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PEARLS OF LABORATORY MEDICINE

Pearl Title: Primary T Cell Immunodeficiencies

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Primary Immunodeficiencies (PID)

- Disorders due to germline mutations of genes that affect the development and/or function of immune cells

as opposed to

secondary immunodeficiencies which are due to another disease, environmental factor(s) or mode(s) of therapy

- PIDs primarily have Mendelian inheritance
- Overall prevalence: 5-10 per 100,000; defined as rare diseases by the US Rare Diseases Act of 2002
- PIDs can affect all aspects of the immune response from innate to adaptive
- They can be classified on the basis of the arm of the immune system that is defective and/or the underlying mechanism of the defect
- Their manifestations range from asymptomatic disorders to diseases associated with severe infections, autoimmunity and malignancy

PIDs: Important developments in the past 25 years

- **Significant increase in the number of identified entities**

- *As of January 2020, the key clinical and laboratory features of 430 conditions have been documented*

- **Broadening of the phenotype of existing entities**

- **“Classical PIDs” versus “PIDs with Narrow Phenotype”**

- **Autoantibody-mediated phenocopies of PIDs**

Primary T Cell Immunodeficiencies

- Genetic disorders affecting the development, growth and survival of T cells
- The defect may affect T cells in isolation or in conjunction with B cells, NK cells or elements of the innate immune system
- The T cell defect may be a component of a broader clinical syndrome
- Different entities may first manifest as early as infancy to as late as adulthood
- The range of manifestations include extreme susceptibility to infection, autoimmunity and/or heightened susceptibility to neoplasia

Major Subsets of Primary T Cell Immunodeficiencies

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- **Defects in Thymus Organogenesis**

- DiGeorge Syndrome, FOXN1 Deficiency, CHARGE Syndrome

- **Severe Combined Immunodeficiency (SCID)**

- Apoptosis of HSC and CLP

- Defective Cytokine-dependent Signalling

- Defective V(D)J Rearrangement

- Defective Signalling through pre-TCR & TCR

- **Atypical ("leaky") SCID**

- Hypomorphic mutations of typical SCID genes; i.e. where the mutation leads to diminished protein expression and/or partial loss of function

