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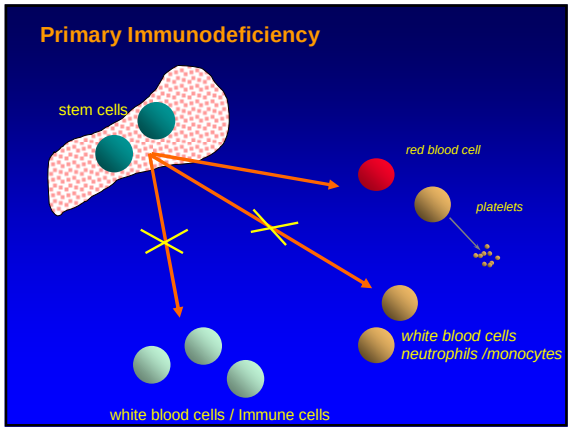
Molecular Diagnosis of Severe Congenital Immunodeficiencies

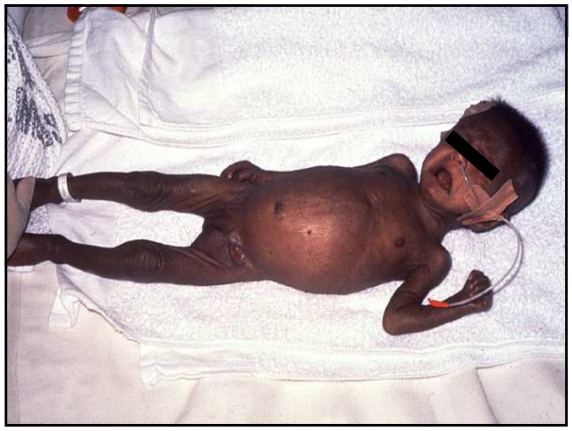
Athens 2010

Bobby Gaspar
Centre for Immunodeficiency
UCL Institute of Child Health/Great Ormond Street NHS Trust

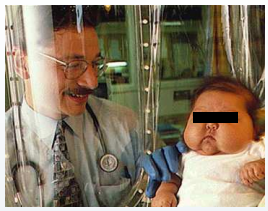


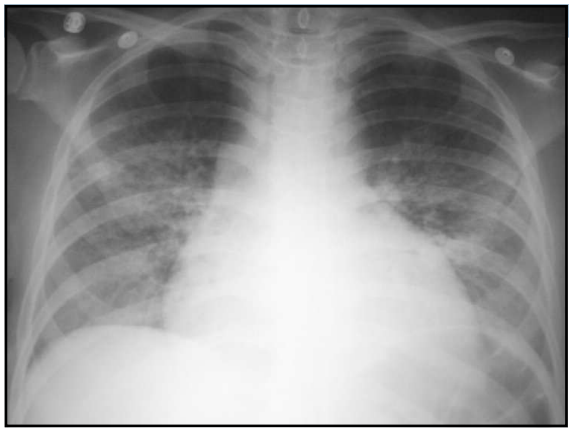
Severe Combined Immunodeficiency (SCID)

- severe defects in cellular and humoral immunity
- different immunological phenotypes
- common clinical presentation
- genetic heterogeneity
- common treatment options

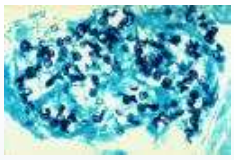


'Bubble babies'

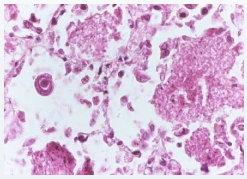




Causes of pneumonitis in SCID

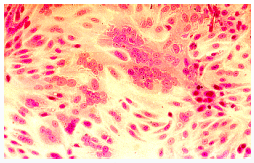


Pneumocystis carnii (PCP)

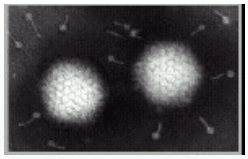


Cytomegalovirus (CMV)

