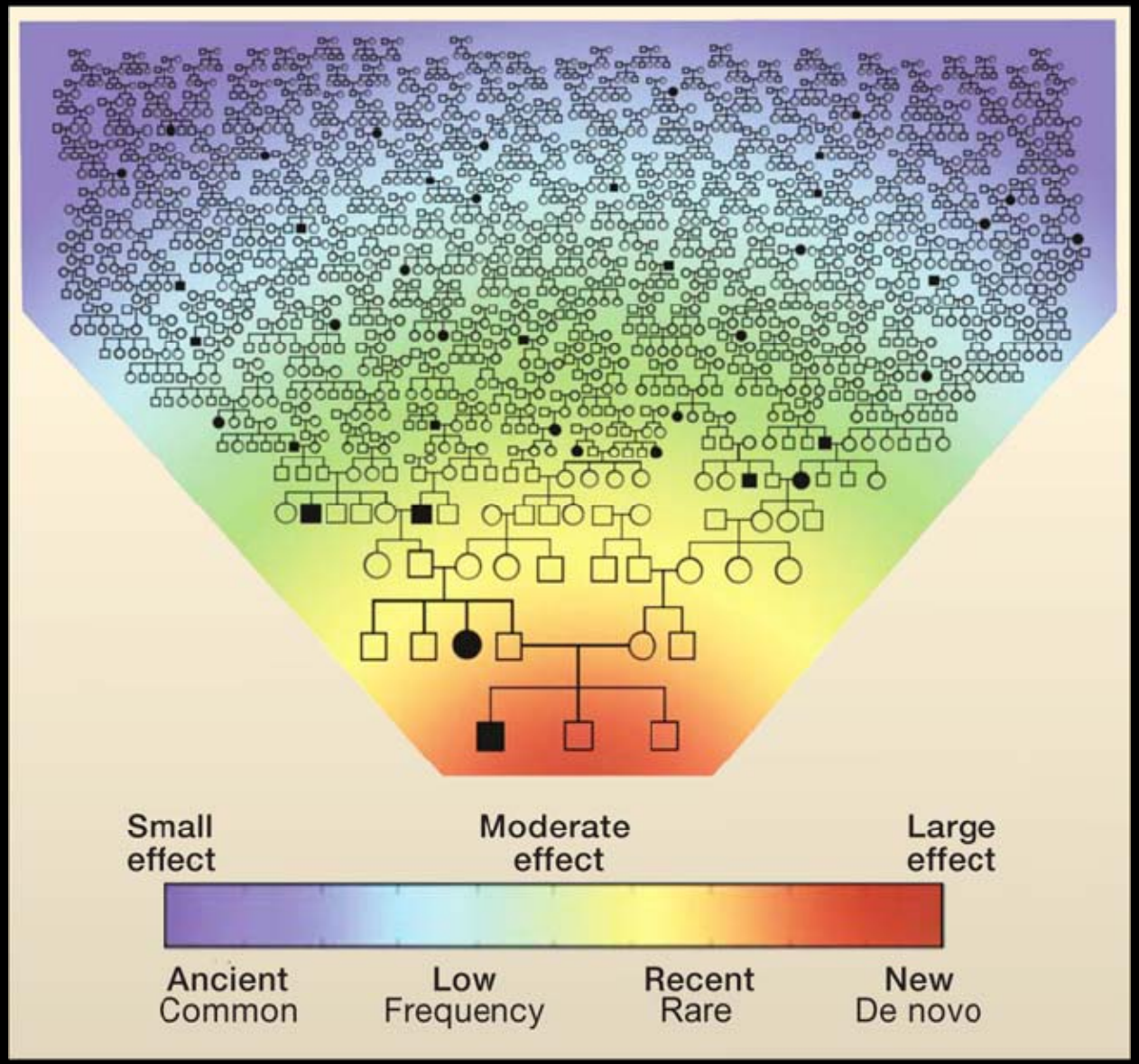
1918 – R.A. Fisher – Concept of variance in genetic studies

1961 – J. Thoday – Genetic mapping techniques to identify specific genes affecting a quantitative trait (QTLs)



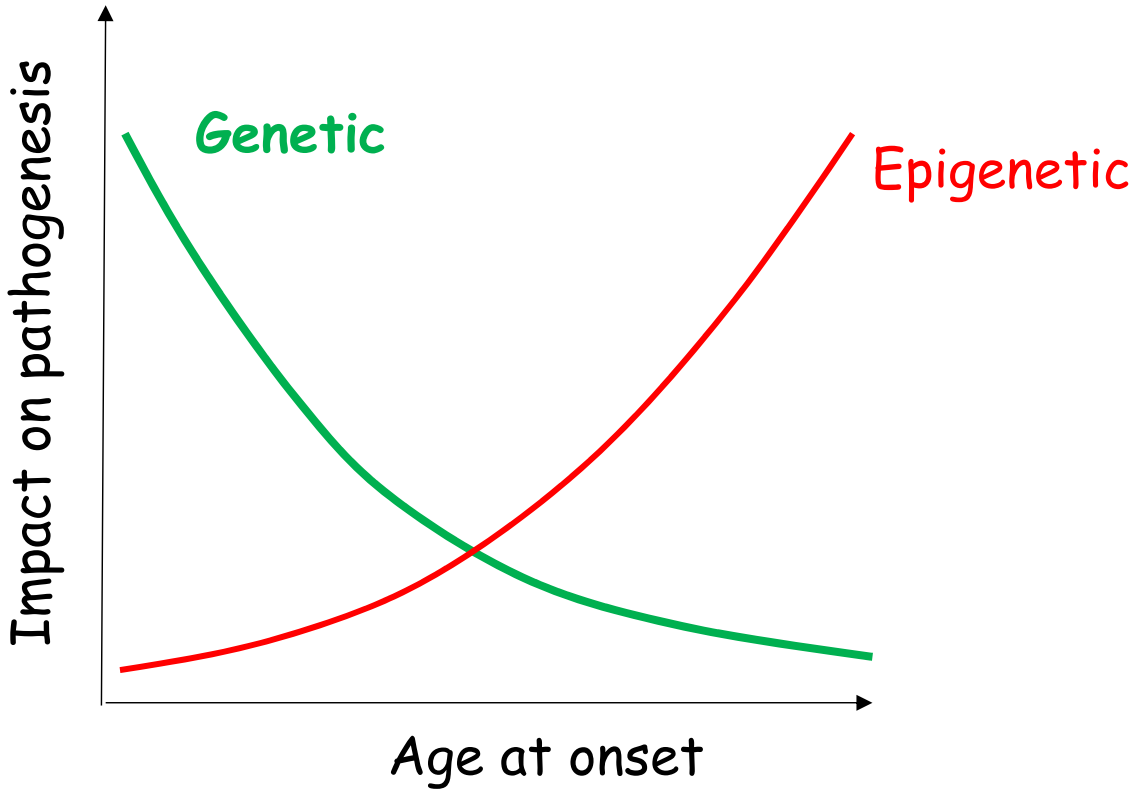


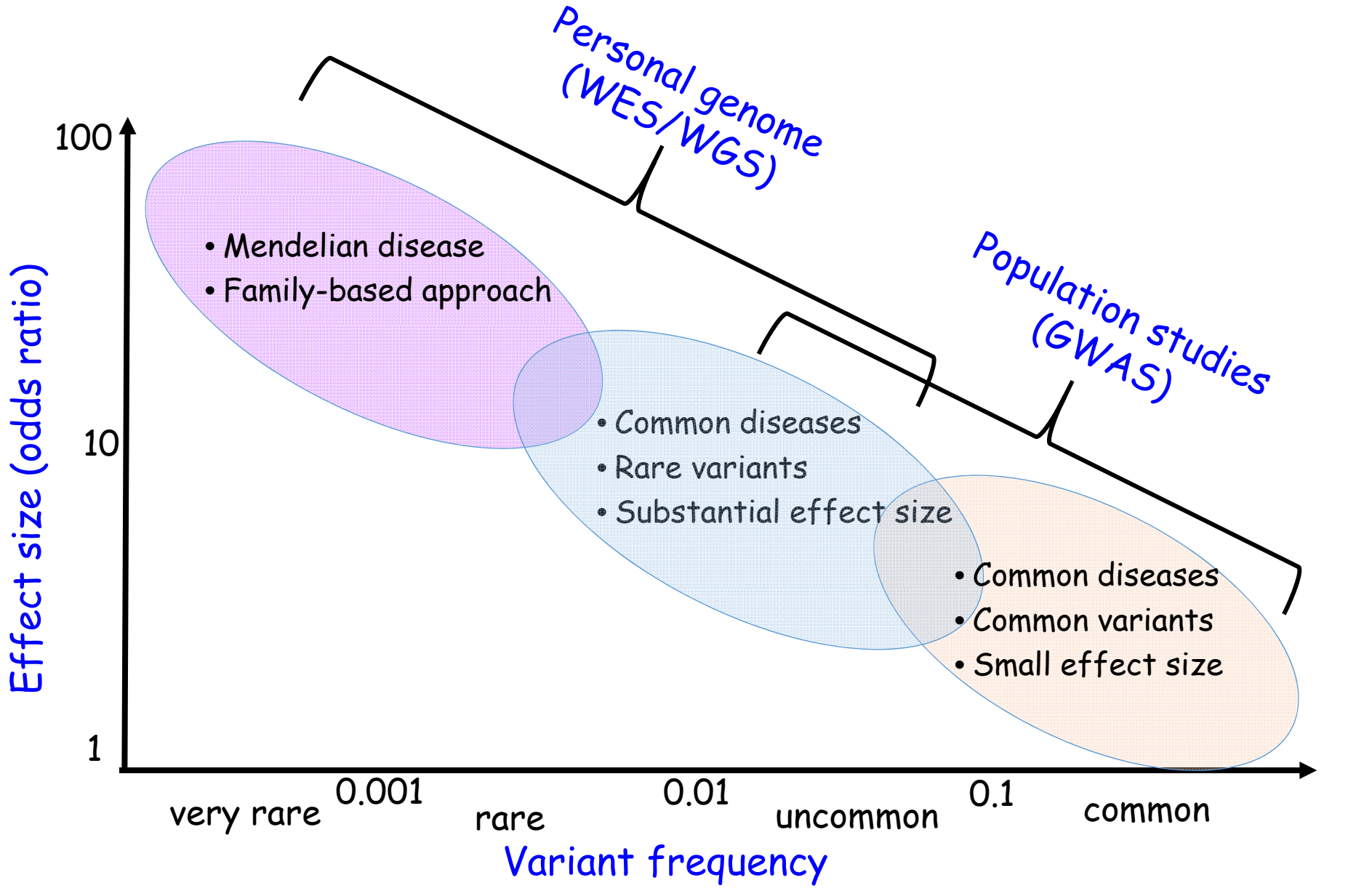
The power of rare variants: from GWAS to WES/WGS



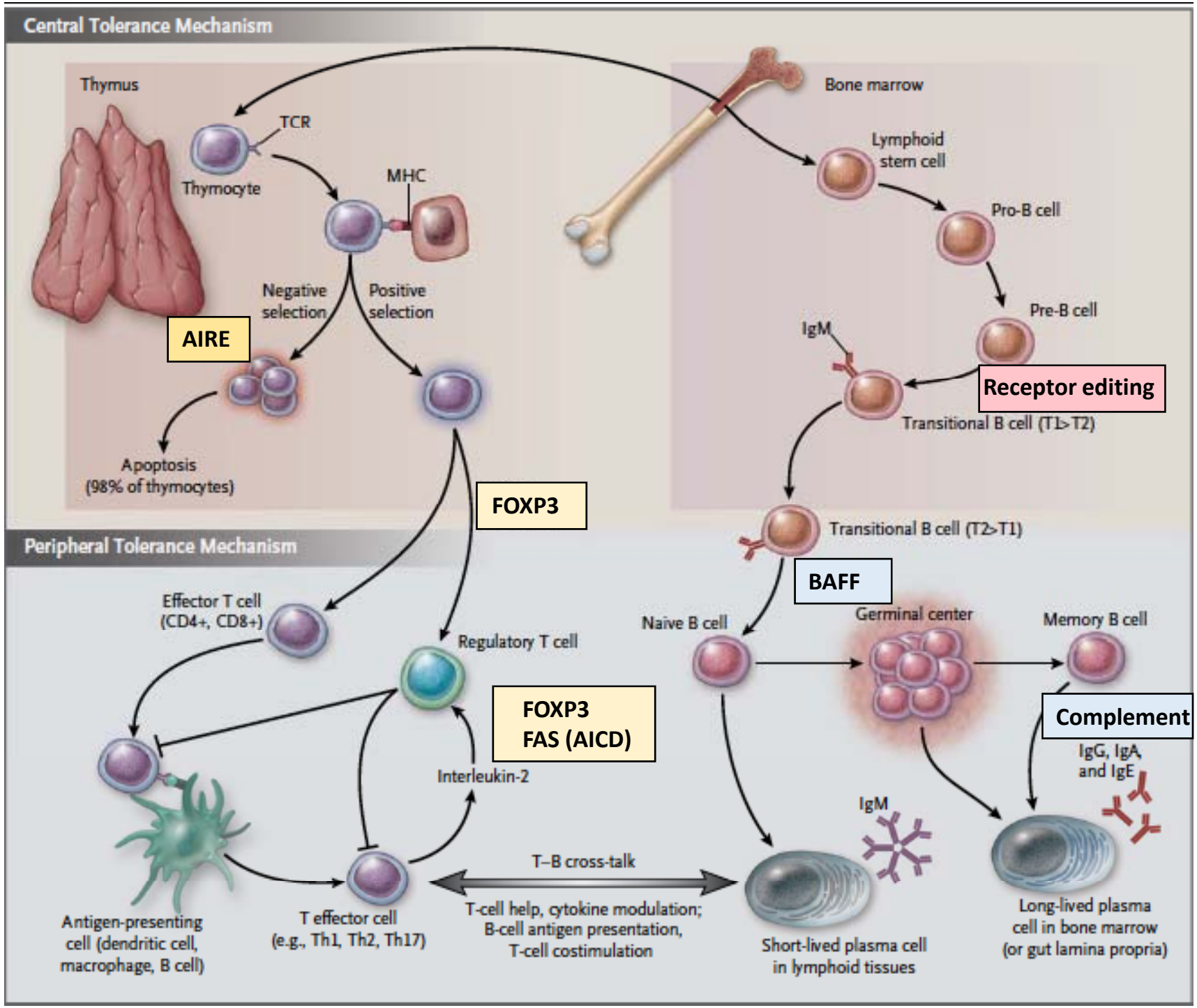
(Lupsky et al., Cell 2011)

Age-dependent role of genetic and epigenetic factors in the pathophysiology of autoimmunity

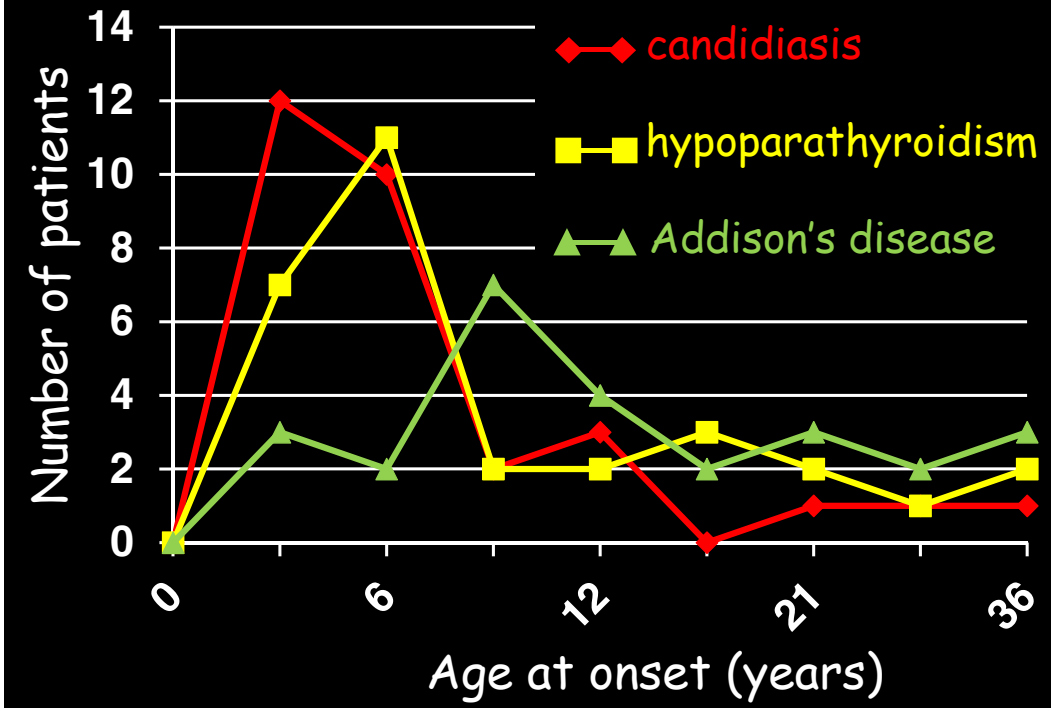


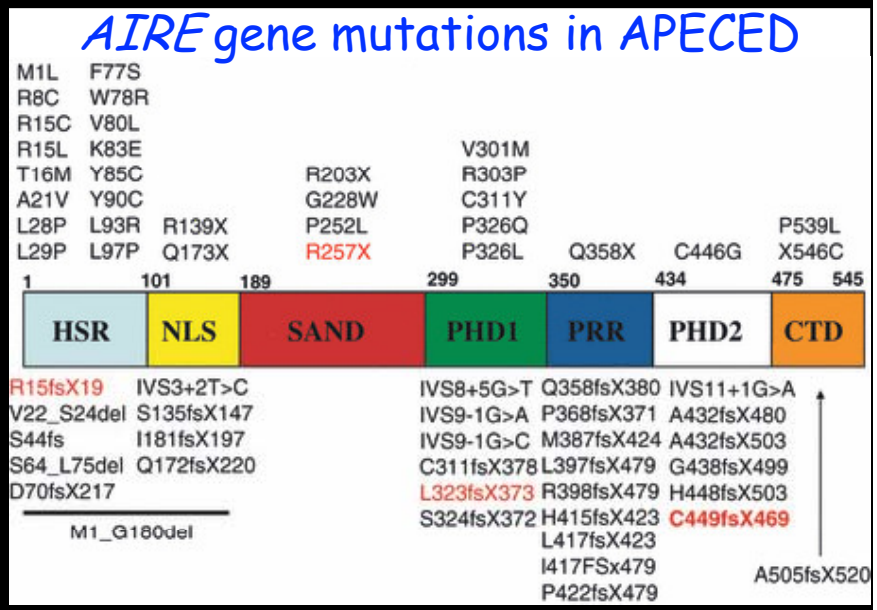


(modified from: Kaiser J, Science, 2012)