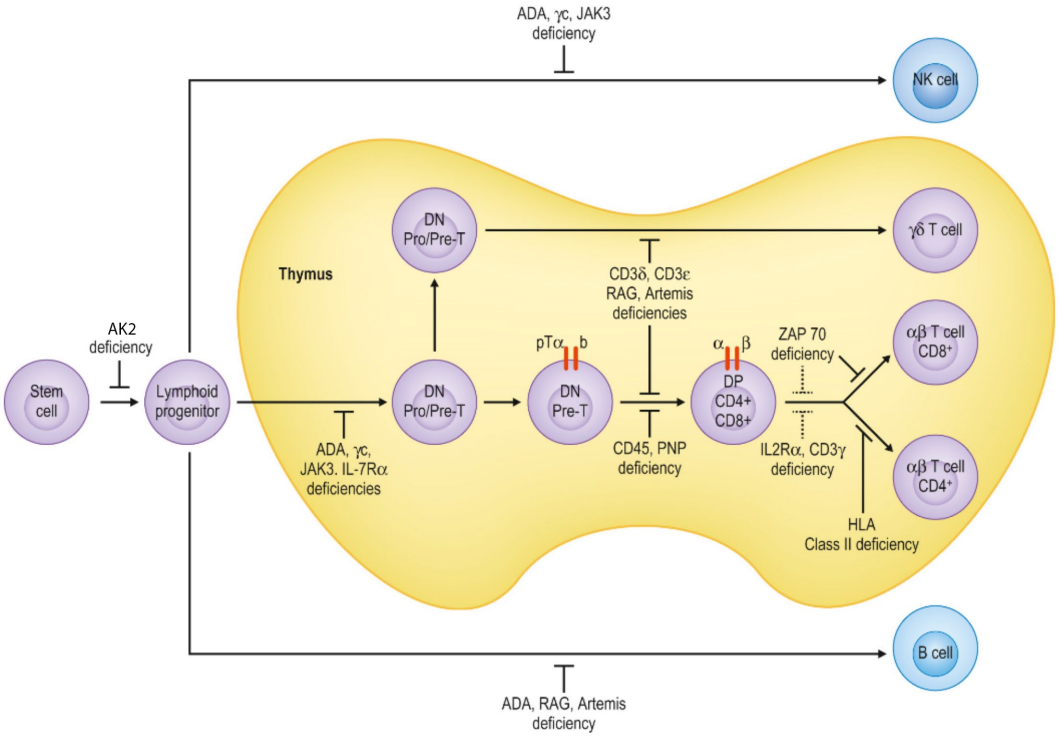
- **Combined Immunodeficiencies (CID)**

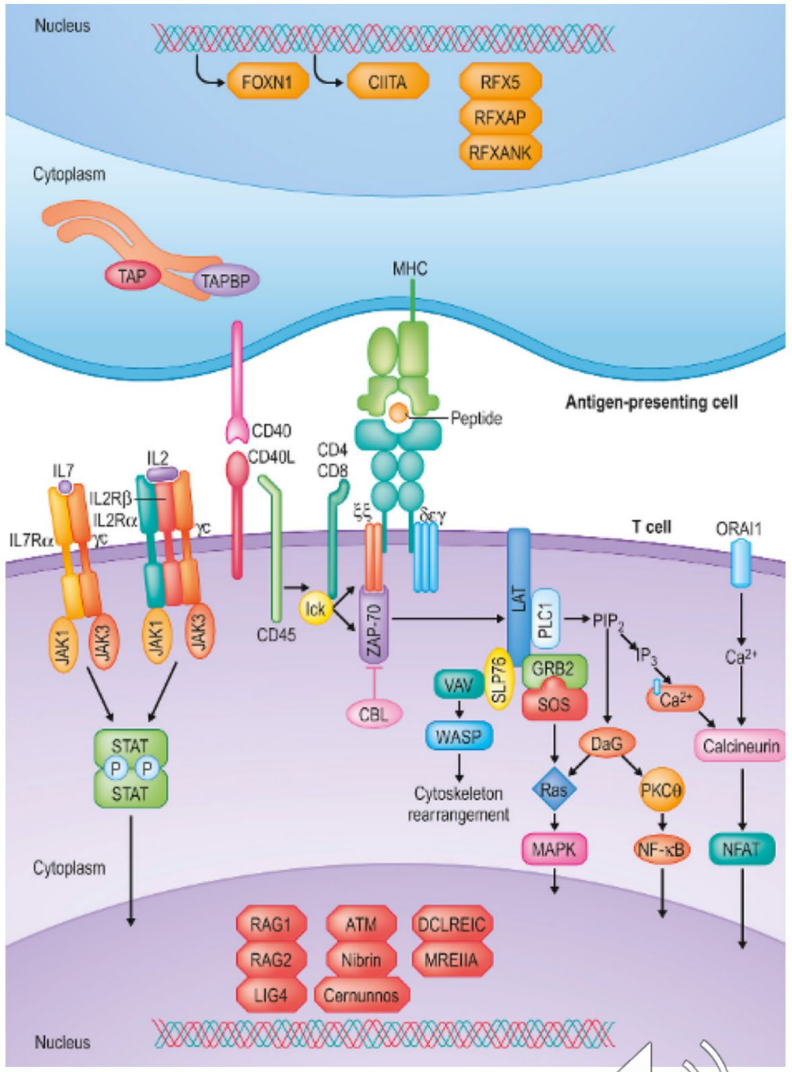
- Defects in Coronin 1A; TCR signalling; NF-κB signalling; Ca^{2+} and Mg^{2+} signalling; Dock proteins; MHC Class I and II