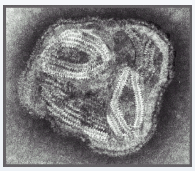
Causes of pneumonitis in SCID



RSV

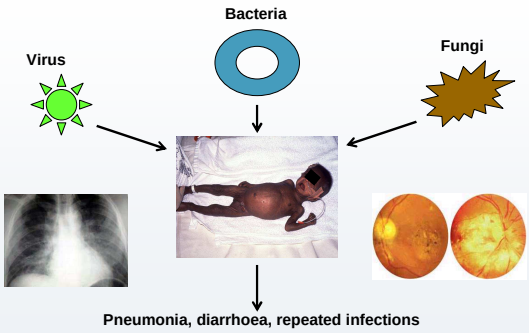


adenovirus

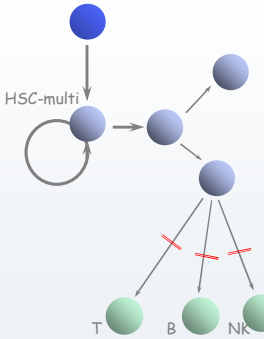


Parainfluenza virus

Severe combined immunodeficiency (SCID)



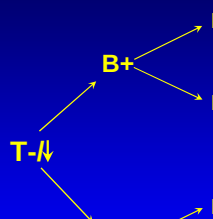
Molecular defects in SCID



SCID/CID

γc, IL7Rα, JAK3, ZAP-70
RAG1/2, artemis, ligase IV, Cernunnos
ADA, PNP
MHC I/II, CD3δ/ε/ζ, CD45, ORAI1, Coronin 1A

Immunophenotypes in SCID



NK- γc/JAK3 SCID

NK+ IL-7Rα deficiency, Coronin-1A, ZAP-70, CD3ε,δ,ζ

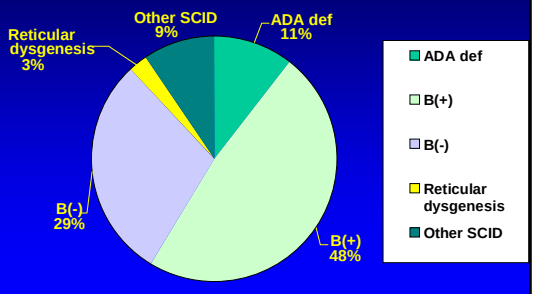
NK- ADA SCID, Reticular Dysgenesis

NK+ RAG1/2 deficiency/Artemis Cernunnos, DNA ligase 4, DNApkcs

Molecular defects in SCID

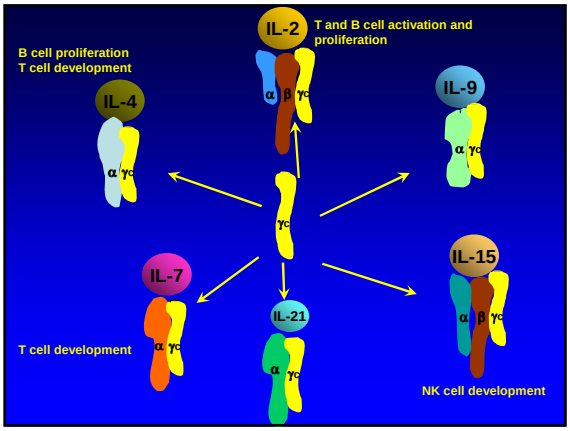
- Cytokine signaling defects – γc, JAK3, IL-7Rα
- TCR defects – CD3δ/ε/ζ, ZAP-70
- VDJ recombination defects – RAG1/2, Artemis, DNA ligase IV, Cernunnos, DNApkcs
- Defects of metabolism – ADA, PNP, AK2 (reticular dysgenesis)
- Other – MHC class II def, Coronin 1A, Ora 1