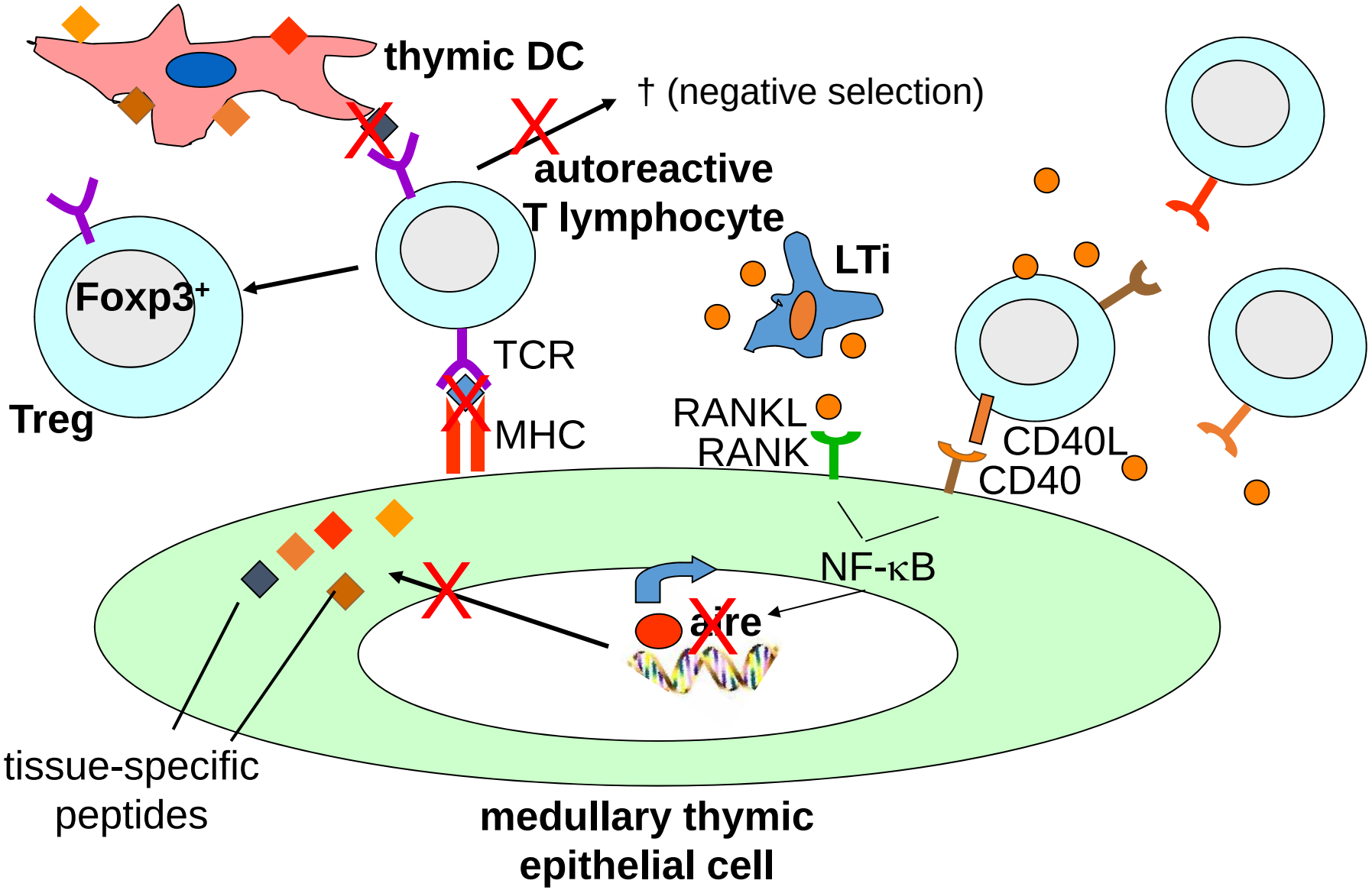

Central Tolerance Mechanism
Thymus
TCR
Thymocyte
MHC
Negative selection
Positive selection
AIRE
Apoptosis (98% of thymocytes)
Bone marrow
Lymphoid stem cell
Pro-B cell
Pre-B cell
IgM
Receptor editing
Transitional B cell (T1>T2)
Transitional B cell (T2>T1)
BAFF
Naive B cell
Germinal center
Memory B cell
Complement
IgG, IgA, and IgE
FOXP3
Peripheral Tolerance Mechanism
Effector T cell (CD4+, CD8+)
Regulatory T cell
FOXP3
FAS (AICD)
Interleukin-2
Antigen-presenting cell (dendritic cell, macrophage, B cell)
T effector cell (e.g., Th1, Th2, Th17)
T–B cross-talk
T-cell help, cytokine modulation; B-cell antigen presentation, T-cell costimulation
IgM
Short-lived plasma cell in lymphoid tissues
Long-lived plasma cell in bone marrow (or gut lamina propria)

Autoimmune PolyEndocrinopathy – Candidiasis – Ectodermal Dystrophy (APECED) syndrome