- CIDs with immune dysregulation

- CIDs with extra-immune features

T Cell Maturation Steps in the Thymus and Signalling Cascades Involved in Primary T Cell Immunodeficiencies





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From Roifman C. M., in *Clinical Immunology 5th Ed.* 2019
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Laboratory Evaluation of Suspected T Cell Defects: *A Tiered Approach*

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- **First Tier Tests:**

- CBC and Differential

- Enumeration and Basic Phenotyping of T Lymphocytes

- **Secondary Tests:**

- Extended Phenotyping of T Lymphocytes

- Lymphocyte Proliferation to mitogens (e.g. PHA), CD3/CD28, and antigens

- Delayed Type Hypersensitivity (DTH)

- **Tertiary Tests:**

- TREC (part of newborn screening in the United States)

- Gene Sequencing (earlier use may be warranted):

Sanger: when one gene is the clear candidate

Gene Panels: when a defined clinical phenotype is investigated

Whole exome: broad clinical phenotypes or negative gene panel approach

Whole genome: when the previous approaches have not been productive

- TCR Vb Repertoire

- Radio-sensitivity Testing; ADA/PNP Levels

- Phosphorylation Assays; Calcium Flux Assays

Severe Combined Immunodeficiency (SCID)

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- A group of at least 20 known, and a number of unknown, inherited disorders of the adaptive immune system
- SCID patients have very low or absent number of T cells and lack adequate T cell function for survival, and absent or non-functional B cells.
- Generally healthy at birth but can soon present with severe opportunistic infections such as CMV infection and *Pneumocytis jiroveci* pneumonia, as well as chronic diarrhoea and failure to thrive.
- Because of B cell dysfunction, they are susceptible to infections with encapsulated bacteria: *Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus*.
- Prior to the use of haematopoietic stem cell transplantation (HSCT), SCID was universally fatal in an early age, making it a medical emergency

Phenotype → Gene

- **T ^{neg} B + NK ^{neg} →** IL2RG (X-Linked), JAK3, STAT5a
- **T ^{neg} B ^{neg} NK ^{neg} →** ADA , AK2
- **T ^{neg} B + NK+ →** IL7R, CD3δ, CD3ε, CD3ζ, CD45, LCK, FOXN1, RMRP, CORO1A, PNP, LIG4
- **T ^{neg} B ^{neg} NK+ →** RAG1, RAG2, DCLRE1C/Artemis, DNA-PKcs, CERNUNNOS/NEHJ1

Available Treatments

- Haematopoietic stem cell transplantation (HSCT)
- Enzyme Replacement (ADA)
- Gene Therapy (ADA, IL2RG)

Definitions of Typical and Leaky SCID (Shearer et al., 2014)

- **Typical SCID:**
- *<300 Autologous T cells/μl*
- *Lymphocyte proliferation to PHA: <10% of the lower limit of normal*
- *Frequent maternal T cell engraftment (proliferation of maternal T cells, originating from maternal-foetal transfusion, in the patient due to the absence of host T cells)*

- **Leaky SCID:**
- *300-1499 autologous T cells/μl (higher if oligoclonal)*
- *Lymphocyte proliferation to PHA: 10-25% of the lower limit of normal*
- *No maternal T cell engraftment*
- *Generally due to hypomorphic mutations in a known SCID gene*

- **Omenn Syndrome:**
- *Presence of oligoclonal T cell populations, eosinophilia and increased serum IgE in combination with laboratory criteria of leaky SCID*
- *Detection of erythroderma, adenopathy and hepatosplenomegaly*