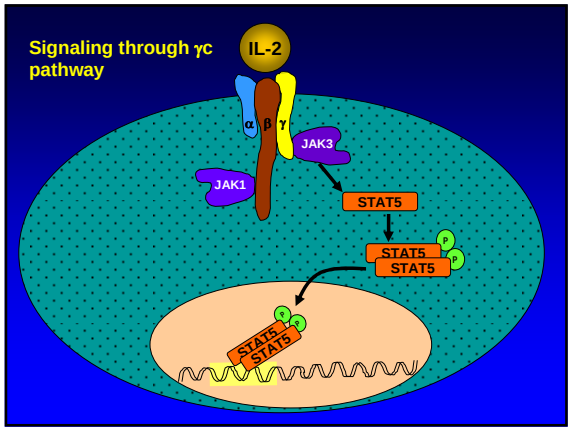
Primary disease in SCID patients (n=477)



X-linked SCID

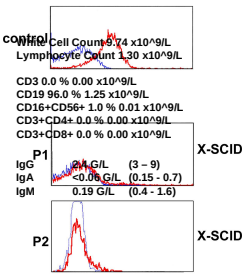
- Most common form of SCID ~ 40-50%
- Classical T-B+NK- phenotype
- Carriers show non-random X-inactivation in T cell lineage
- Defect in common γ chain (Noguchi et al. 1993)
- Common component of IL-2, IL-4, IL-7, IL-9, IL-15 cytokine receptors

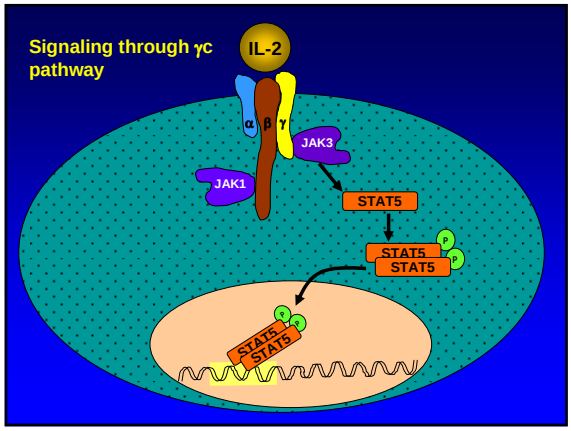




Flowcytometric analysis of γc expression in XSCID patients

Diagnosis of T-B+NK- SCID



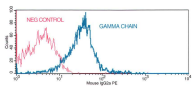


Diagnosis of T-B+NK- SCID

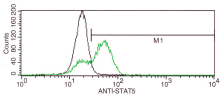
γc expression

Phospho STAT5

C



C



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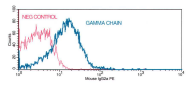

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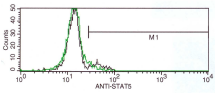


Mutation (γc) R298X
Diagnosis: X-SCID

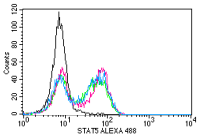
Defective *IL7R* expression in T-B+NK+ severe combined immunodeficiency

Anne Puel, Steven F. Zeigler, Rebecca H. Buckley & Warren J. Leonard

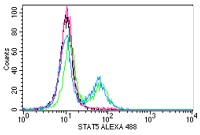
- Defects in IL-7Rα
- Classical presentation at 2-4mths of age
- Treated by HSCT

T-B+NK+ SCID 3

Control



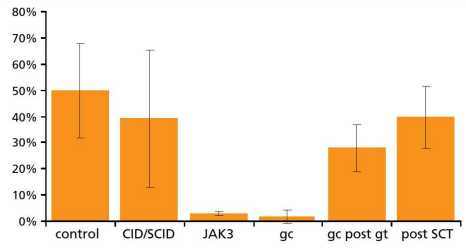
T-B+NK+ SCID 3



IL-2
IL-7
IL-15

- Normal γc expression
- Normal STAT5 ptyr to IL-2 and IL-15
- Abnormal STAT5 ptyr to IL-7
- Sequenced IL-7Ra :
  - c.83-2A>G/heterozygote
  - p.Lys128X/heterozygote