AIRE controls negative selection of self-reactive T cells in the thymus

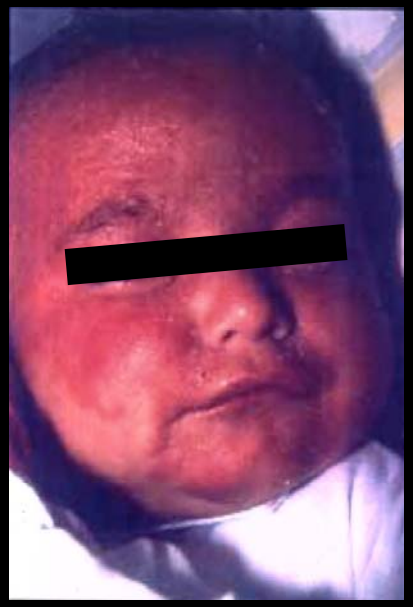


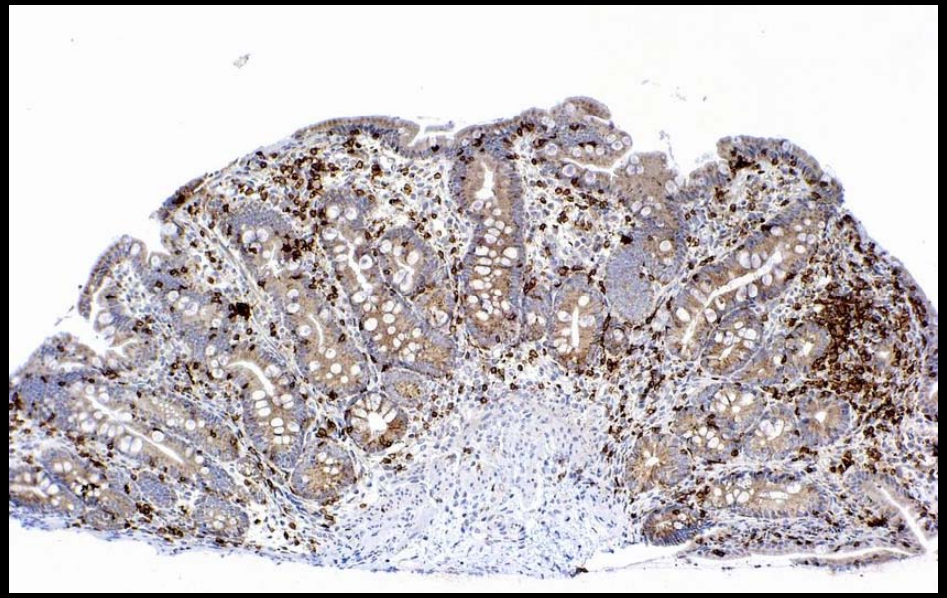
I **I**mmune deficiency/dysregulation

P **P**olyendocrinopathy

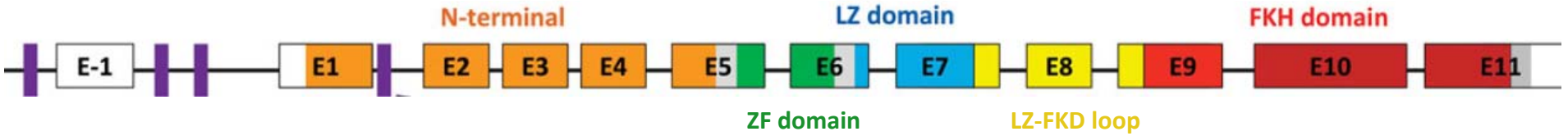
E **E**nteropathy

X **X**-linked inheritance

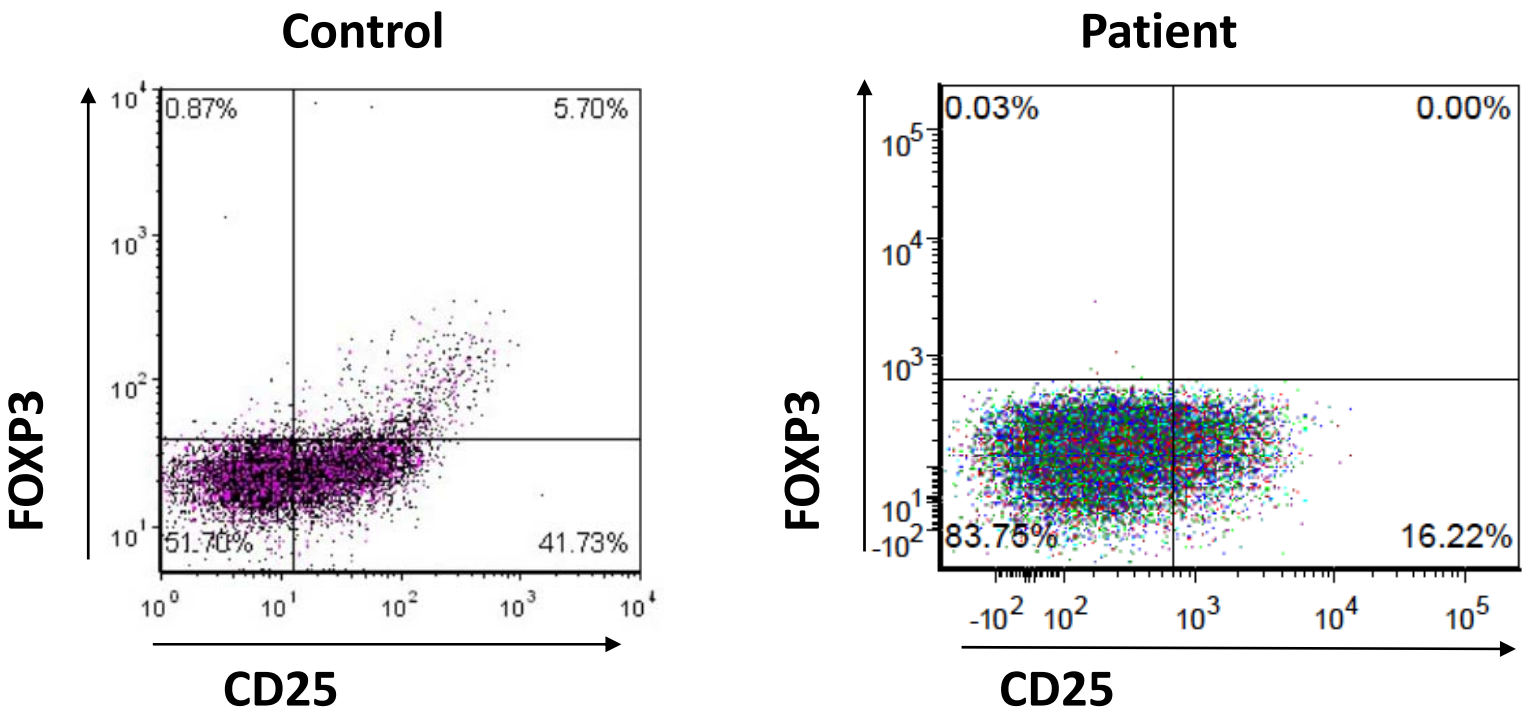




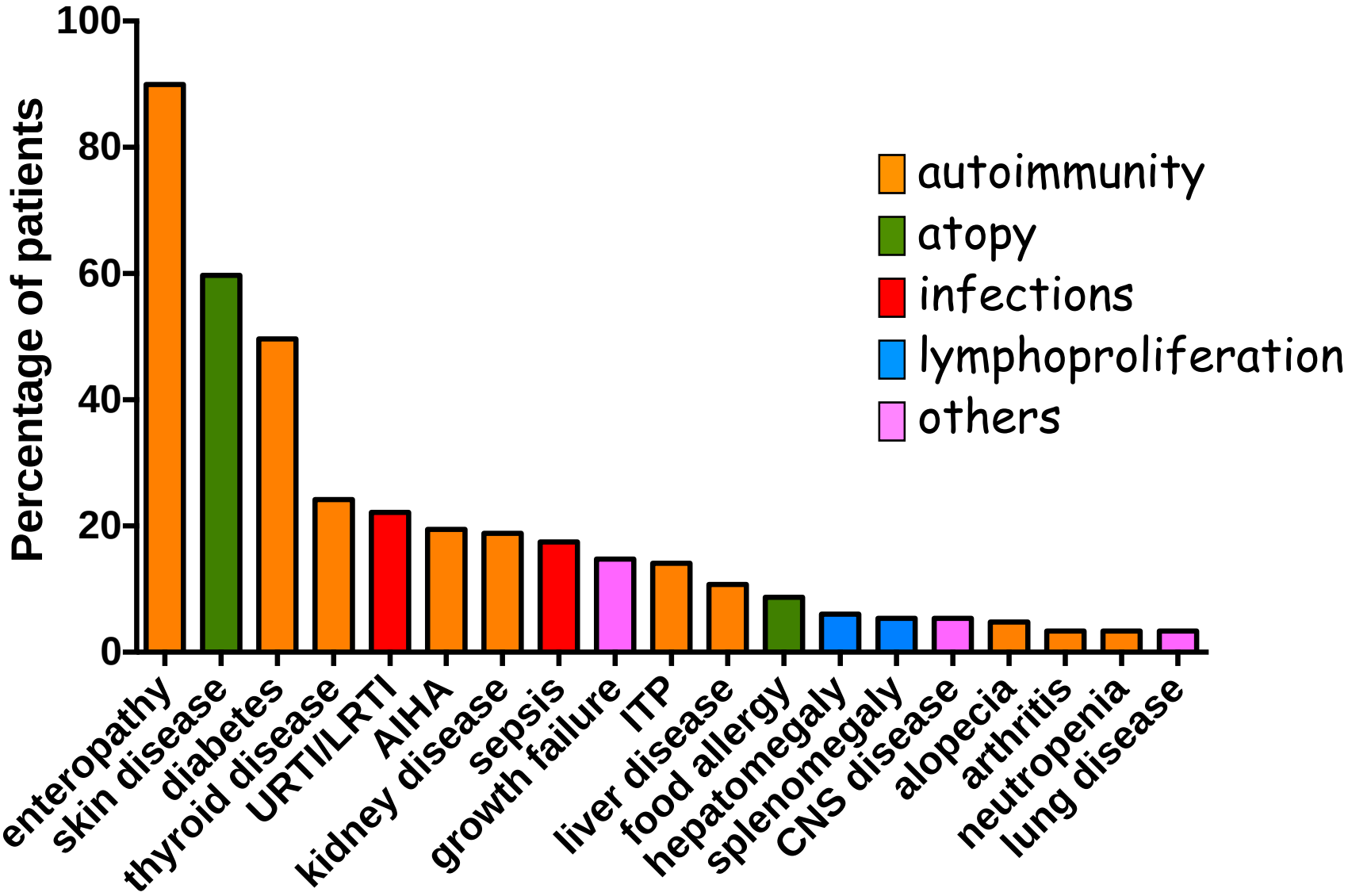
FOXP3 gene and protein



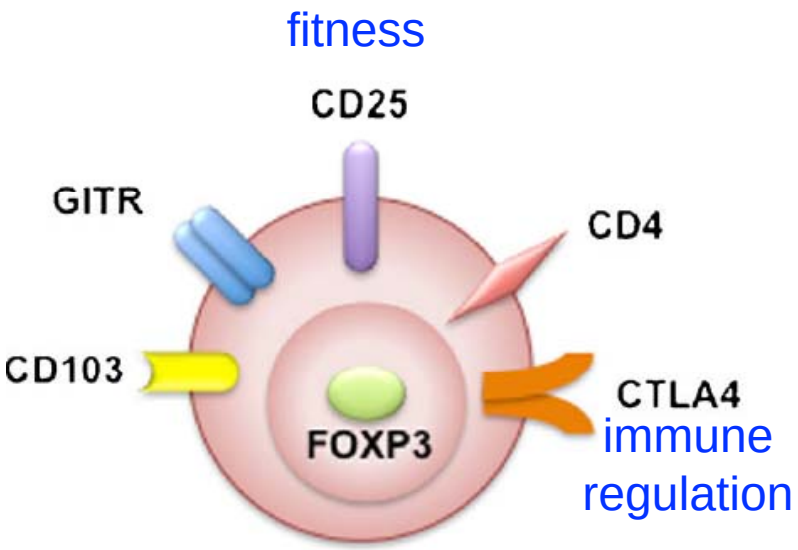
Gated on CD4⁺ cells

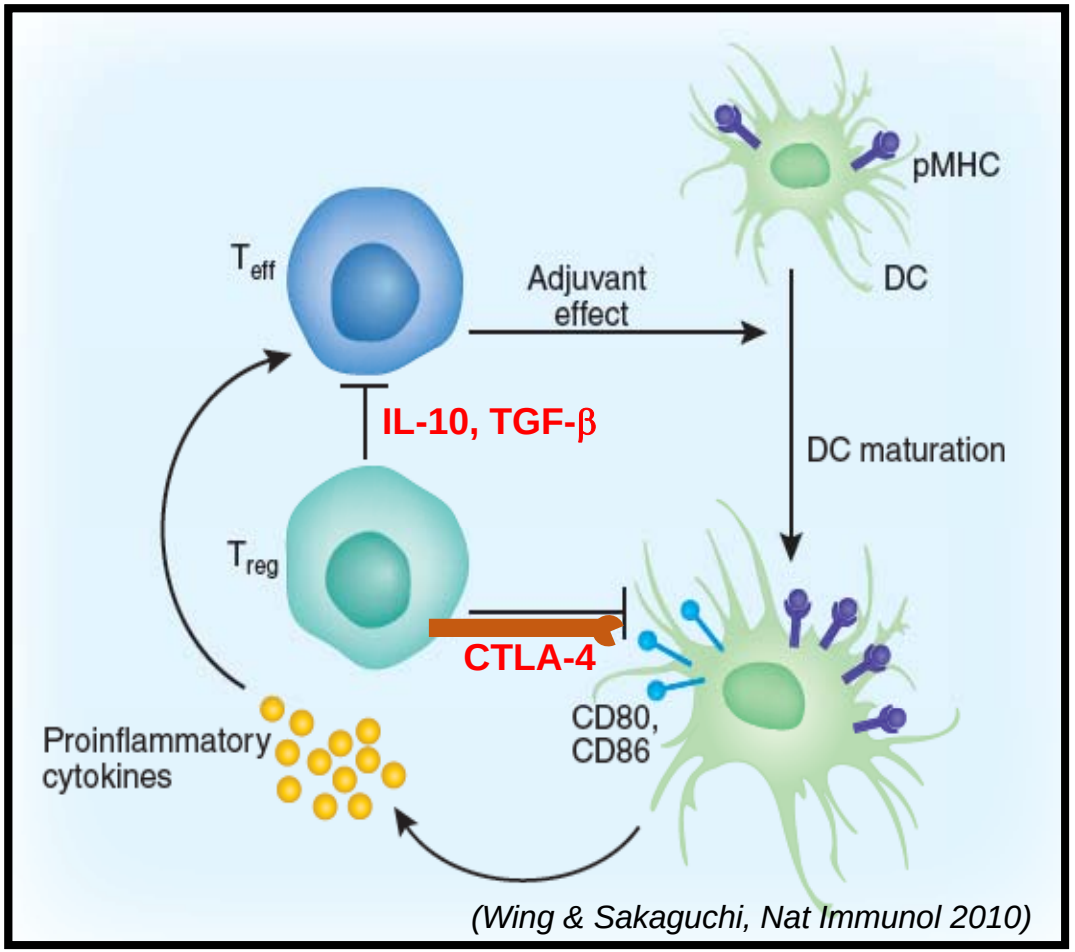


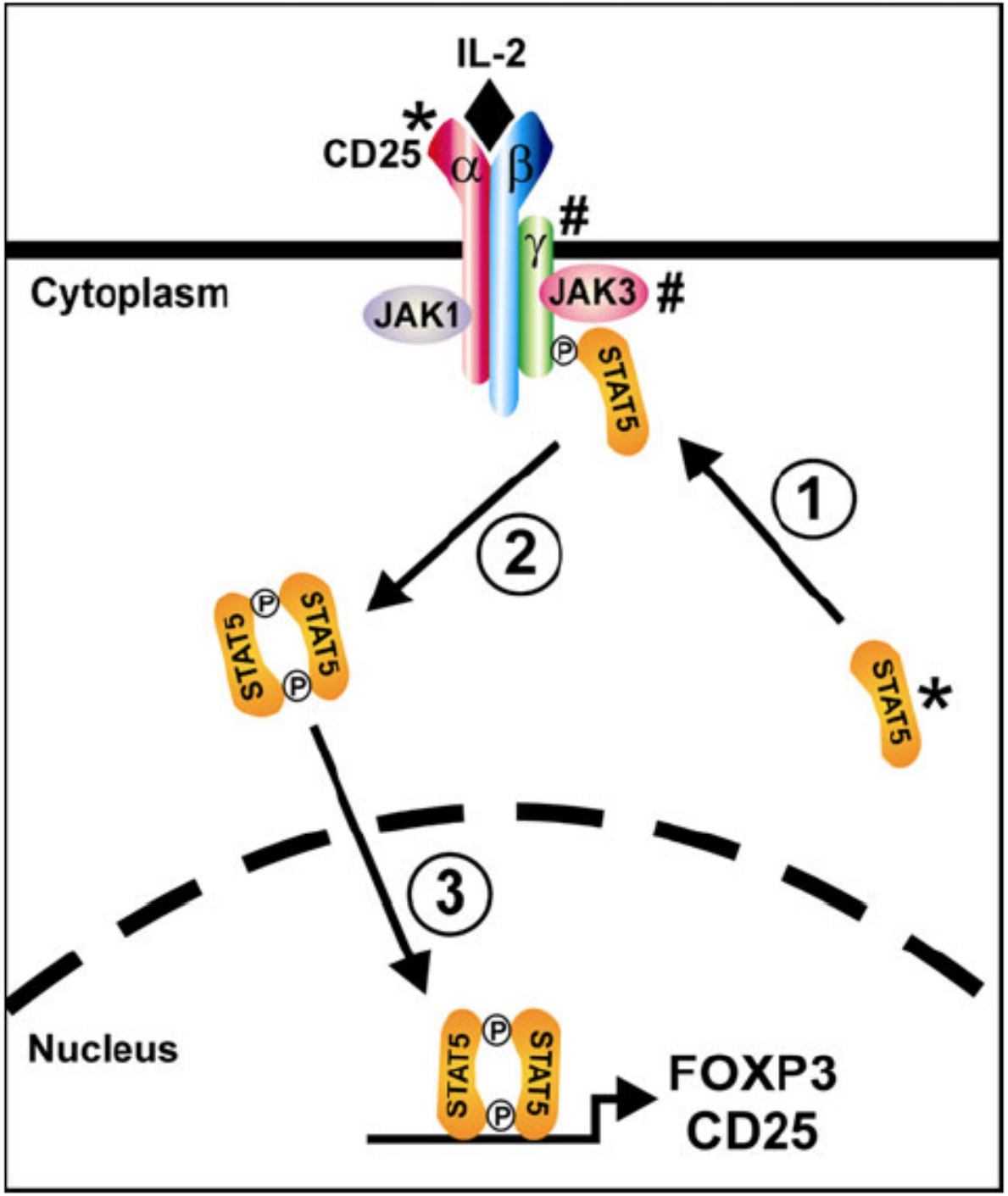
IPEX: Clinical features (n=149 patients from 52 publications)



T_{reg} cells: a key player in immune homeostasis







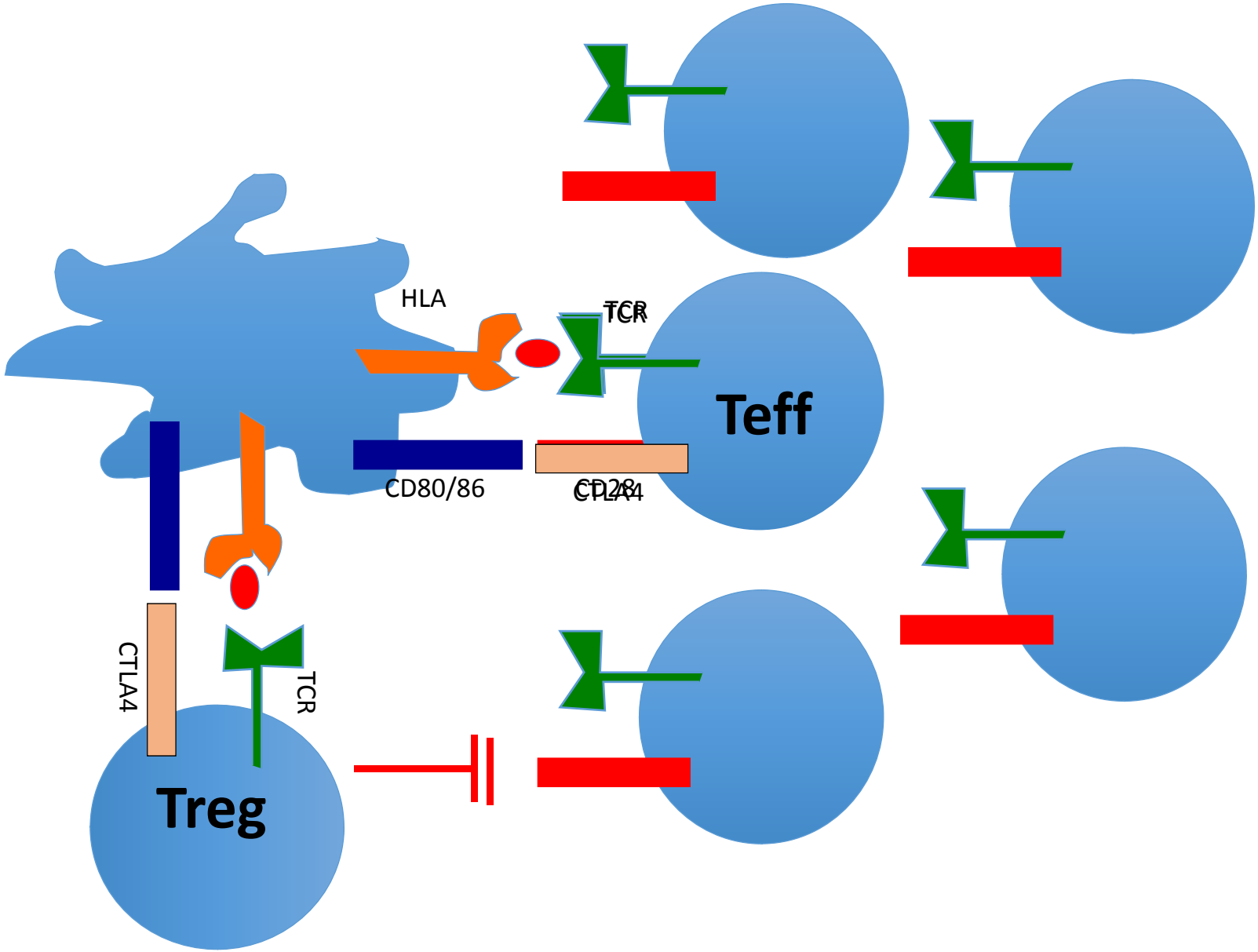
IL2RA deficiency

Enteropathy
Recurrent/chronic viral inf.
Hematomegaly
Lymphadenopathy
Eczema
Candidiasis
Normal IgE
AR inheritance

STAT5B deficiency

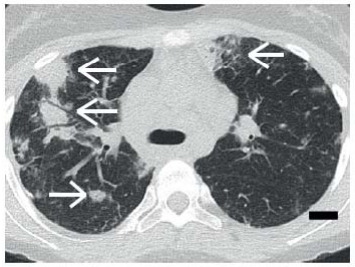
Markedly short stature
Chronic lung disease
Eczema
Diarrhea
Autoimmune hepatitis
Autoimmune hypothyroidism
Recurrent viral infections
Normal GH, very low IGF-1
AR inheritance

CTLA4 helps extinguish T cell activation

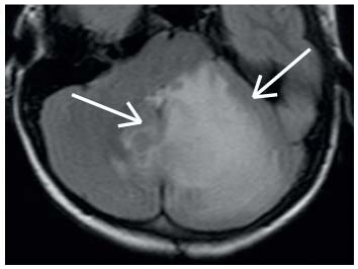


CTLA4 Deficiency

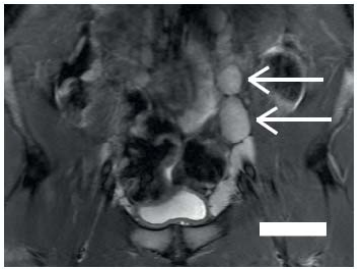
- Progressive lung disease with bronchiectasis and lymphocytic infiltrates

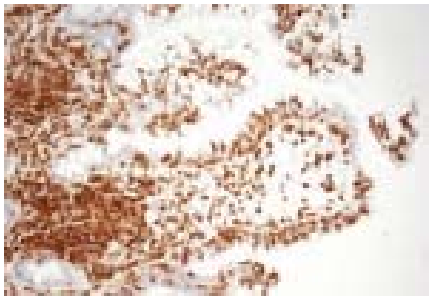


- Lymphoid aggregates in the brain



- Lymphadenopathy and infiltration of lymphocytes in mucosal tissues

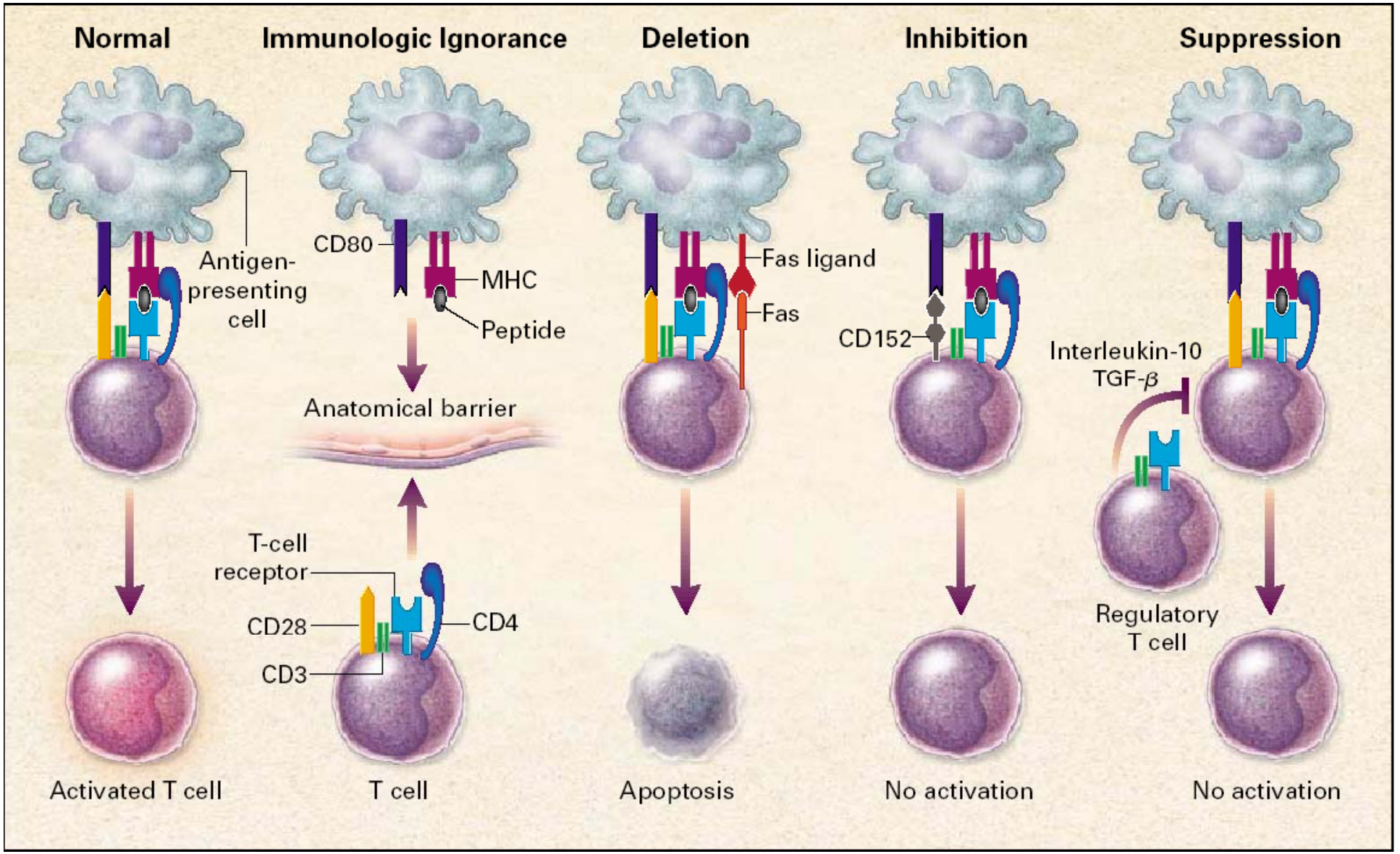




- Autoimmune cytopenias
- Hypogammaglobulinemia
- T cell lymphopenia with activated T cells
- Autosomal dominant inheritance, incomplete penetrance

(Kuehn et al., Science 2014; Schubert et al., Nat. Med. 2014)

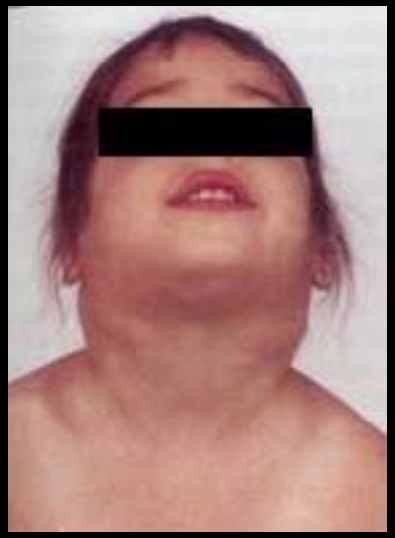
Peripheral Mechanisms of T Cell Tolerance



Autoimmune LymphoProliferative Syndrome (ALPS)

Clinical features

non malignant chronic lymphoproliferation
autoimmune cytopenias
increased risk of lymphoma (10%)

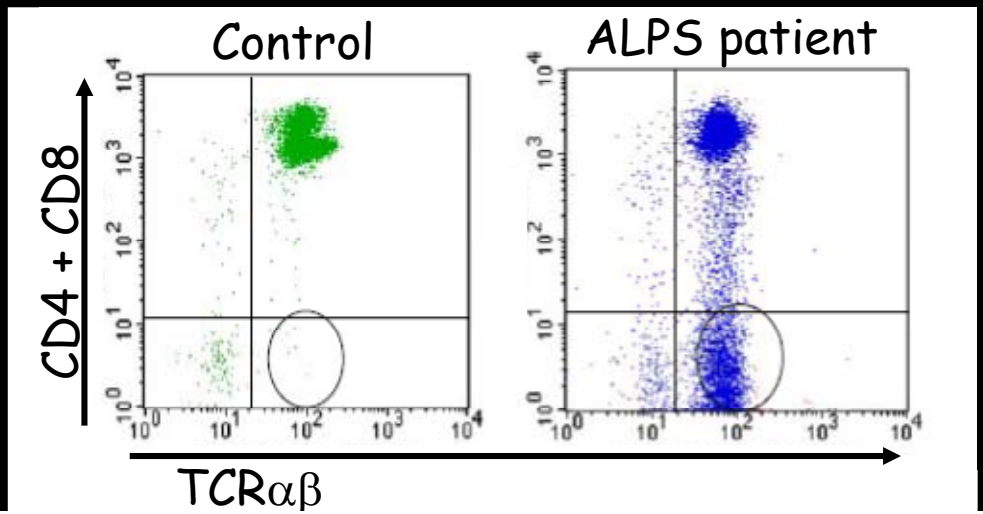


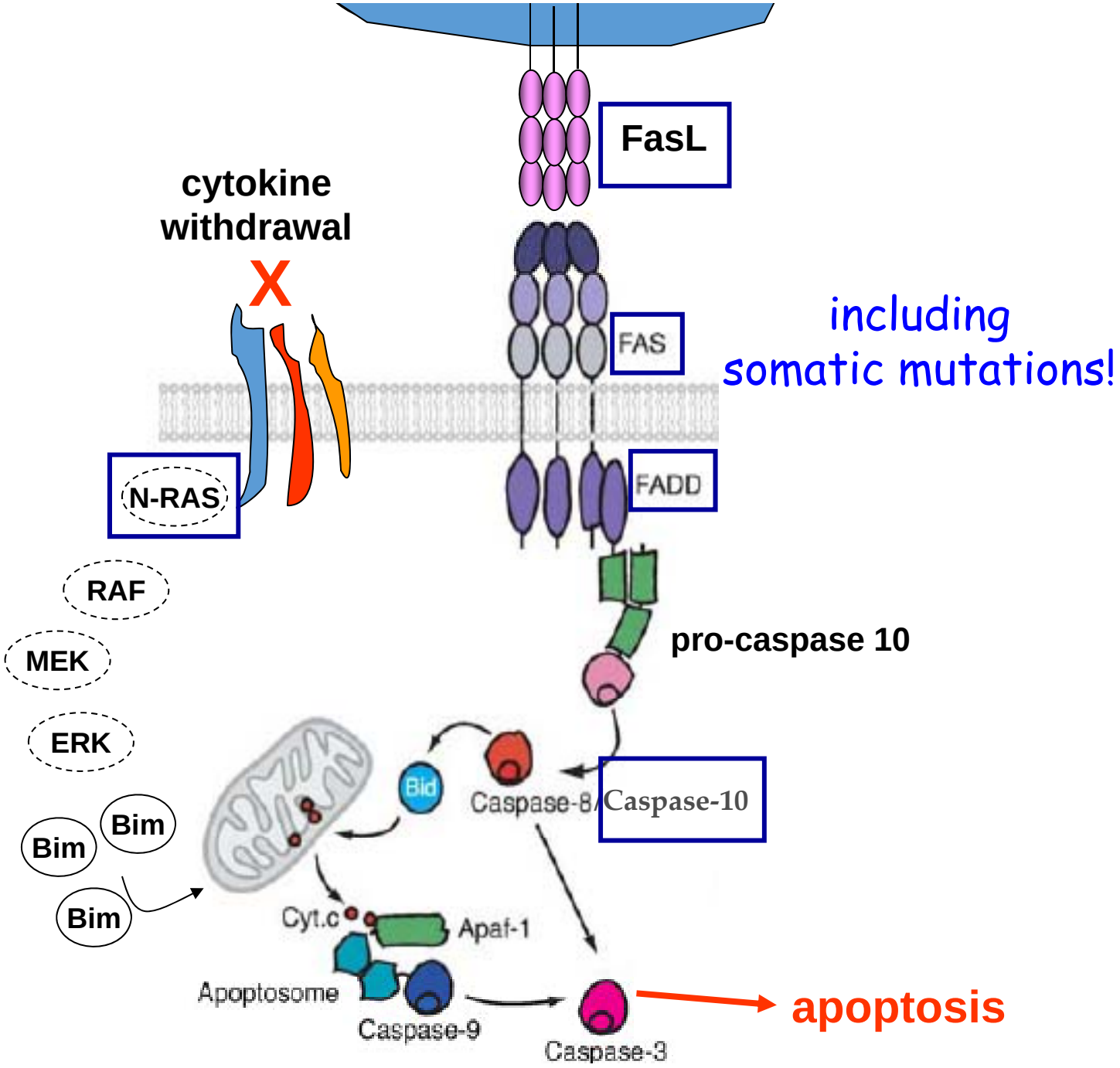
Laboratory features

↑ TCR$\alpha\beta^+$ $CD4^-$ $CD8^-$ (DN) T cells
impaired Fas-mediated apoptosis (most cases)
↑ FasL, ↑ IL-10
↑ vit. B12