Newborn Screening for SCID

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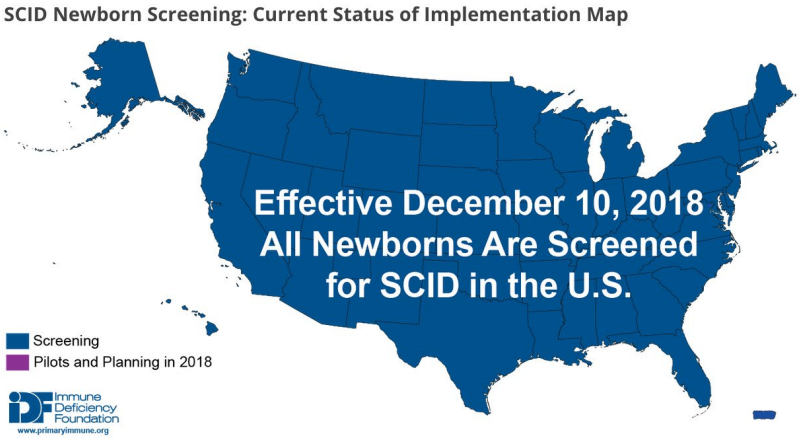
- *Rearrangement of the T cell receptor α (TCR-α) chain in developing T cells leads to the excision of the TCR-δ locus, creating the δ REC-ψJα TREC (T cell receptor excision circle).*
- *The TREC assay is based on a qPCR method targeting the δ REC-ψJα signal joint region. Amplifying a reference gene, such as albumin, in parallel with TREC serves as the internal control for the assay.*
- *The assay quantifies TREC copy numbers in DNA extracted from dry blood spots collected from each newborn, allowing a population-based assessment of T cell developmental defects.*
- *The DNA extraction method and the calibrator used can lead to variation in the TREC copy numbers reported by different laboratories.*
- *Lack of amplification in both the TREC and the reference gene can be due to insufficient DNA or the presence of inhibitors in the sample.*



As of December 10, 2018

2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Wisconsin	Massachusetts	California	Delaware	Colorado	Minnesota	Illinois	Arkansas	Alaska	Arizona	Nevada
		New York	Michigan	Connecticut	Ohio	Iowa	Hawaii	Georgia	Kansas	Alabama
				Florida	Pennsylvania	Maine	Montana	Idaho	Missouri	Indiana
				Mississippi	Utah	Nebraska	New Hampshire	Kentucky	North Carolina	Louisiana
				Texas		New Jersey	Oklahoma	Maryland		
				Wyoming		New Mexico	Puerto Rico	North Dakota		
						Oregon	South Carolina	Tennessee		
						Rhode Island	South Dakota	Vermont		
						Washington	Virginia			
						Washington, DC				
						West Virginia				

Timeline of SCID Newborn Screening implementation in U.S.

Also screening: District of Columbia, Navajo Nation, Puerto Rico

Using TREC in Newborn Screening: Kwan et al. (2014) Epidemiological Highlights and Distribution of Outcomes