Change in percent STAT5 tyrosine phosphorylated cells post IL-2 stimulation shown by diagnosis/therapy



Details of pre-therapy γc and JAK3 SCIDs are in the Table.
Terms used: CID/SCID (combined immunodeficiency/severe combined immunodeficiency) excludes γc and JAK3 SCIDs but includes MHC II SCIDs, IL-7Rγ SCIDs and undefined T-B+ SCIDs.
gc=gamma chain (γc) SCIDs. gc post gt=γc SCIDs post gene therapy. Post SCT=non-γc SCIDs post stem cell transplant.

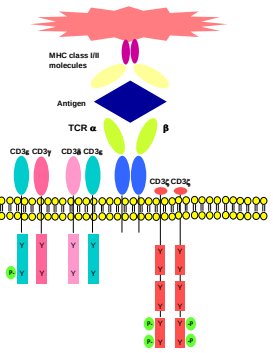
TCR defects

Effect of CD3δ Deficiency on Maturation of α/β and γ/δ T-Cell Lineages in Severe Combined Immunodeficiency

Harjit K. Dadi, Ph.D., Amos J. Simon, Ph.D., and Chaim M. Roifman, M.D.

Inherited and Somatic *CD3ζ* Mutations in a Patient with T-Cell Deficiency

Frédéric Rieux-Laucat, Ph.D., Claire Hivroz, Ph.D., Annick Lim, B.S., Véronique Mateo, Ph.D., Isabelle Pellier, M.D., Françoise Selz, B.S., Alain Fischer, M.D., Ph.D., and Françoise Le Deist, M.D., Ph.D.

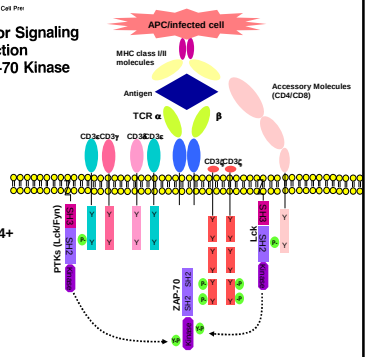


T-↓B+NK+ SCID

TCR defects

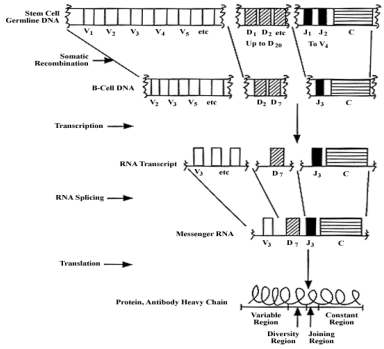
Cell, Vol. 76, 947–958, March 11, 1994, Copyright © 1994 by Cell Press

Defective T Cell Receptor Signaling and CD8+ Thymic Selection in Humans Lacking Zap-70 Kinase



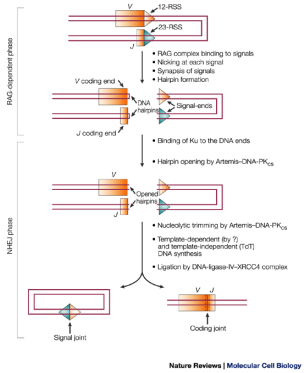
T↓B+NK+ SCID
CD8 absent/v low
Low poorly functional CD4+

The Genetic Basis of Antibody Diversity



Non-radiosensitive

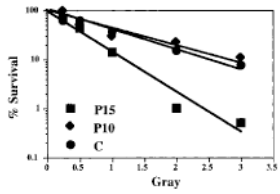
Radiosensitive



Nature Reviews | Molecular Cell Biology

A Human Severe Combined Immunodeficiency (SCID) Condition with Increased Sensitivity to Ionizing Radiations and Impaired V(D)J Rearrangements Defines a New DNA Recombination/Repair Deficiency