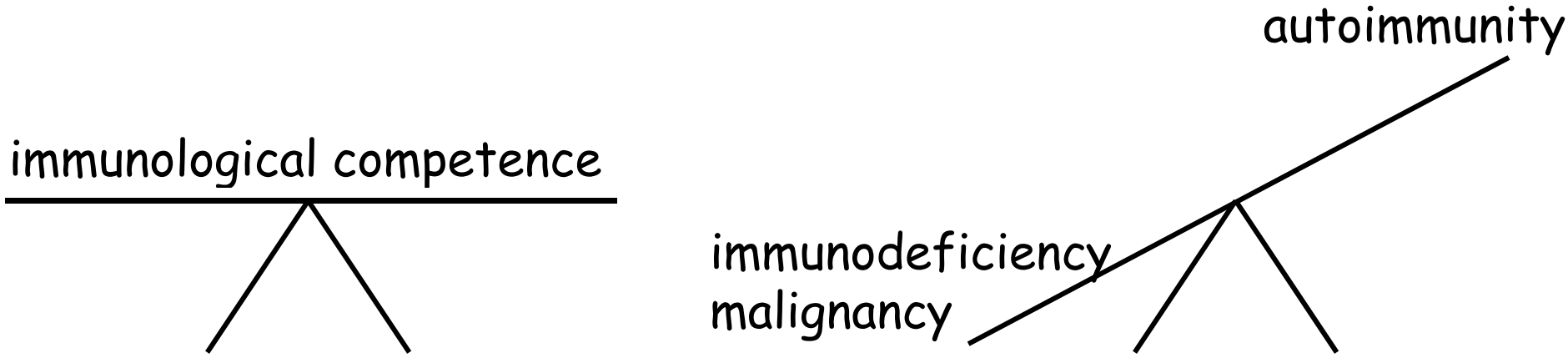
Genetics

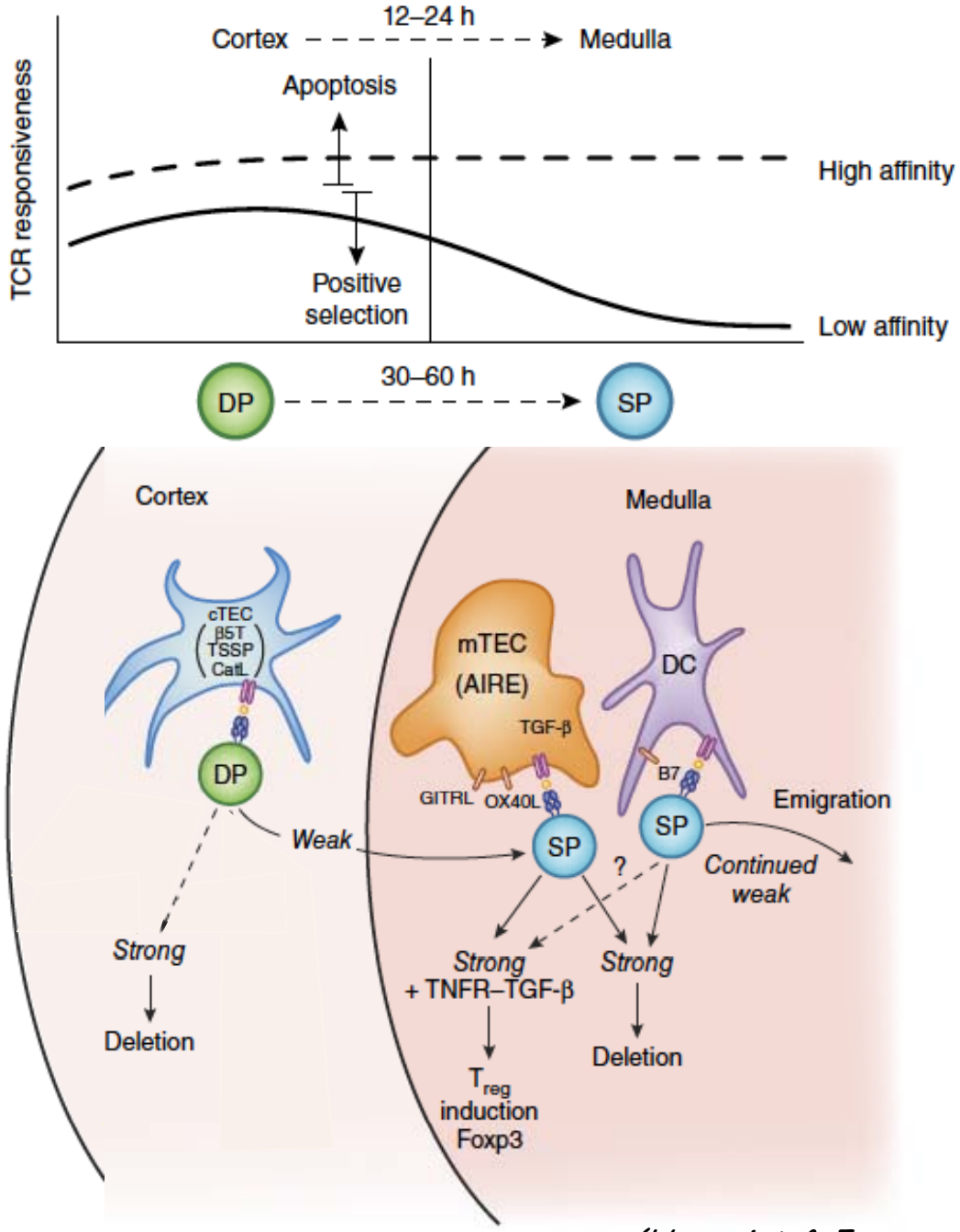
AD or sporadic
AR (more rare)
somatic mutations




FasL
cytokine
withdrawal
X
FAS
including
somatic mutations!
N-RAS
FADD
RAF
MEK
ERK
pro-caspase 10
Bid
Caspase-8/Caspase-10
Bim
Bim
Bim
Cyt.c
Apaf-1
Apoptosome
Caspase-9
Caspase-3
apoptosis

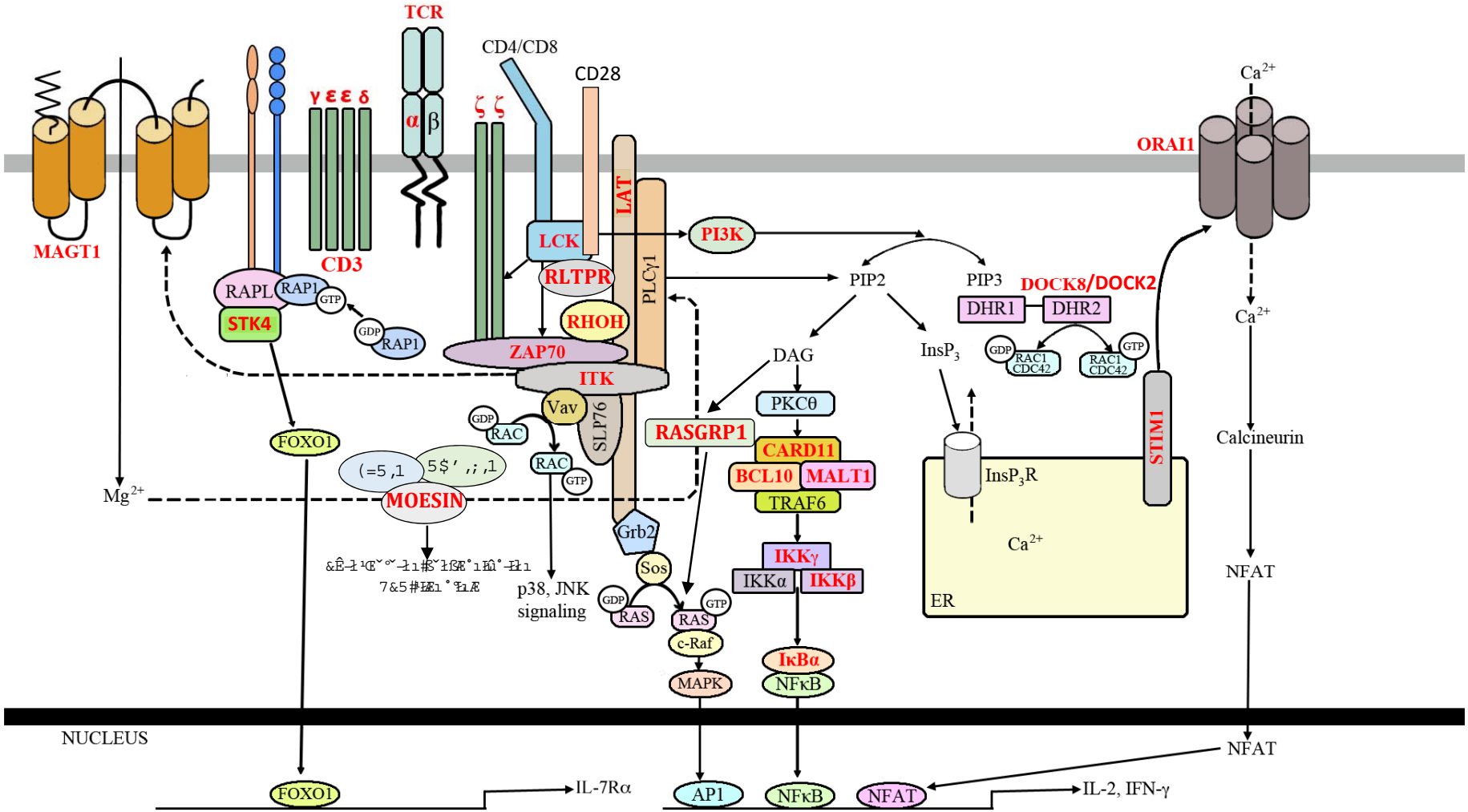
The seesaw model of immunological competence





(Hogquist & Jameson, Nat Immunol 2014)

Defects of T cell signaling



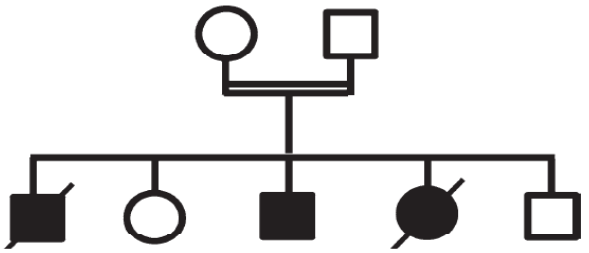
→ cell survival
→ cell proliferation
→ cytokine production

→ effector mechanisms
→ immune regulation

J Exp Med 2016; 13:1185-99

Early onset combined immunodeficiency and autoimmunity in patients with loss-of-function mutation in *LAT*

Baerbel Keller,[1*] Irina Zaidman,[2*] O. Sascha Yousefi,[1,3,4*] Dov Hershkovitz,[5] Jerry Stein,[6] Susanne Unger,[1] Kristina Schachtrup,[1] Mikael Sigvardsson,[7] Amir A. Kuperman,[8,9] Avraham Shaag,[10] Wolfgang W. Schamel,[1,3**] Orly Elpeleg,[10**] Klaus Warnatz,[1**] and Polina Stepensky[10,11**]



P1 P2 P3

P1

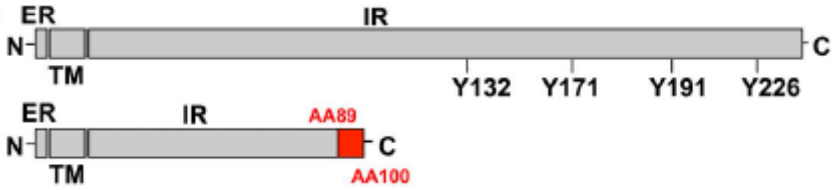
pneumonias
bronchiectasis
EBV, CMV
AIHA, ITP
lymphadenopathy
splenomegaly
progressive hypoγglob.
progress. lymphopenia

P2

congenital toxoplasmosis
pneumonias
VZV, CMV, adenovirus
candida
AIHA, ITP
lymphadenopathy
splenomegaly
hypo- to hyper-γglob.
progress. lymphopenia
elevated γδT cells

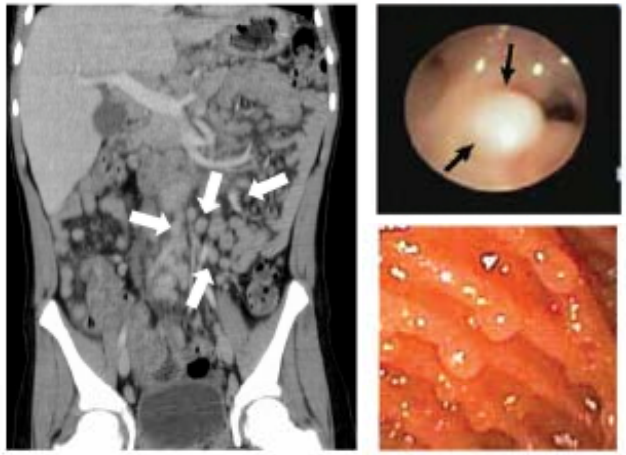
P3

pneumonias
UTIs
CMV
TTP
lymphadenopathy
splenomegaly
hyper-γglobul.
elevated γδT cells