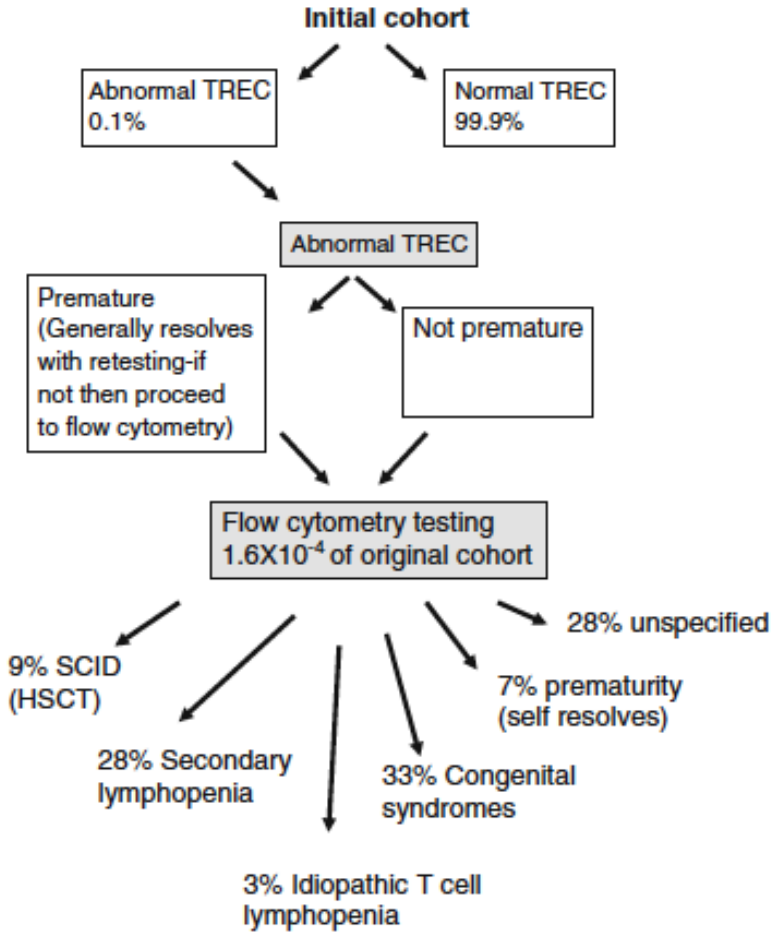
- **Higher Incidence of SCID than Previously Estimated:**
  - ***3,030,083 screened newborns:***

  52 Cases of SCID identified

  Incidence increasing from 1:75000 prior to screening to 1:58000 afterwards

  IL2RG 19% (10), RAG1 15% (8), IL7RA 12%(6), ADA 10% (5), JAK3 6% (3), RMRP 4% (2), others 2% (1 each), Unknown 12% (no mutation detected)
- **Changes in the Perceived Proportion of Different SCID Genotypes:**

  - Easier identification of rare autosomal recessive genotypes
  - *The proportion of X-linked SCID is cut by half*
  - *This signifies the higher absolute number of autosomal recessive genotypes than previously appreciated*



Secondary lymphopenia	Congenital syndromes
26% Heart disease	57% 22q11.2 del/DiGeorge syndrome
19% Multiple congenital anomalies	15% T21
13% Third space	3% Ataxia telangiectasia
13% Glanomalies	3% T18
3% Neonatal leukemia	2% CHARGE

From Jyonouchi S. et al., J Clin Immunol 2017;37:363-74
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Using TREC in Newborn Screening: Amatuni et al. (2019) Distribution of Outcomes in California (2010-2017)

- ***3,252,156 screened newborns*:**
  - ***562 required flow cytometry***
  - ***213 had T cell lymphopaenia**, i.e. <1500 T cells/μl:*

***SCID, n= 50:** IL2RG (14), ADA (9), RAG1 (8), IL7RA (6), JAK3 (3), RAG2 (3), RMRP (1), BCL11B (1), Unknown (5) (no mutation identified)*

***Syndromes, n= 72:** DiGeorge (47), Trisomy 21 (8), Ataxia-Telangiectasis (5), CHARGE syndrome (3), Diabetic embryopathy (3), CLOVES syndrome (1), EXTL3 deficiency (1), Fryns syndrome (1), Nijmegen syndrome (1), Noonan syndrome (1), Rac2 deficiency (1)*

***Secondary, n= 25:** Congenital heart disease (6), Hydrops (6), Gastroschisis (4), Chylothorax (2), Maternal immunosuppression (2), Third space fluid leakage (2), Intestinal atresia (1), Meconium ileus (1), Teratoma of the thymus (1)*

Pre-term birth and low birth weight, n= 33

Idiopathic, n= 33

Assessment of TREC, CD4 Recent Thymic Emigrant (CD4RTE), Proliferation and TCRVβ Spectratype in a SCID Patient