By Nathalie Nicolas,* Despina Moshous,* Marina Cavazzana-Calvo,* Dora Papadopoulo,‡ Régina de Chasseval,* Françoise Le Deist,* Alain Fischer,* and Jean-Pierre de Villartay*



Cell, Vol. 105, 177–186, April 20, 2001, Copyright ©2001 by Cell Press

Artemis, a Novel DNA Double-Strand Break Repair/V(D)J Recombination Protein, Is Mutated in Human Severe Combined Immune Deficiency

Despina Moshous,* Isabelle Callebaut,† Régina de Chasseval,* Barbara Corneo,* Marina Cavazzana-Calvo,* Françoise Le Deist,* Ilhan Tezcan,‡ Ozden Sanal,‡ Yves Bertrand,§ Noel Philippe,§ Alain Fischer,* and Jean-Pierre de Villartay*

is flanked by recombination signal sequences (RSSs) composed of conserved heptamers and nonamers separated by random sequences of either 12 or 23 nucleotides. V(D)J recombination can be roughly divided into three steps. First, the RAG1 and RAG2 proteins initiate the rearrangement process through the recognition of

- novel defect in T-B- SCID
- radiosensitive in comparison to RAG

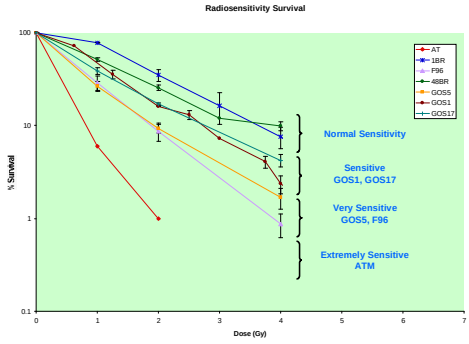
Radiosensitive forms of SCID

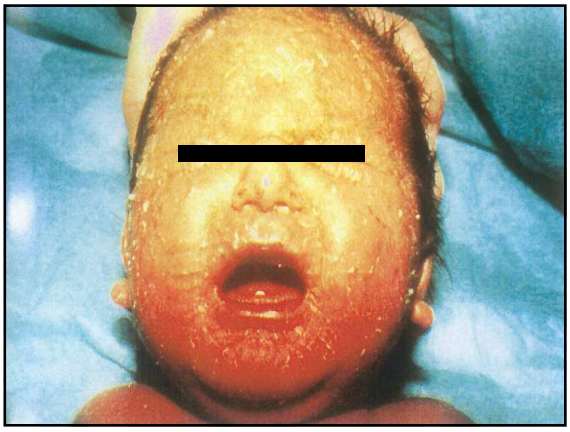
Defective gene	Mutation	Radiosensitivity	Immune defect	Growth delay	Microcephaly	Cancer predisposition	Refs
Artemis	Null	Yes	SCID	No	No	?	[8]
	Hypomorphic	Yes	SCID	No	No	Yes	[9]
DNA-LigIV	Hypomorphic	Yes	Variable from no deficiency to SCID	Yes	Variable	Yes	[10–14]
Cernunnos	Hypomorphic?	Yes	SCID	Yes	Yes No	?	[16–17**]

SCID, severe combined immunodeficiency.