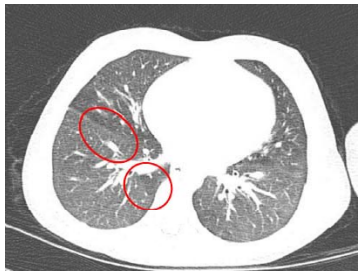


Activated PI3-kinase Cδ syndrome (APDS): Clinical phenotype

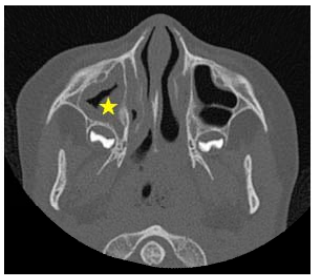
nodular lymphoid hyperplasia



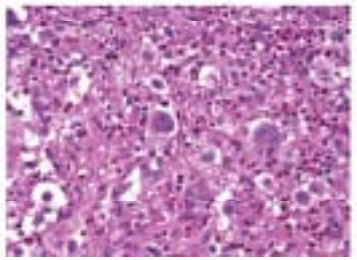
bronchiectasis



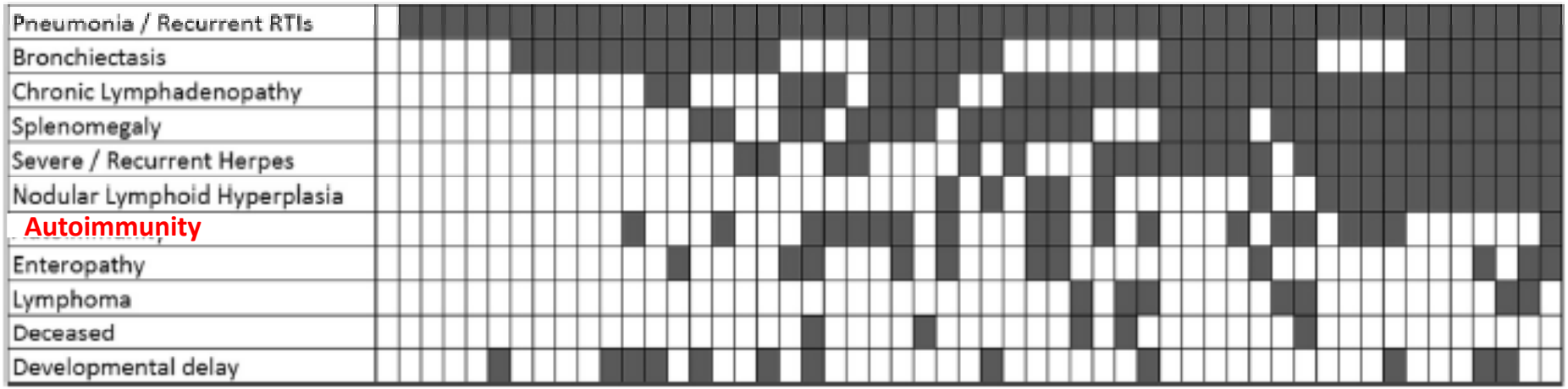
recurr. URTI



B cell lymphoma

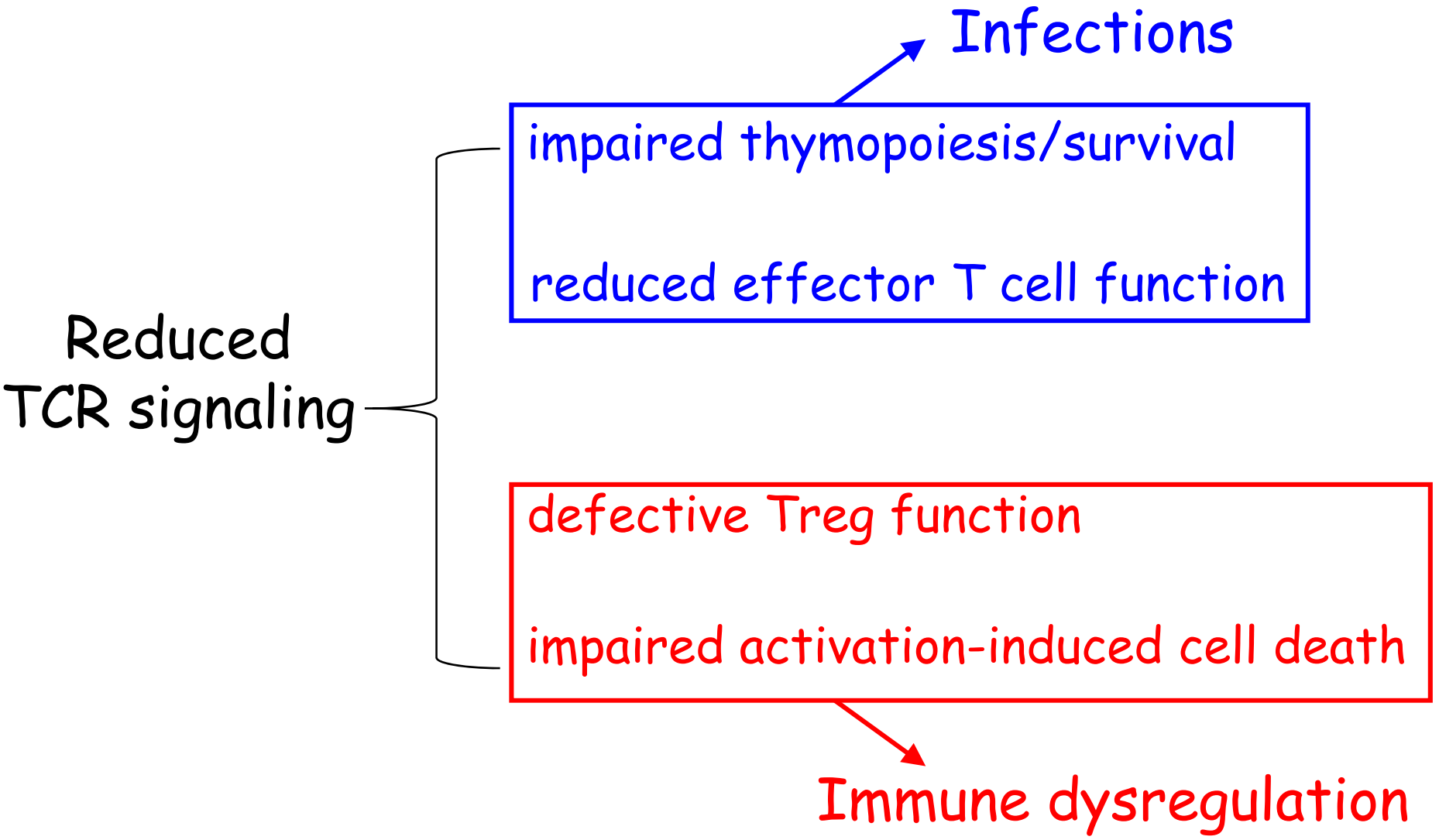


chronic/recurrent viremia (CMV, EBV, VZV, HSV)
sclerosing cholangitis



(Coulter et al., JACI 2016)

Defects of T cell signaling: When immunodeficiency and immune dysregulation go hand-in-hand

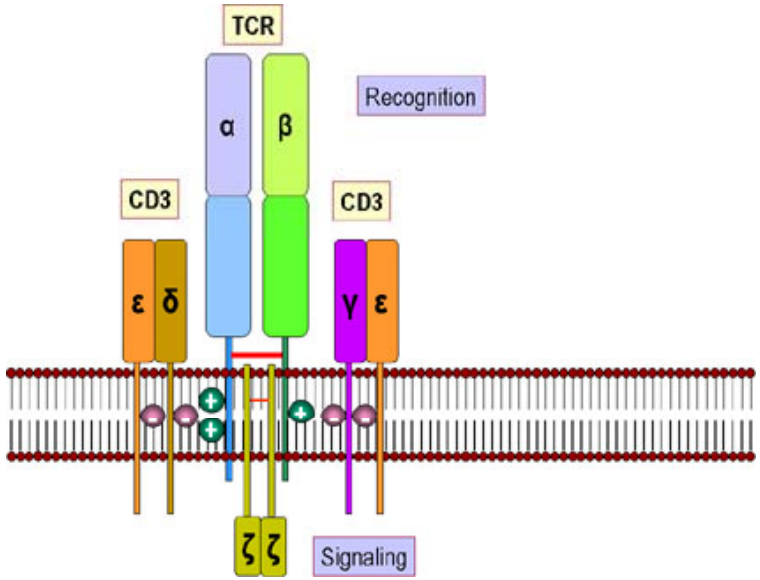


Combined immunodeficiencies with dysfunctional T lymphocytes: how to diagnose them?

Laboratory features

- T cell lymphopenia (variable severity)
- Altered distribution of T cell subsets (often increase of T_{EM} and T_{EMRA})
- Reduced number of naïve T cells
- Impaired T cell function
- Immunoglobulin serum levels are variable (from low to high)
- Antibody production is variably affected
- Autoantibodies may be present

Critical role of TCR signaling in T cell development



CD3δ deficiency: T⁻ SCID

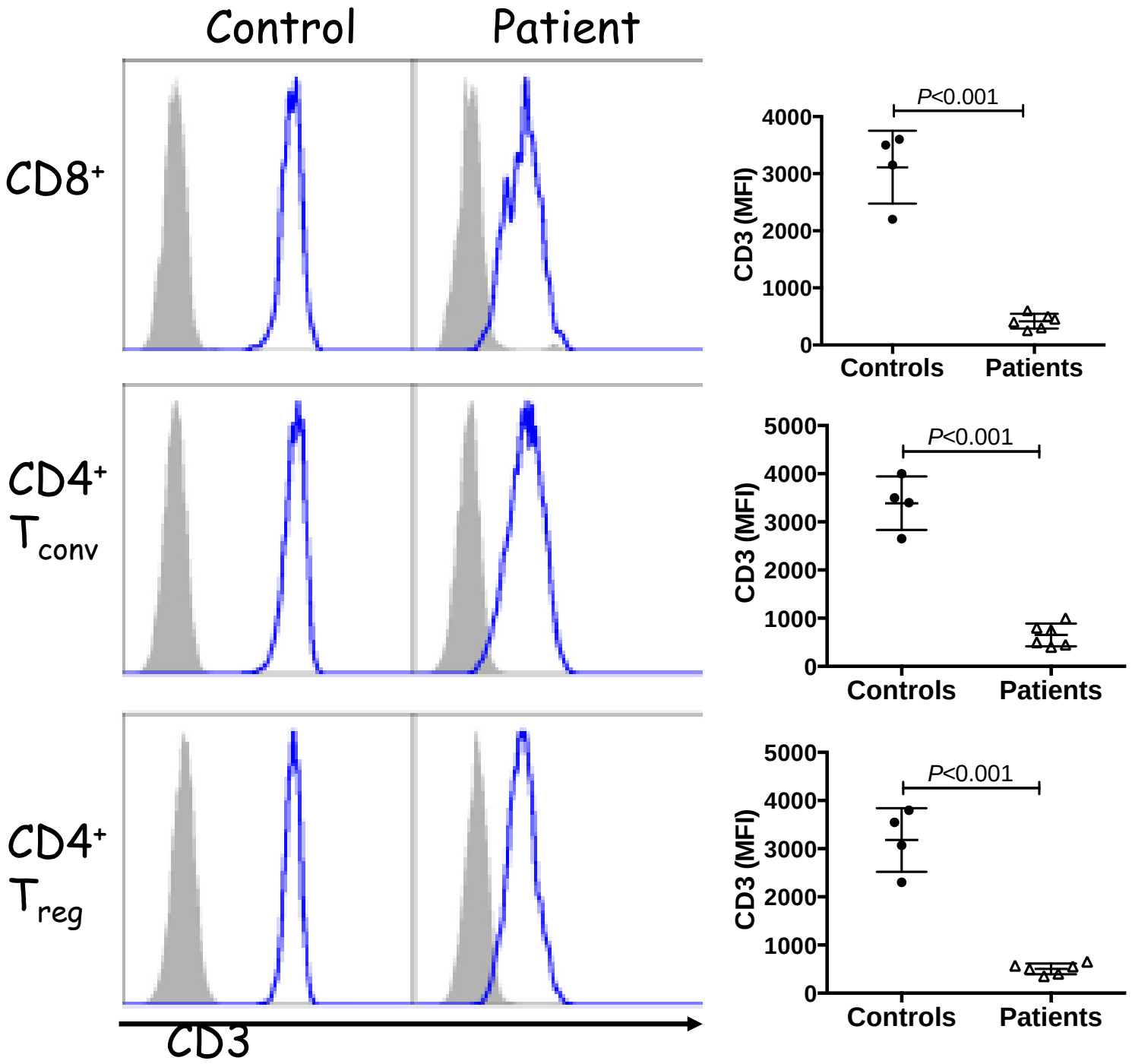
CD3ε deficiency: T⁻ SCID

CD3ζ deficiency: T⁻ SCID

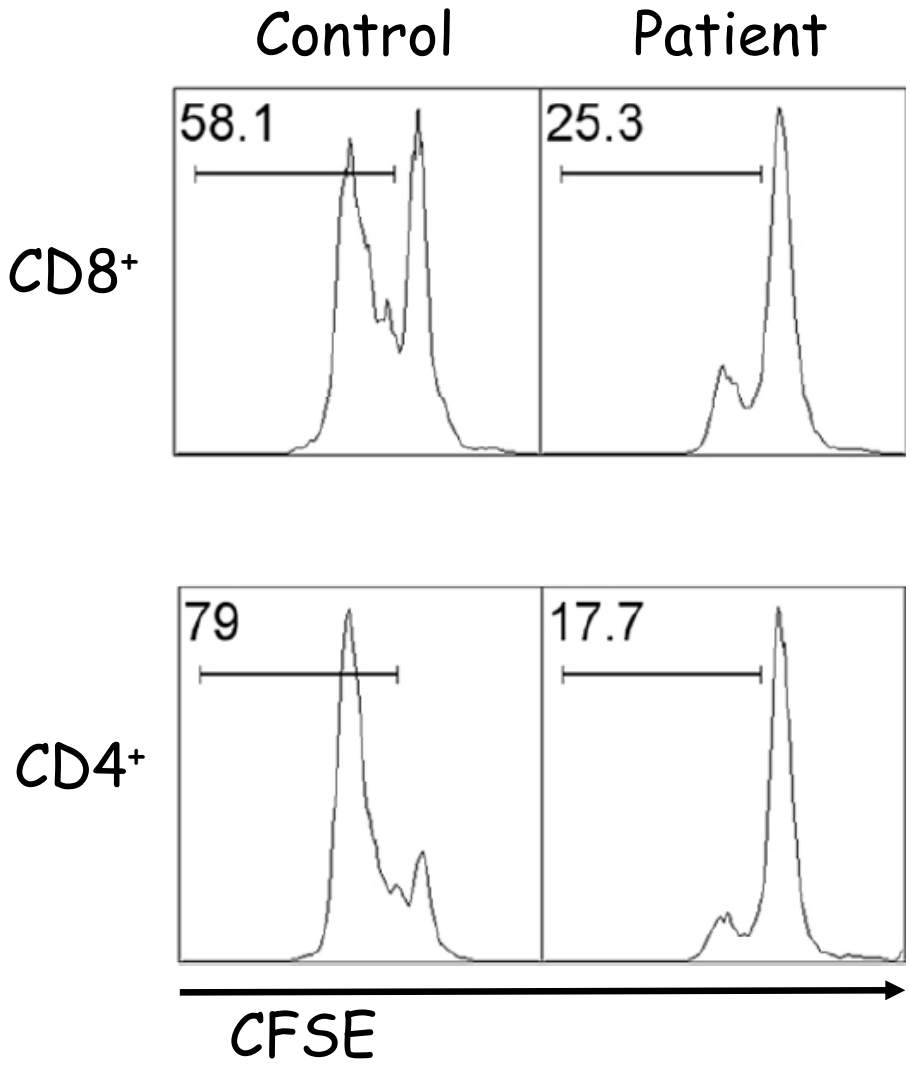
CD3γ deficiency: $T^{+/lo}$ CID with autoimmunity

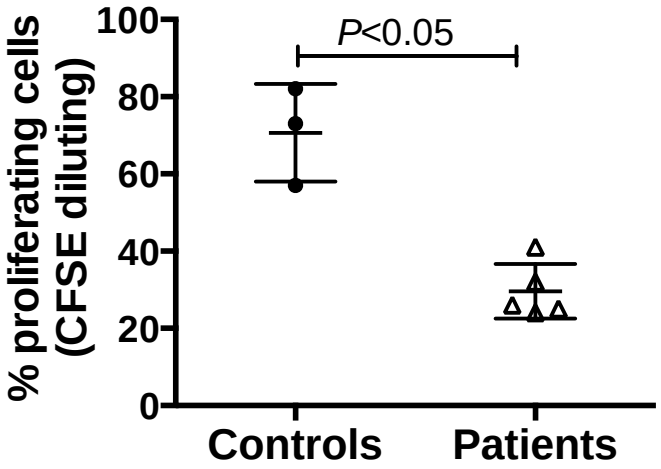
6 patients with CD3γ deficiency

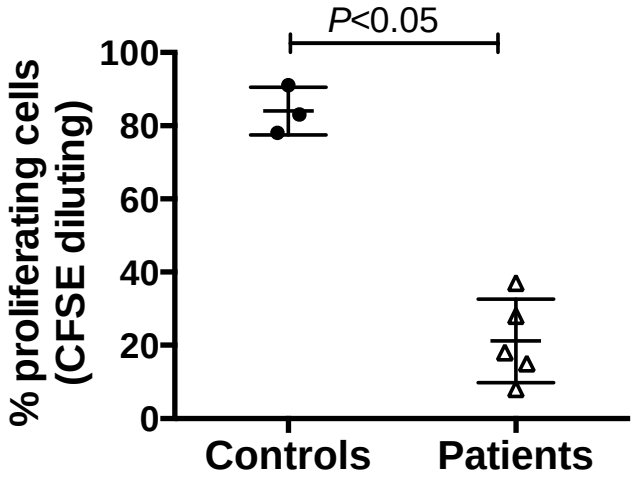
	Age (y)	CD3/μL	IgG (mg/dL)	Infections	Autoimmunity
P1	2	2018	338	pneumonia, EBV	enteropathy, AIHA
P2	11	630	1240	recurrent RTI	thyroiditis
P3	1.1	2800	881	none	thyroiditis
P4	12	380	491	recurrent RTI	thyroiditis, AIHA, ITP
P5	20	1200	1660	bronchiectasis	thyroiditis, AIHA, NS
P6	34	1051	632	abscesses, viral meningitis	none


Control
Patient
CD8+
CD4+ Tconv
CD4+ Treg
CD3
P<0.001
CD3 (MFI)
4000
3000
2000
1000
0
Controls
Patients
5000

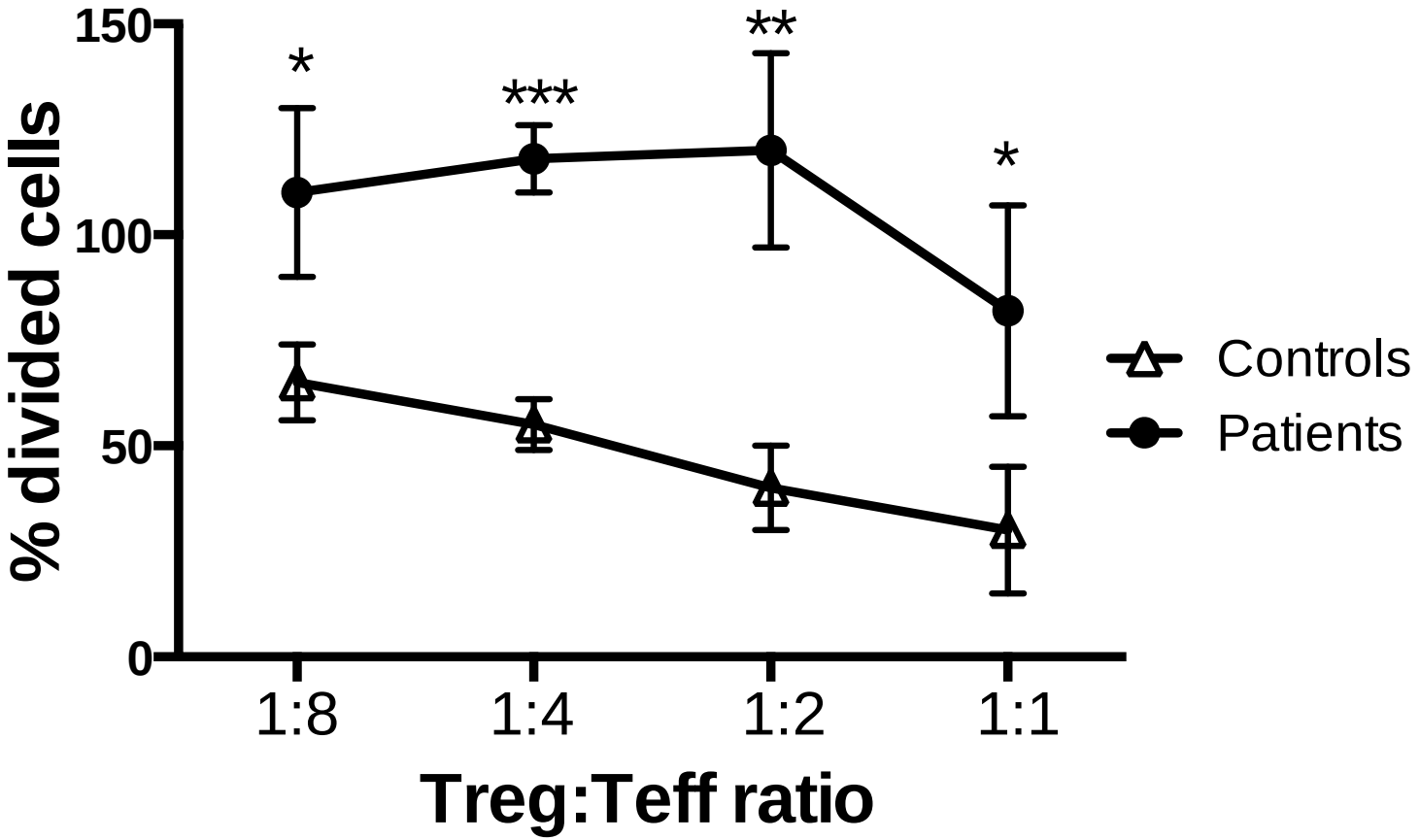
Impaired in vitro proliferation to PHA of T lymphocytes from *CD3G*-mutated patients