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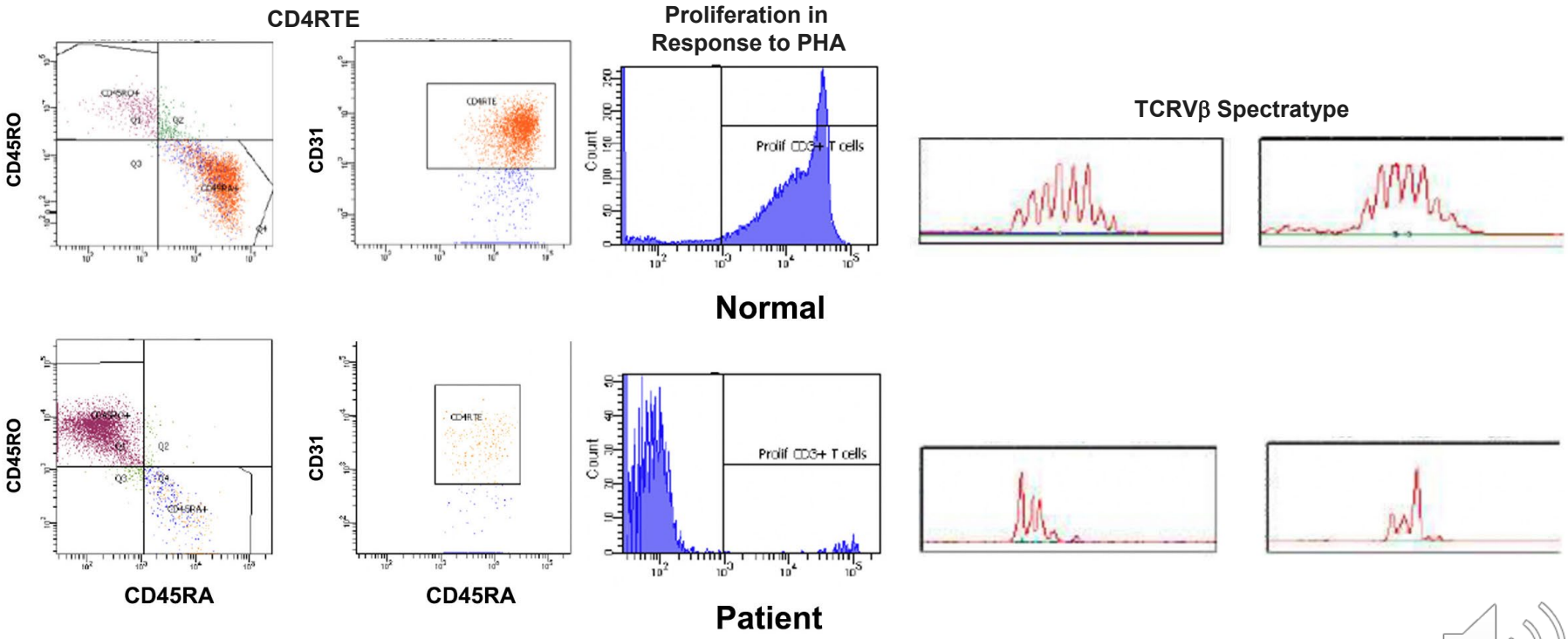
Variable	Result	Reference Range
CD3+ T cells	841	1484-5327 cells/mL
CD4+ T cells	60	733-3181 cells/mL
CD8+ T cells	724	370-2555 cells/mL
TREC	Below LOD	>=6794/10^6 CD3+ T cells