DNApkcs recently described

Fibroblast radiation sensitivity in GOSH patients





Patient with florid rash, lymphadenopathy, FTT and pneumonitis

LYMPHOCYTE PANEL

HAEM RESULTS:- WBC 8.3 ABS LY 5.64
CD3 48 %
CD19 45 %
CD16+CD56+ 0 %
CD4 4 %
CD8 42 %

PHA STIMULATION

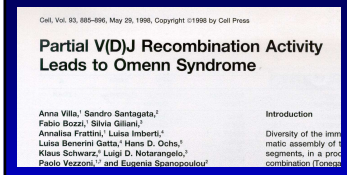
PHA 0 ug/ml 295 MEAN DPM
PHA 1.0 ug/ml 312 MEAN DPM
PHA 2.0 ug/ml 336 MEAN DPM
PHA 4.0 ug/ml 403 MEAN DPM
PHA 8.0 ug/ml 441 MEAN DPM
Stimulation Index 1.49

Immunoglobulins

IgG	4.1 G/L	(3 – 9)
IgA	<0.06 G/L	(0.15 - 0.7)
IgM	0.09 G/L	(0.4 - 1.6)
Total IgE	826 IU/ML	

Omenn's Syndrome

- Clinical presentation of SCID
- Erythroderma, eosinophilia, hepatosplenomegaly, lymphadenopathy
- Increased IgE, activated T cells, low/absent B cells
- Restricted TCR Vβ usage



Cell, Vol. 93, 885–896, May 29, 1998, Copyright ©1998 by Cell Press

Partial V(D)J Recombination Activity Leads to Omenn Syndrome

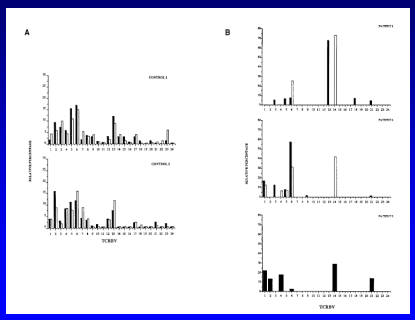
Anna Villa,[1] Sandro Santagata,[2] Fabio Bozzi,[1] Silvia Giliani,[3] Annalisa Frattini,[1] Luisa Imberti,[4] Luisa Benerini Gatta,[1] Hans D. Ochs,[5] Klaus Schwarz,[6] Luigi D. Notarangelo,[3] Paolo Vezzoni,[1,7] and Eugenia Spanopoulou[8]

Introduction

Diversity of the imm… matic assembly of … segments, in a proc… combination (Tonega…

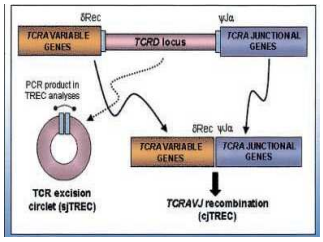
- Mutations found in RAG1 and RAG2
- Mainly missense mutations with stable protein expression
- Partial V(D)J activity retained
- OS mutants affect the formation of RSS/RAG 1/RAG 2 complex

Restricted TCRVβ clonality in Omenn's syndrome



Rieux-Laucat et al. (1998) *J Clin Invest* 102 (2): 312-321

TRECs



Peggs and Mackinnon, BJH 2004

UCL

Results

	Patient	Diagnosis	TRECs per million cells
	MD	SCID ADA Deficiency	0
	SR	SCID X-linked	0
*	LV	CID	0
	JH	Gamma Chain SCID	0
	LR	SCID T low B+ NK+	0
	MH	SCID T low NK+ B+ IL-7 R def.	0
	SC	Gamma Chain SCID	0
	HB	Gamma Chain SCID	0
	AA	SCID T low NK+ B low	0
	NL	Gamma Chain SCID	0
	OC	Gamma Chain SCID	0
	MM	Gamma Chain SCID	0
	TA	RAG SCID	0
	ZK	SCID T- B- NK+	0
*	AC	Evans CID	0
	RR	Gamma Chain SCID	0
	IM	SCID ADA Deficiency	0
*	JA	Gamma Chain SCID	0
	MH	SCID ADA Deficiency	0
	MD	?ADA SCID	0

All SCID DBS TREC samples are zero

Includes patients with maternal engraftment and atypical feature CID/Evans

Omenn's Syndrome

- Clinical and immunological syndrome
- Heterogeneous molecular basis
- Defects in RAG1/2, Artemis, gamma c, ADA, CHH, IL-7Rα etc
- Restricted TCR Vβ usage
- Low TRECs