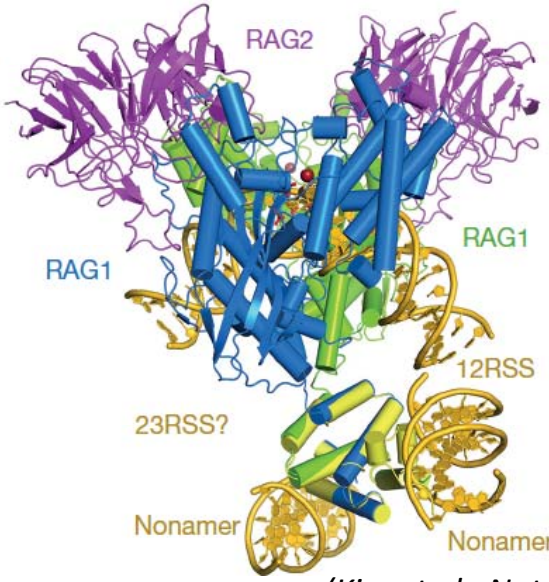


In patients with CD3G mutations, altered TCR signaling is associated with defective T_{reg} suppressive activity

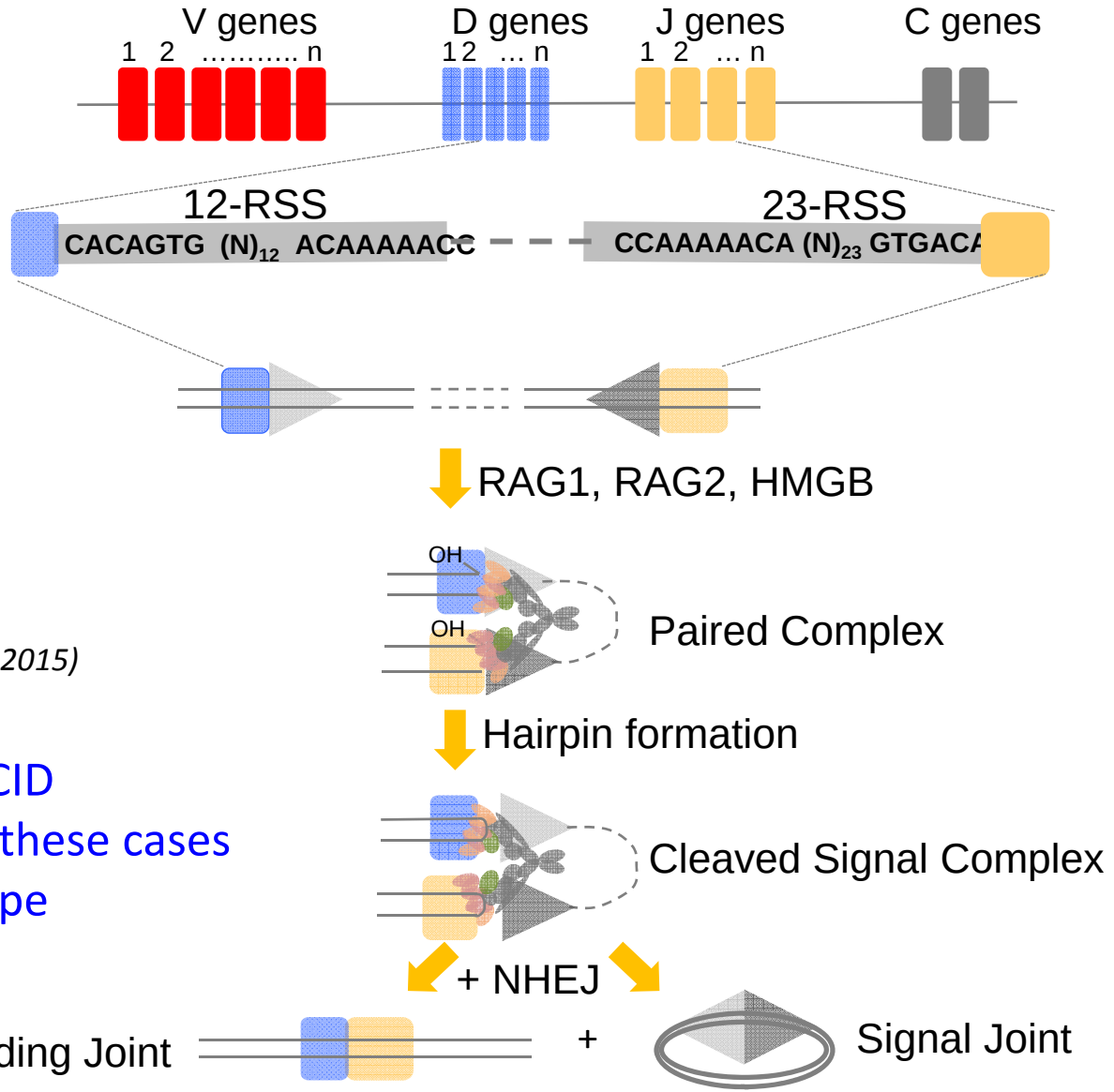


RAG1/2 Proteins: Key Players of V(D)J Recombination

RAG1/RAG2 heterotetramer



(Kim et al., Nature 2015)



RAG deficiency: 20% of all SCID

RAG1 mutated in 65-80% of these cases

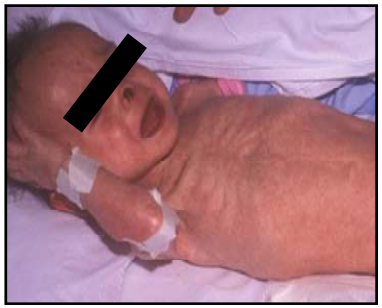
SCID is not the only phenotype

Phenotypic heterogeneity of SCID: RAG defects as an exemplum

INFECTION — AUTOIMMUNITY

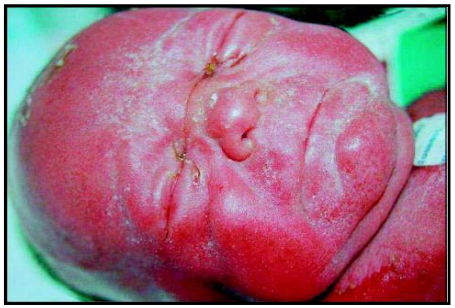
SCID ($T^-B^-NK^+$)

Early onset
Severe infections
Failure to thrive
Lack of T and B cells
Absent Ig



Omenn syndrome

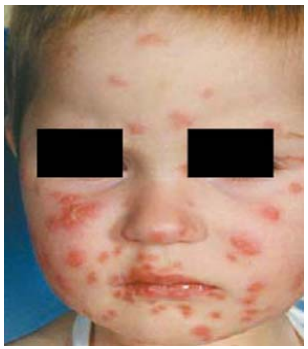
Early onset
Oligoclonal T cells
B cell lymphopenia
↓↓IgG, but ↑ IgE
Eosinophilia

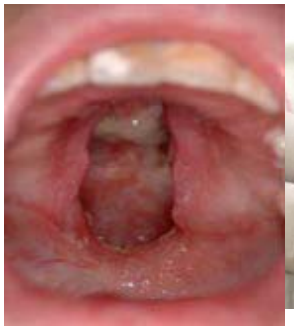


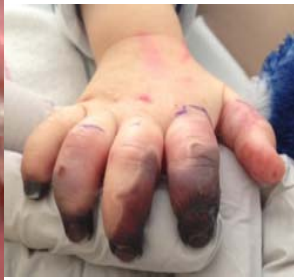
CID + Granuloma/Autoimmunity

Delayed onset
Infections
Autoimmunity
Granulomas

Variable T/B cell counts
↓, normal or ↑ Igs
Autoantibodies

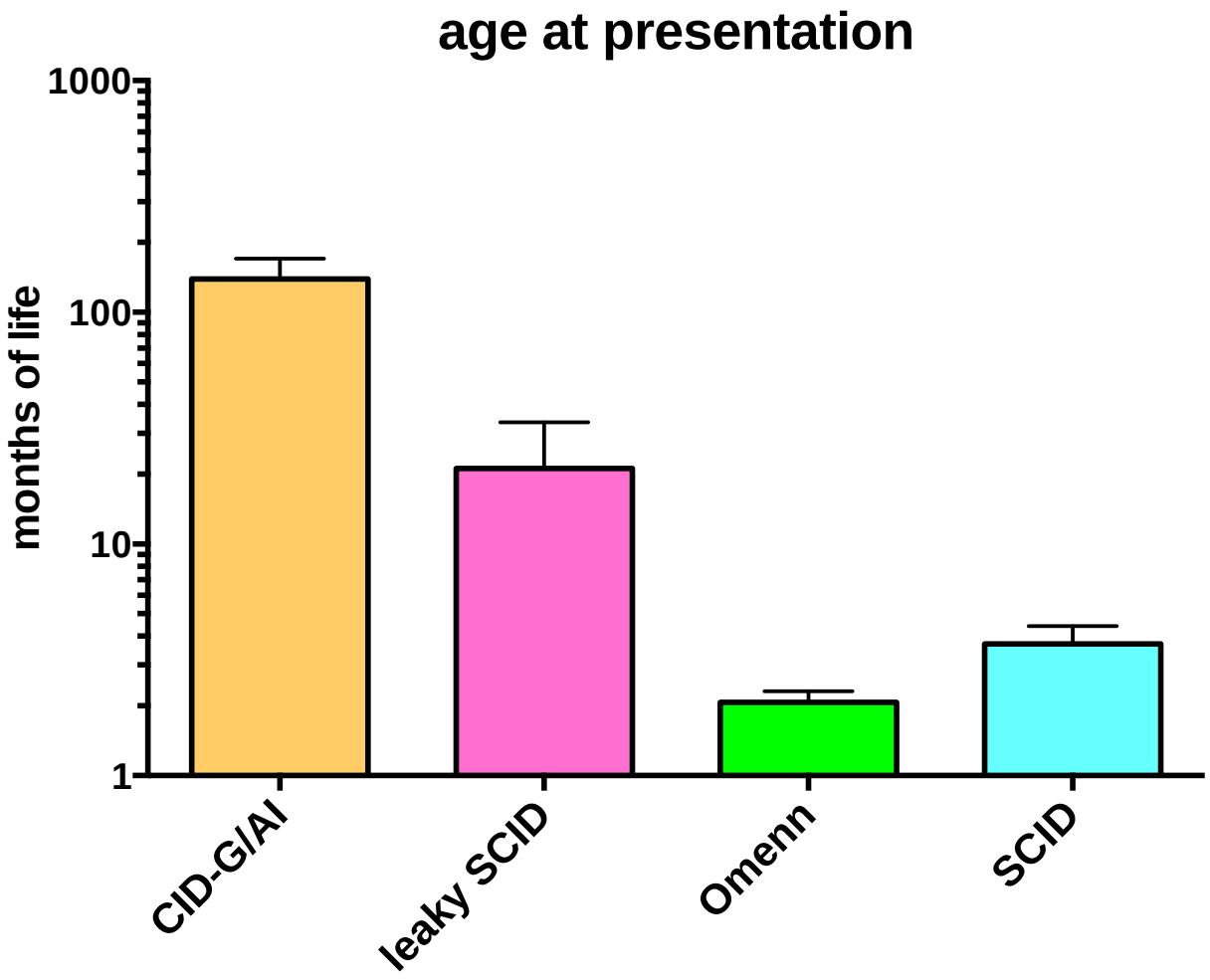




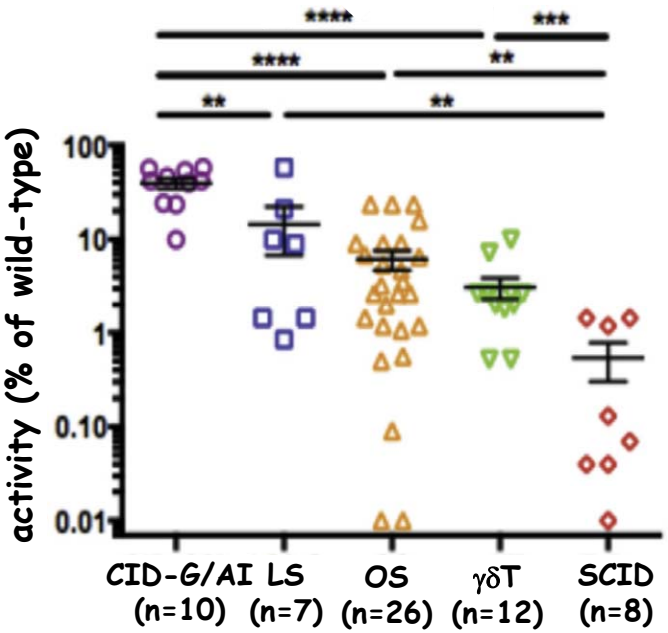


Additional phenotypes: Ab deficiency, CVID-like, XLA-like

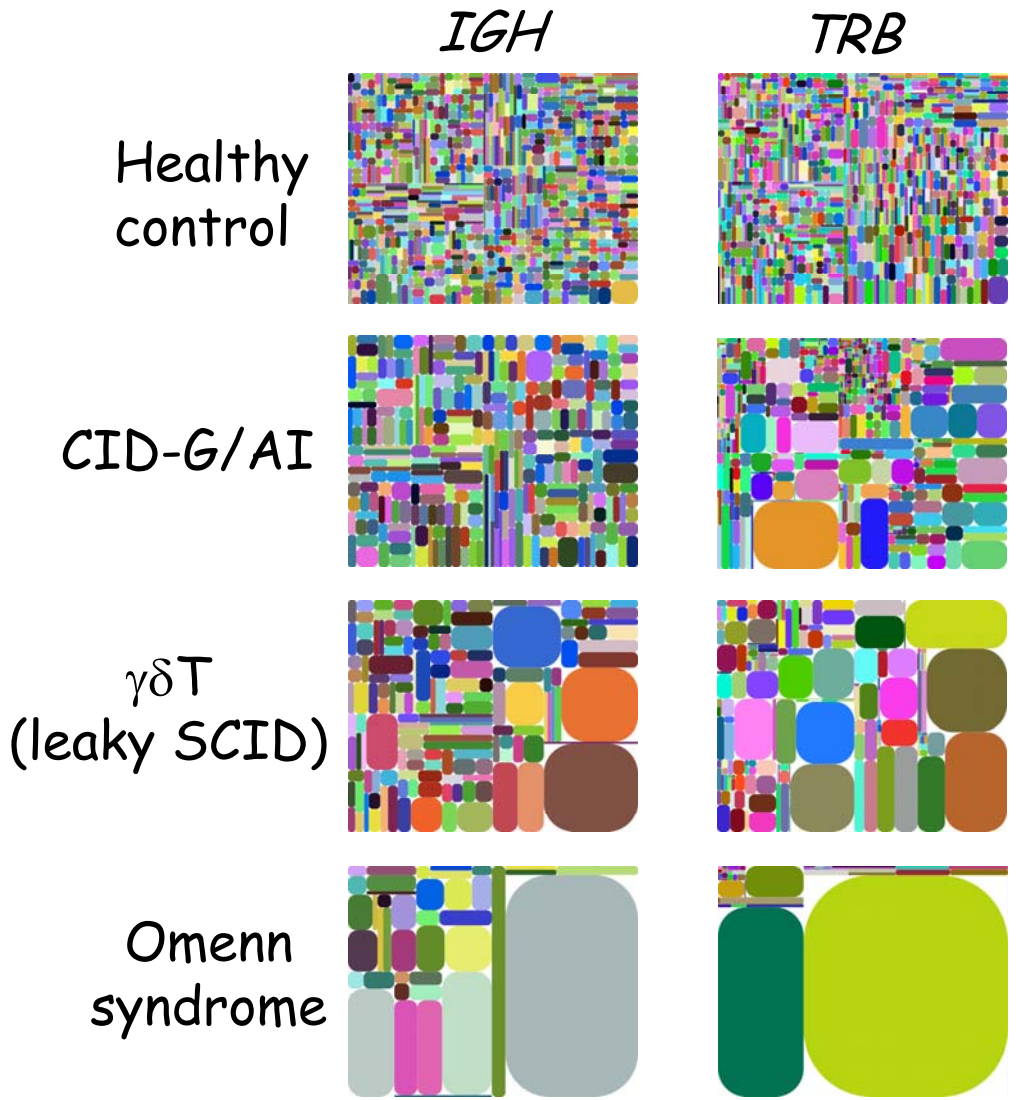
Immunological features in patients with various RAG deficiency phenotypes



The clinical phenotype of human RAG deficiency correlates with the degree of recombination activity and with diversity of T and B cell repertoire



(Lee et al, JACI 2014)



(Lee et al, Science Immunol 2016)

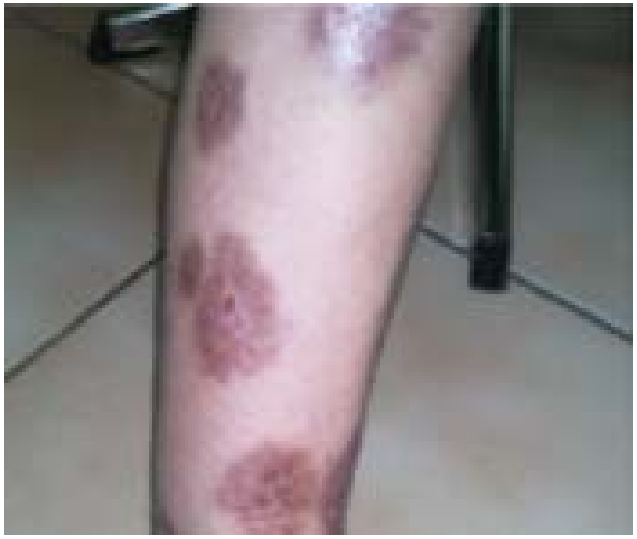
Immune dysregulation in RAG deficiency

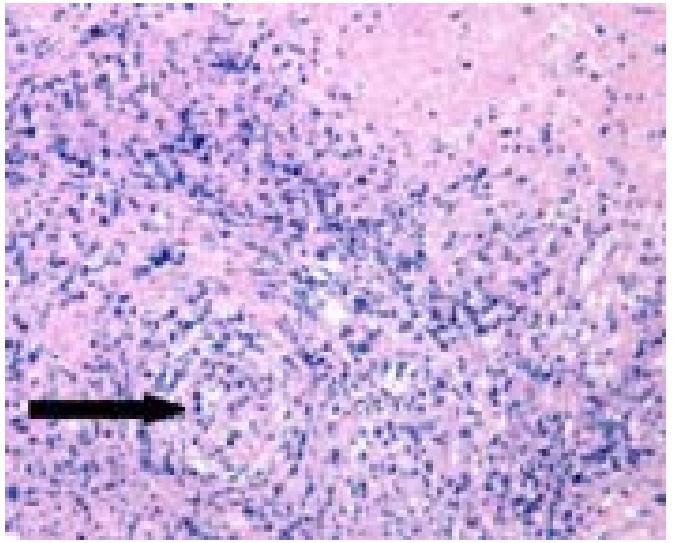
	RAG1 def. (n= 103)		RAG2 def. (n=87)		**Total RAG (n=190)**	
Manifestation	*N*	*%*	*N*	*%*	***N***	***%***
Autoimmunity	22	21.3	12	13.8	**34**	**17.9**
Granulomas	10	9.7	5	5.7	**15**	**7.9**
Skin rash	41	39.8	40	48.2	**81**	**42.6**
Hepatomegaly	15	14.6	22	25.3	**37**	**19.5**
Lymphadenopathy	11	10.7	19	22.9	**30**	**15.8**
Splenomegaly	8	7.8	23	26.4	**31**	**16.3**

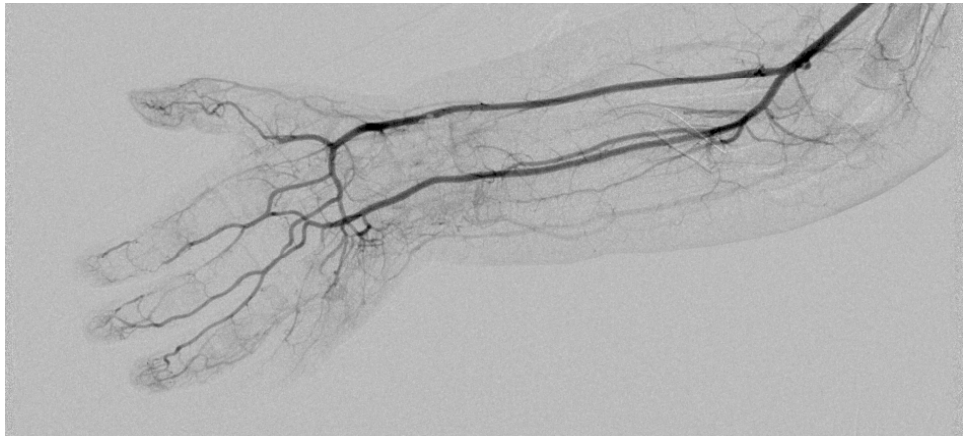
Which autoimmune manifestations?