Monitoring Allo-HCT in a Patient with SCID

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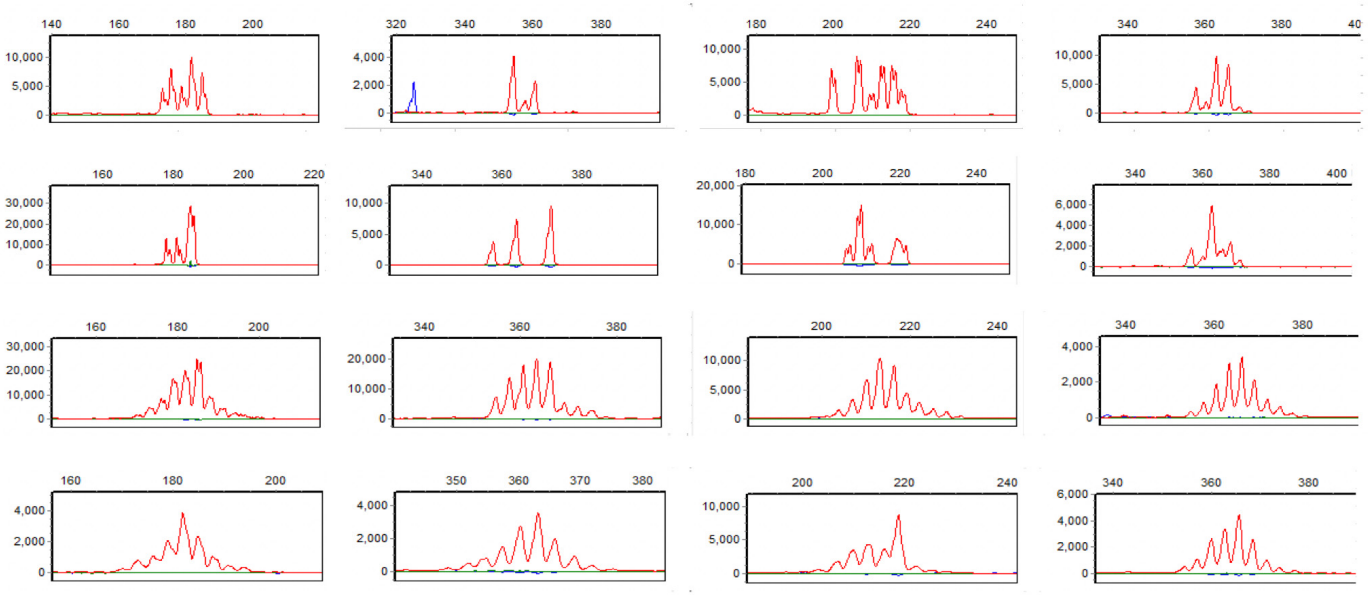
Variable	FEB 2017	MAY 2017	NOV 2017	NOV 2018	DEC 2019	Reference Range
CD3+ T cells	148	610	543	2257	1760	1484-5327 cells/mL
CD4+ T cells	137	498	285	1360	997	733-3181 cells/mL
CD8+ T cells	7	99	183	711	623	370-2555 cells/mL
TREC	Below LOD	3358	4578	25195	19532	>=6794/10^6 CD3+ T cells