2 month old child with severe FTT, diarrhoea and recurrent cough

Specimen Type: Serum
IMMUNOGLOBULINS

IgG 7.70 G/L 5.2 - 18
IgA **<0.06** G/L 0 - 0.02
IgM 0.20 G/L 0.02 - 0.2

white Cell Count 4.48 x10^9/L
Lymphocyte Count 0.09 x10^9/L

CD3 1.0 %	**0.00 x10^9/L**
CD19 9.0 %	**0.01 x10^9/L**
CD16+CD56+2.0 %	**0.00x10^9/L**
CD3+CD4+ 0 %	**0.00 x10^9/L**
CD3+CD8+ 0 %	**0.00 x10^9/L**

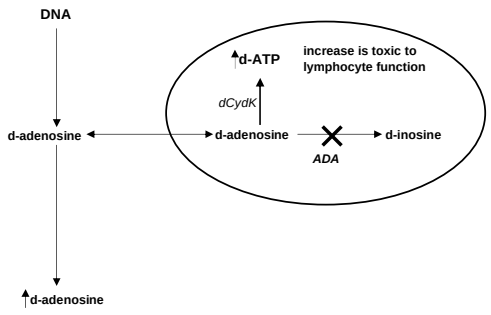
Specimen Type: Blood (heparin)
PHA STIMULATION

PHA 0 ug/ml 1753 MEAN DPM
PHA 1.0 ug/ml 1974 MEAN DPM
PHA 2.0 ug/ml 2505 MEAN DPM
PHA 4.0 ug/ml 2321 MEAN DPM
PHA 8.0 ug/ml 1194 MEAN DPM
Stimulation Index 1.4

ADA deficiency

- Enzyme expressed in all body cells - purine salvage pathway
- Deficiency results in abnormalities of lymphocyte function and proliferation
- ~10-15% of all cases of SCID
- Variability in clinical presentation

Biochemical defect in ADA deficiency

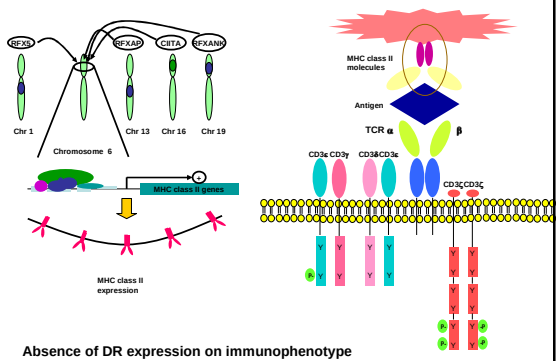


Immunodeficiency in ADA deficiency

- Cell mediated and humoral abnormality
- ↓T, ↓B, ↓NK
- 85-90% present in the first year of life
- Pneumonitis, diarrhoea, skin infection, FTT

- 15-20% delayed onset/partial ADA deficiency
- Present 2-3 years of life
- less severe infective episodes
- less severe immunophenotype and metabolic abnormalities

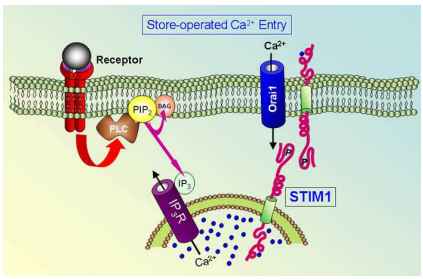
Defects in MHC class II expression



Absence of DR expression on immunophenotype

A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function

Stefan Feske[1,2+], Yousang Gwack[1,2+], Murali Prakriya[3], Sonal Srikanth[1,2], Sven-Holger Puppel[1], Bogdan Tanasa[1], Patrick G. Hogan[1], Richard S. Lewis[3], Mark Daly[4,5] & Anjana Rao[1,2]