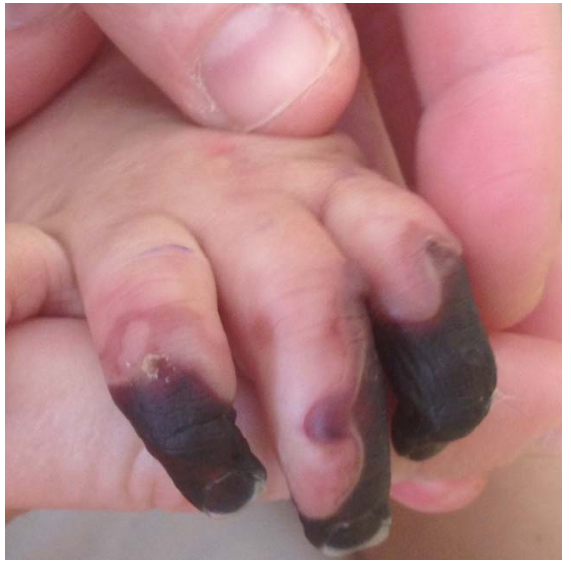
Manifestation	*N*	*%*
Cytopenias	22	11.6
AIHA	16	8.4
neutropenia	8	4.2
ITP	7	3.7
Vasculitis	3	1.6 each
IBD	3	
Vitiligo	3	
Alopecia	3	
Hypothyroidism	3	

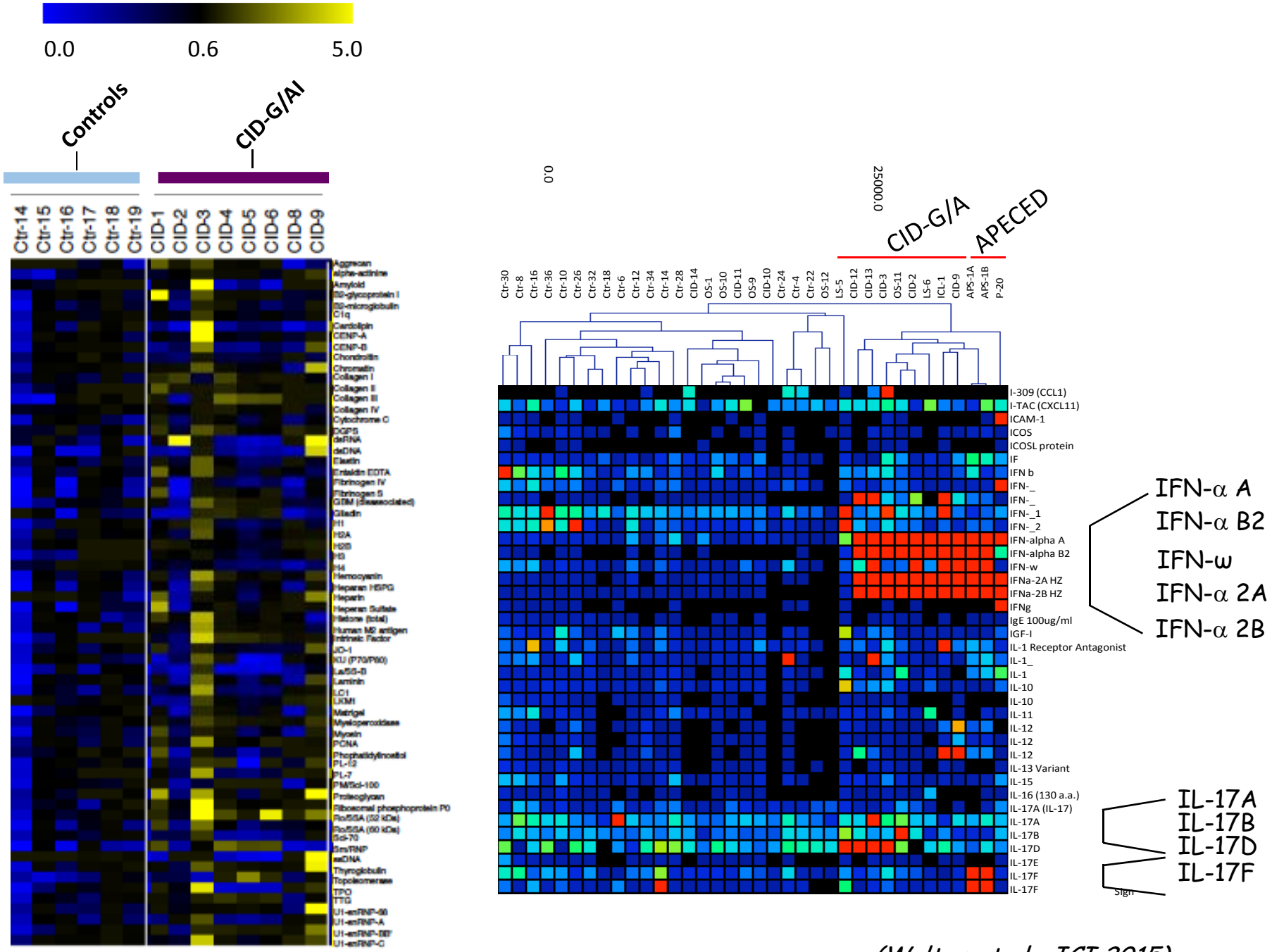
Psoriasis, focal glomerulosclerosis, biliary cirrhosis, myasthenia gravis, arthritis, have also been reported











(Walter et al., JCI 2015)

PLoS Medicine July 2006

Anti-Interferon Autoantibodies in Autoimmune Polyendocrinopathy Syndrome Type 1

Anthony Meager[1*], Kumuthini Visvalingam[1], Pärt Peterson[2], Kaidi Möll[2], Astrid Murumägi[3], Kai Krohn[3], Petra Eskelin[4,5], Jaakko Perheentupa[5], Eystein Husebye[6,7], Yoshihisa Kadota[8¤], Nick Willcox[8]

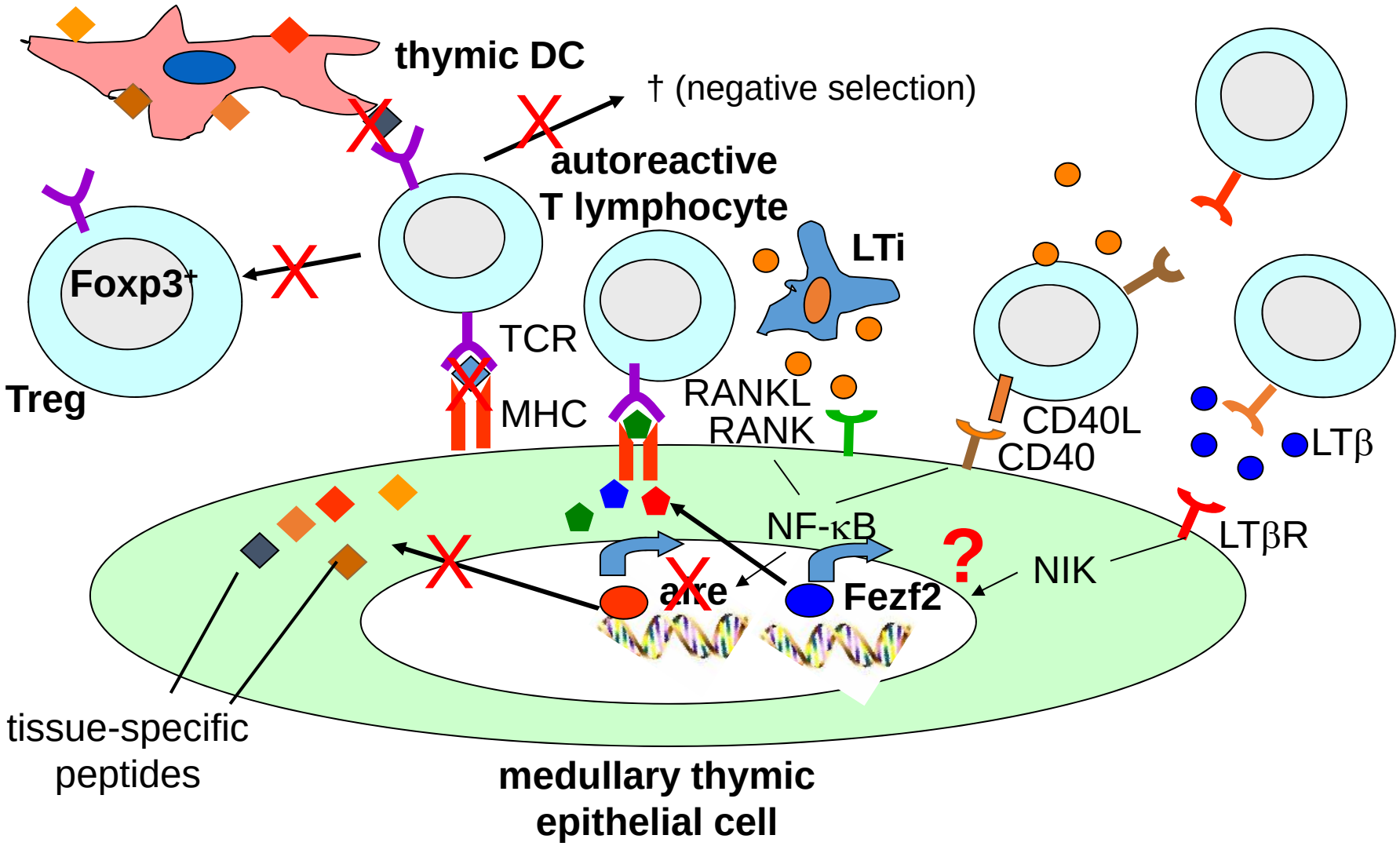
Clin Exp Immunol 2003; **132**:128–136

Anti-cytokine autoantibodies in autoimmunity: preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis

A. MEAGER*, M. WADHWA*, P. DILGER*, C. BIRD*, R. THORPE*, J. NEWSOM-DAVIS† & N. WILLCOX† *Division of Immunobiology, The National Institute for Biological Standards and Control, South Mimms, Herts, and †Neurosciences Group, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK

These conditions are characterized by defective AIRE expression

Deletional and non deletional mechanisms of central tolerance are impaired in Omenn syndrome



BLOOD, 26 AUGUST 2010 • VOLUME 116, NUMBER 8

Hypomorphic Rag mutations can cause destructive midline granulomatous disease

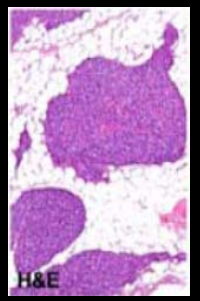
Suk See De Ravin,[1] Edward W. Cowen,[2] Kol A. Zarember,[1] Narda L. Whiting-Theobald,[1] Douglas B. Kuhns,[3] Netanya G. Sandler,[4] Daniel C. Douek,[4] Stefania Pittaluga,[5] Pietro L. Poliani,[6] Yu Nee Lee,[7] Luigi D. Notarangelo,[7] Lei Wang,[7] Frederick W. Alt,[7] Elizabeth M. Kang,[1] Joshua D. Milner,[1] Julie E. Niemela,[8] Mary Fontana-Penn,[9] Sara H. Sinal,[9] and Harry L. Malech[1]

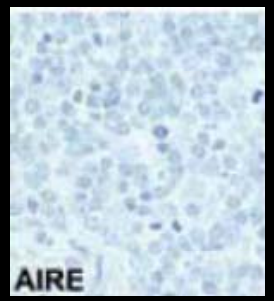
14y old male with loss of uvula and
nasal septum destruction
granulomatous skin lesions
myasthenia gravis

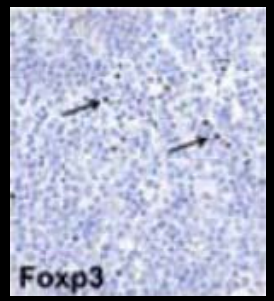
Sister: autoimmune cytopenias
ANA + collagen vascular disease
palpebral ptosis

RAG1: p.W522C; p.L541Cfs*30

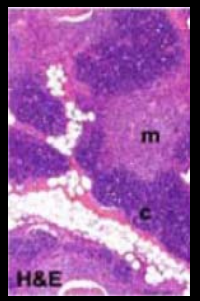
Patient

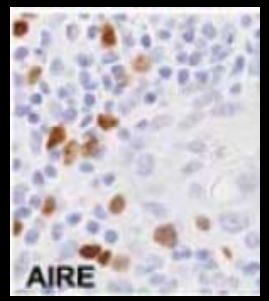


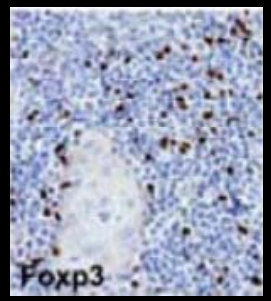


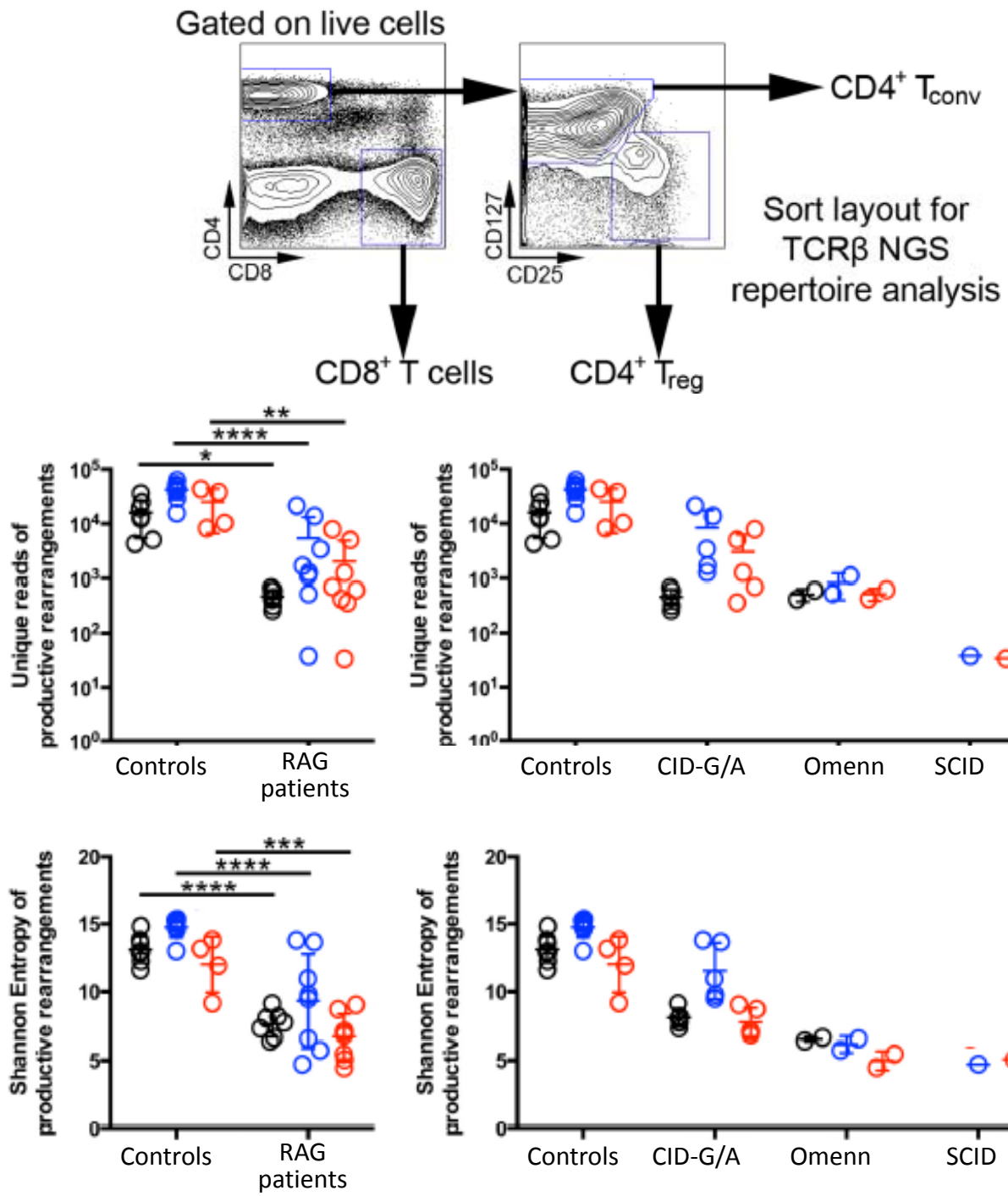


Control

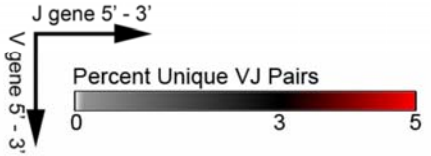







Gated on live cells
CD4
CD8
CD127
CD25
CD4+ Tconv
Sort layout for
TCRβ NGS
repertoire analysis
CD8+ T cells
CD4+ Treg
Unique reads of productive rearrangements
Controls
RAG patients
CID-G/A
Omenn
SCID
Shannon Entropy of productive rearrangements

Analysis of *TRBV-TRBJ* gene pairing demonstrates restriction of T cell repertoire and clonotypic expansions in patients with CID-G/A and Omenn