FEB 2017

NOV 2017

NOV 2018

DEC 2019

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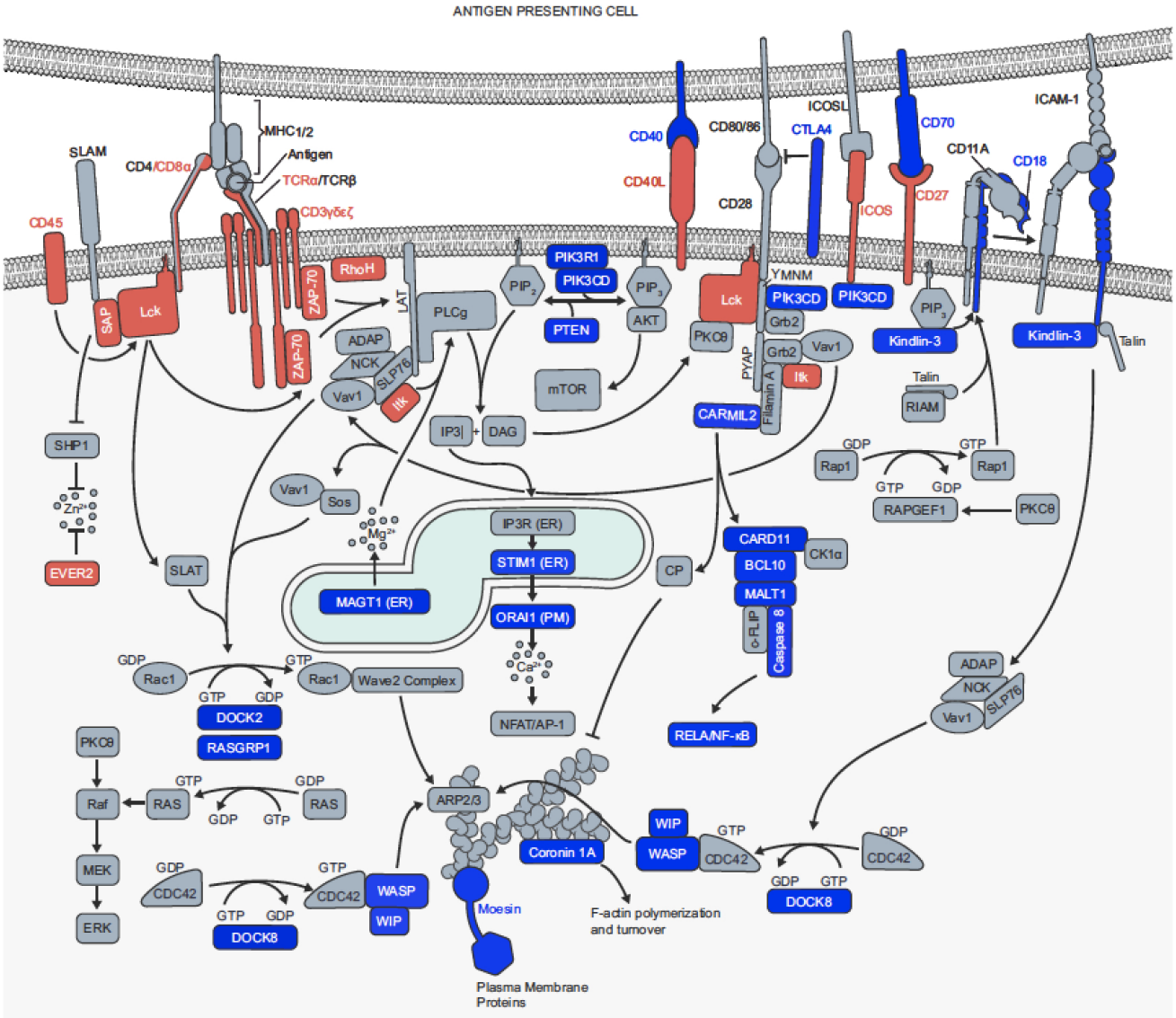
T Cell Signalling Defects Associated with Immunodeficiency



From Lenardo MJ, Holland SM. Immunol Rev 2019;287:5-8.
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Defective T Cell Signalling: *Immunodeficiency and Immune Dysregulation*

- ***Susceptibility to Infection:***
  - *Impaired T cell development and/or survival in the thymus*
  - *Reduced effector T cell function*

- ***Immune Dysregulation:***
  - *Impaired negative selection of autoreactive T cells*
  - *Impaired generation and/or function of regulatory T cells (Tregs)*
  - *Defective activation-induced cell death*

Laboratory Features of Combined Immunodeficiencies with Defective T Cell Signalling

- *Variable degrees of T cell lymphopaenia*
- *Decreased number of naïve T cells*
- *Changed distribution of T cell subpopulations, with potential increase in effector memory (T_{EM}) and effector memory RA (T_{EMRA}) subsets*
- *Variable effect on serum immunoglobulin levels (from hypo- to hyper-gammaglobulinaemia)*
- *Potential presence of autoantibodies*

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