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The actin regulator coronin-1A is mutated in a thymic egress deficient mouse strain and in a T-B+NK+ SCID patient

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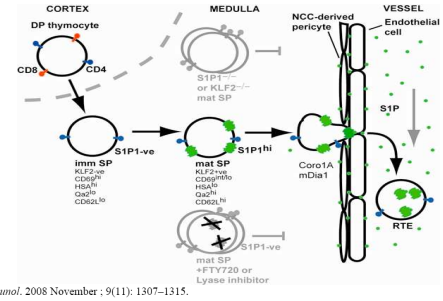

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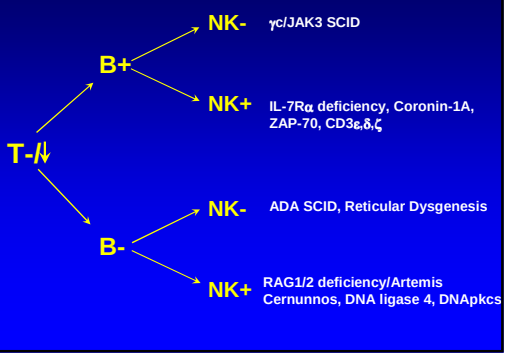
Immunophenotypes in SCID



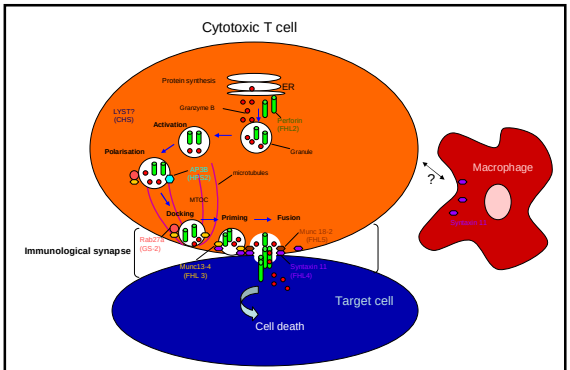


Figure 1.

Figure 2

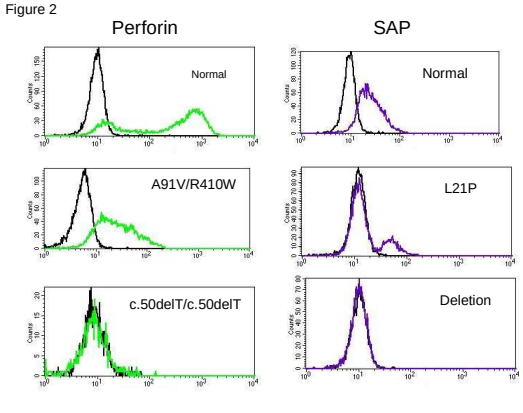


Figure 3a

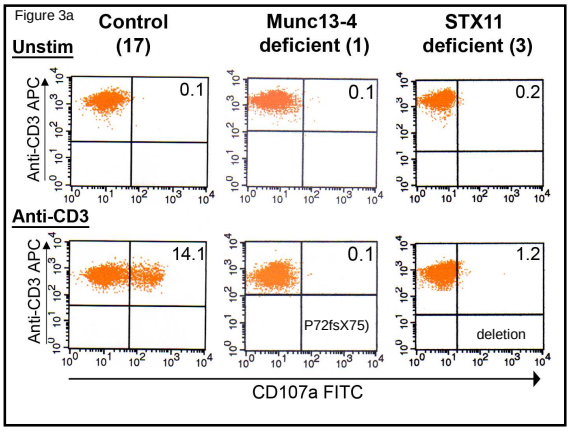
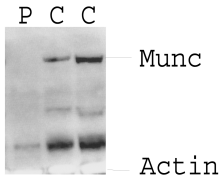


Figure 3b



Munc 13-4 mutation in patient: P72fsX75)