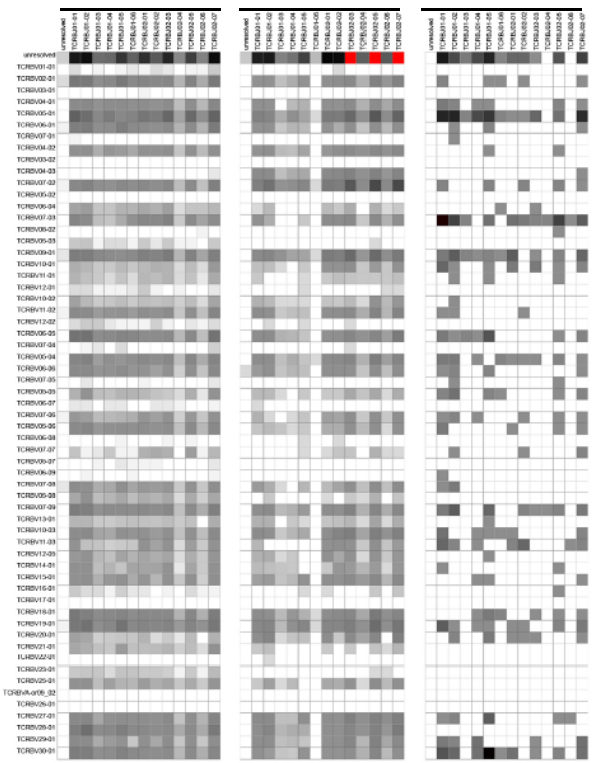


T_{reg}

Control CID-G/A Omenn

T_{conv}

Control CID-G/A Omenn



$CD8^+$

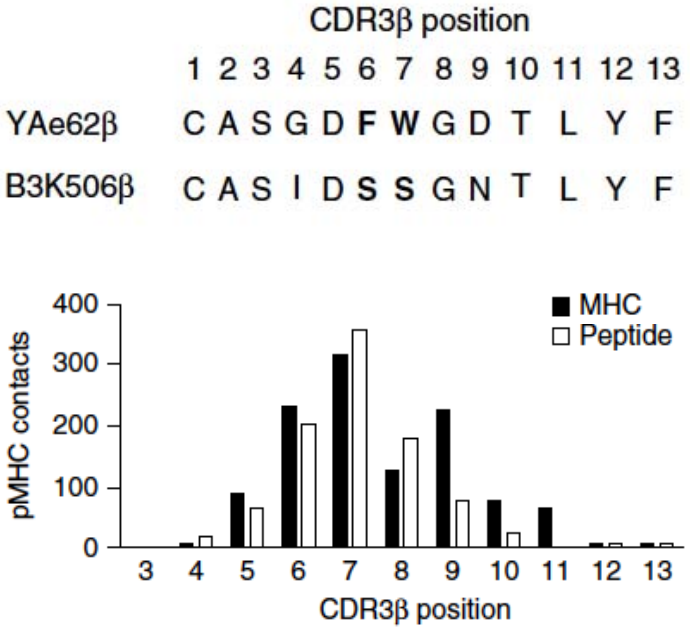
Control CID-G/A Omenn

VOLUME 17 NUMBER 8 AUGUST 2016 NATURE IMMUNOLOGY

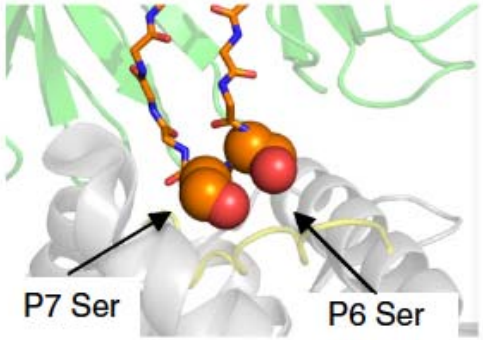
Hydrophobic CDR3 residues promote the development of self-reactive T cells

Brian D Stadinski[1], Karthik Shekhar[2], Iria Gómez-Touriño[3], Jonathan Jung[1], Katsuhiro Sasaki[1], Andrew K Sewell[4], Mark Peakman[3], Arup K Chakraborty[5–10] & Eric S Huseby[1]

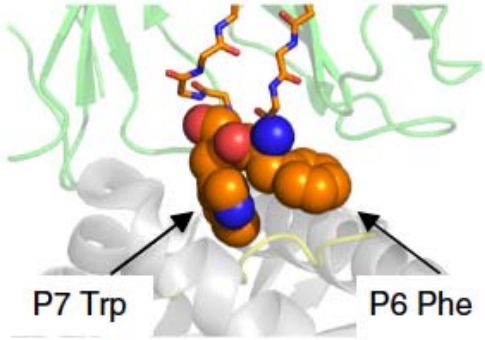
a



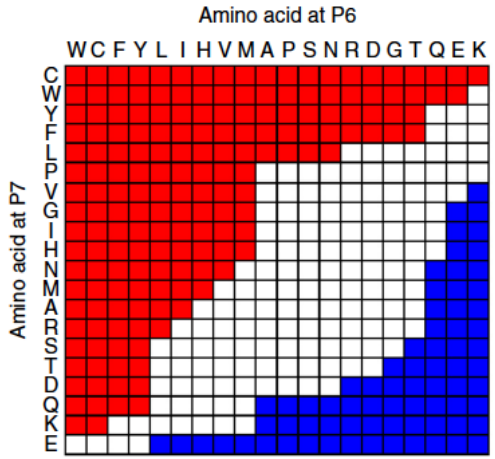
b



c



P6-P7 doublet AA composition

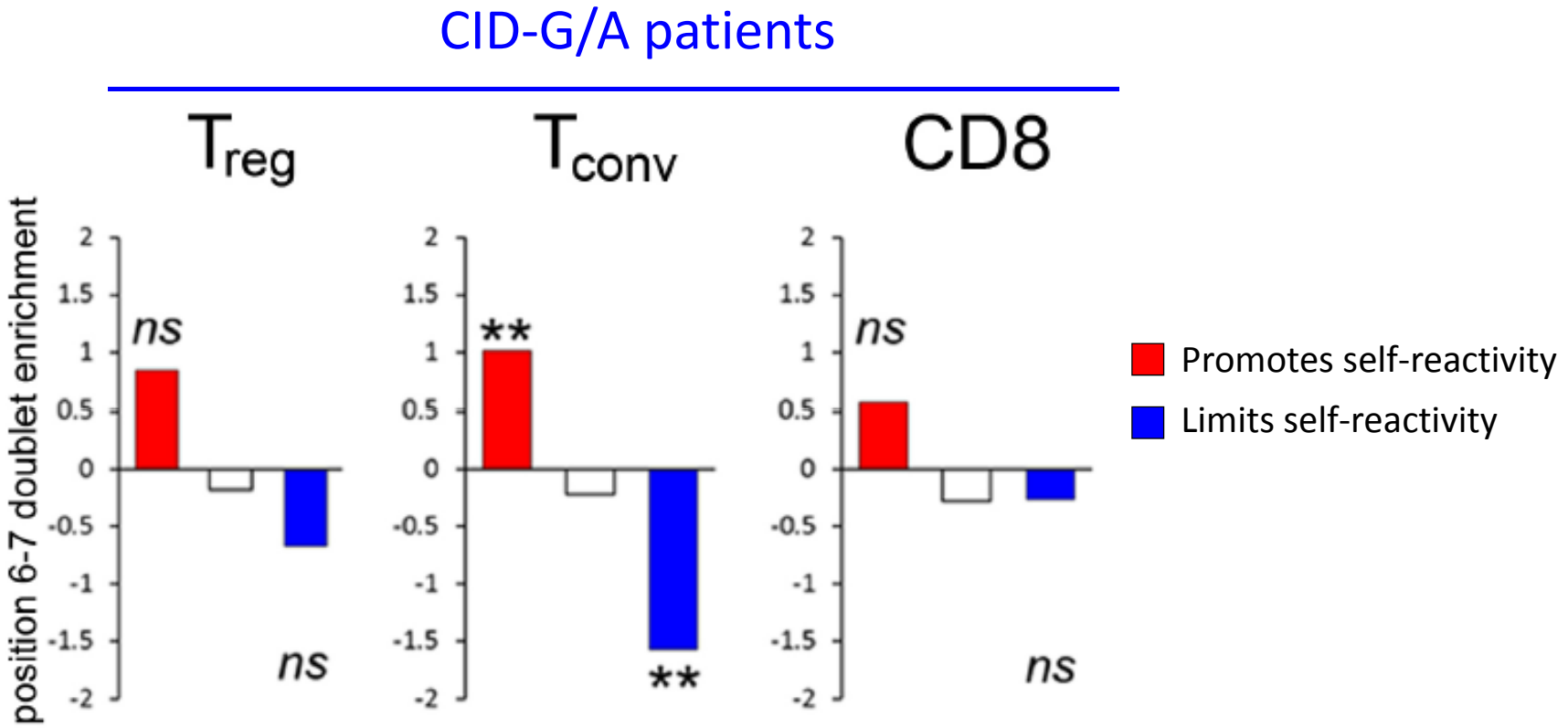


Promotes self-reactivity

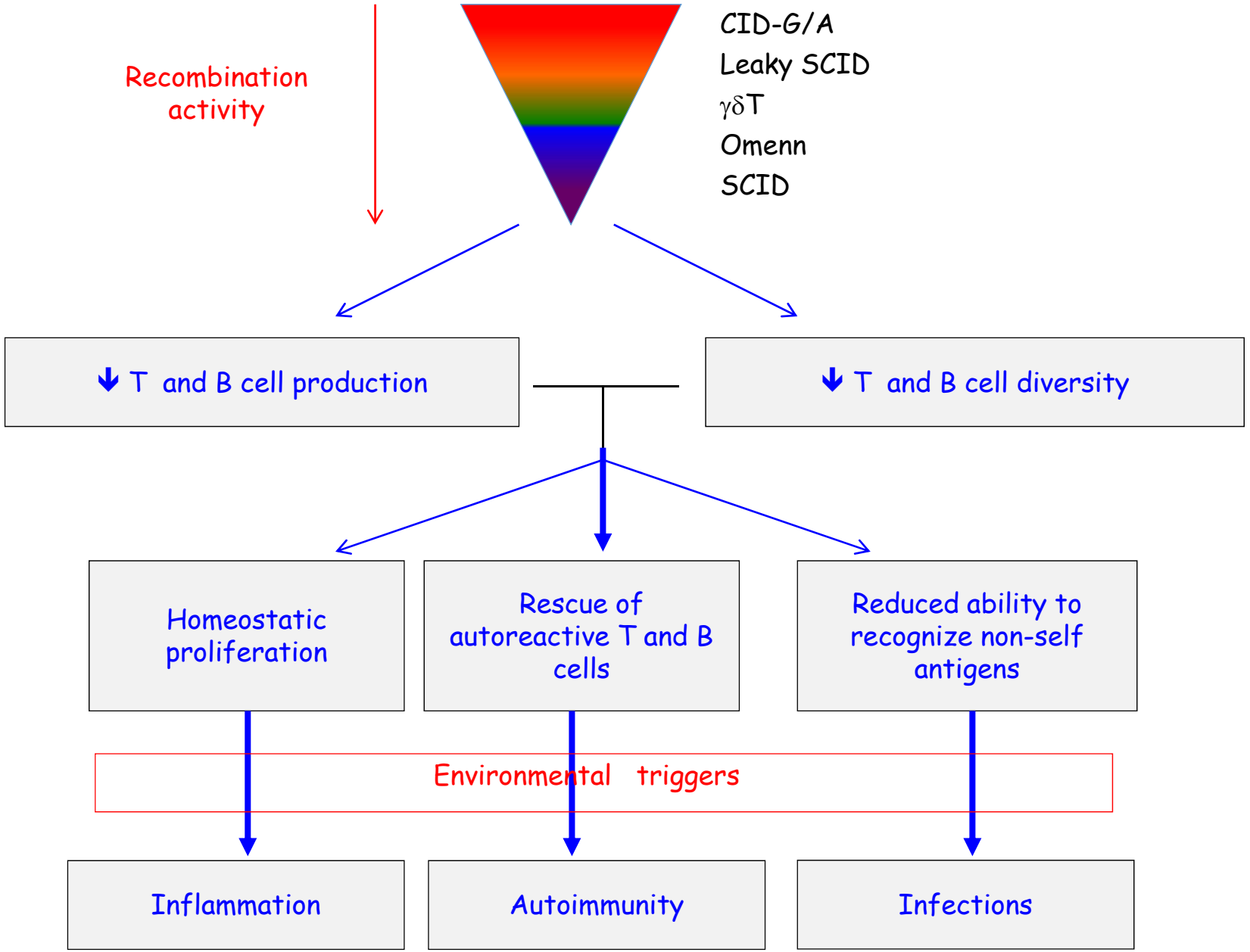
Limits self-reactivity

Abnormalities of CDR3β composition in CID-G/A

Increased self-reactivity in T_{conv} cells from CID-G/A patients



Mechanisms accounting for phenotypic heterogeneity



PID with immune dysregulation: challenges and needs

- Phenotypic heterogeneity
- Need to identify biomarkers predictive of complicated clinical course
- Multiple therapeutic options, but lack of a clear path

✓Need of a prospective/retrospective registry
✓Must involve multiple specialists (immunologists, transplanters, ID, pulmonolists, GI doctors, Hem/Onc doctors)
✓ Need of prospective clinical trials

YuNee Lee

Jolan Walter

John Manis

Ottavia Delmonte

Jared Rowe

UMass

Brian Stadinski

Eric Huseby

Huang Chiung-Hui

M. T. de la Morena

Suk See de Ravin

Rakesh Goyal

Anthony Hayward

Steven Holland

Maria Kanariou

Sevgi Keles

Alejandra King

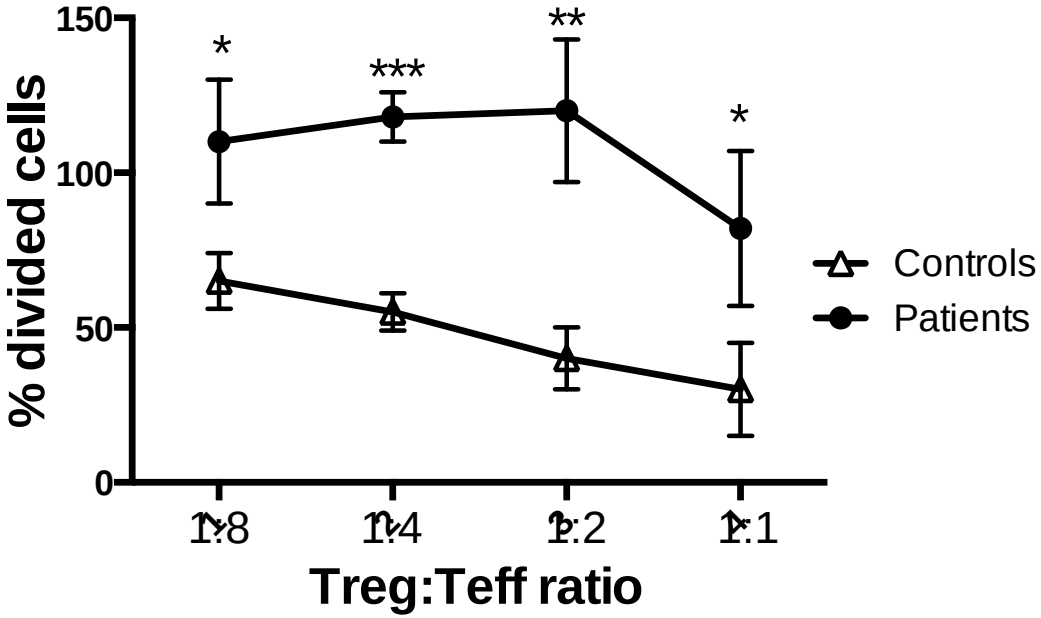
Taco Kuijpers

Benedicte Neven

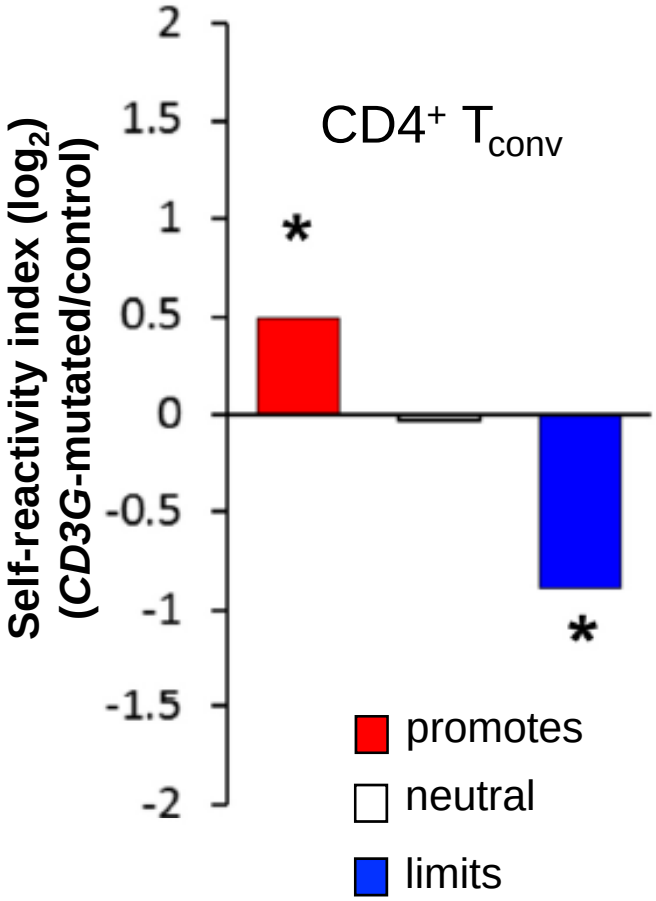
Jian Yi Soh

In patients with CD3G mutations, altered TCR signaling Is associated with anomalies of T_{reg} and T_{conv} cells

T_{reg} cells from *CD3G*-mutated patients have impaired suppressive activity

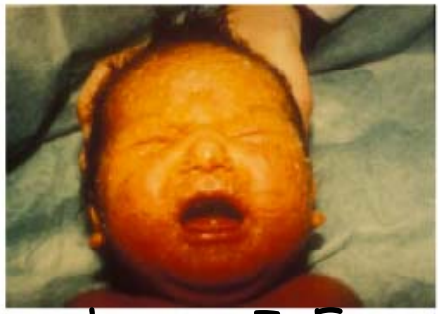


$CD4^+$ T_{conv} cells from *CD3G*-mutated patients have a self-reactivity molecular signature



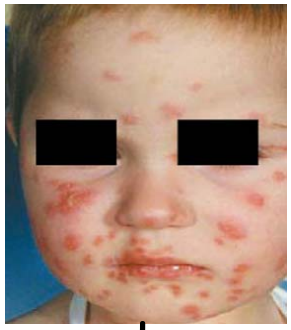
Immune dysregulation of RAG deficiency

Omenn syndrome



hyper-IgE
eosinophilia
Th2 skewing

CID-G/AI



granulomas
EBV, VZV

autoimmunity
inflammation

Spectrum of autoantibodies in RAG deficiency

A total of 31 patients with proven *RAG* mutations:

- 15 patients with CID-G/AI or idiopathic CD4 lymphopenia
- 5 patients with leaky SCID
- 7 patients with Omenn syndrome
- 4 patients with SCID