Laboratory evaluation of primary immunodeficiencies

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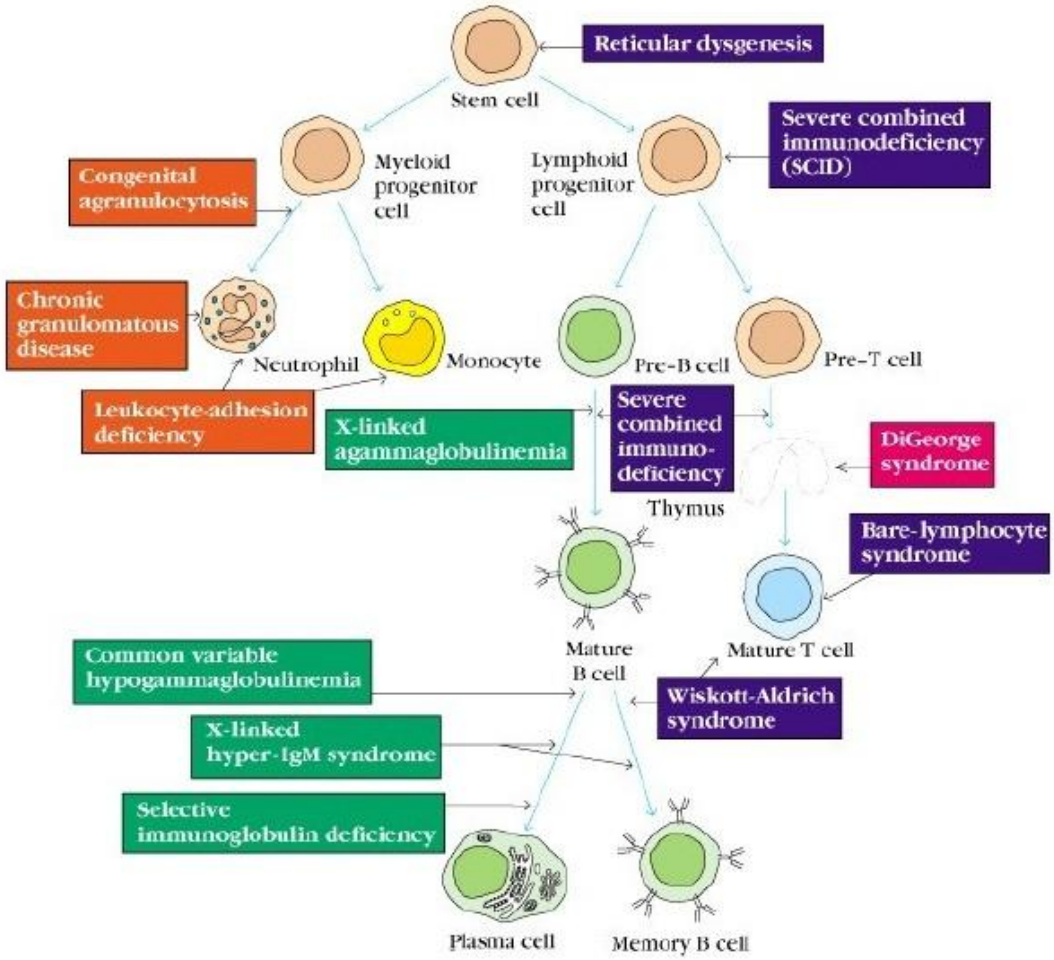
More than 200 primary immunodeficeincies (PID) have been described



Cant A. 2013

Chapel H. Classification of primary immunodeficiency diseases by the International Union of Immunological Societies (IUIS) Expert Committee on Primary Immunodeficiency. 2011.

Basic evaluation of the immune system

- Available at all the University Hospitals
- Can often be performed on day to day basis, if indicated

  - Quantitative
    - Blood cell count (leukocytes)
    - S-immunoglobulin's (Igs)
    - Isoagglutinins (anti-A and anti-B)
    - CH50 and AH50 (complement function)
    - Immunophenotyping (enumeration of leukocyte subpopulations)
    - Neutrophil oxidase function (intracellular killing)
  - Qualitative
    - Specific antibody response (post immunization)
    - T-cell proliferation assays

Advanced evaluation of the immunesystem

- Second line test except for known familial cases or very suspicious clinical presentation
- Often only performed in a few labs in Denmark
- May require shipping of samples to labs outside DK
- Please ask you local immunologist who will be delighted to help you

Examples:

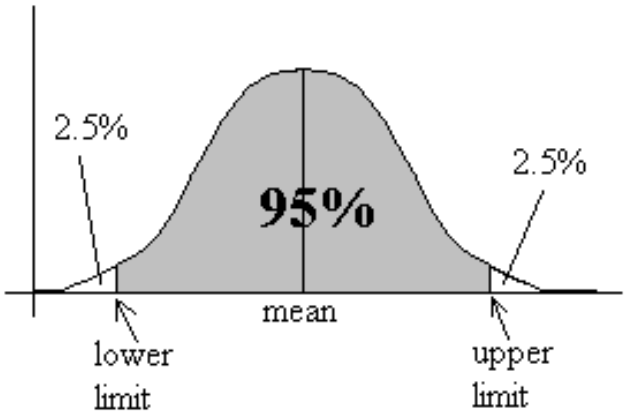
- Special B- and T-cell subpopulations
- NK-cell function
- Antibodies against neutrophil surface antigens
- Specific activation assays (e.g. TLR function)
- Immunophenotyping for specific intra or extracellular molecules (e.g. IFNGR1, BTK)
- Genetic testing (e.g. del22q11.2, TACI, B-cell somatic hypermutation,TNFRSF1)

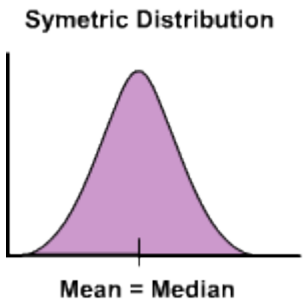
Laboratory evaluation of primary immunodeficiencies. Oliveira JB, Fleisher TA. 2010
Primary immunodeficiencies update. J Allergy Clin Immunol. 2010
Common variable immunodeficiency - an update. Salzer U, Warnatz K, Peter HH 2012

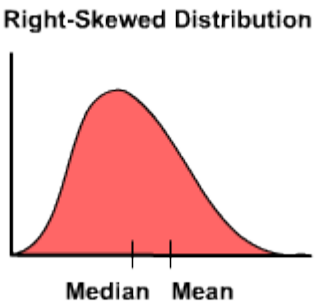
Some (very) general aspects of laboratory testing

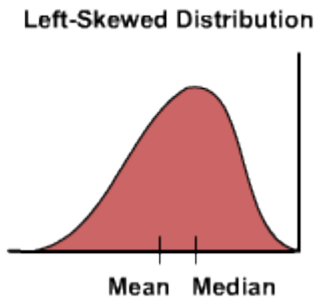
Normal range = 95% confidence interval
95% CI, 5/95 percentiles, prediction intervals

Never rely on lab values only
Always repeat an abnormal lab test that do not match the clinical presentation



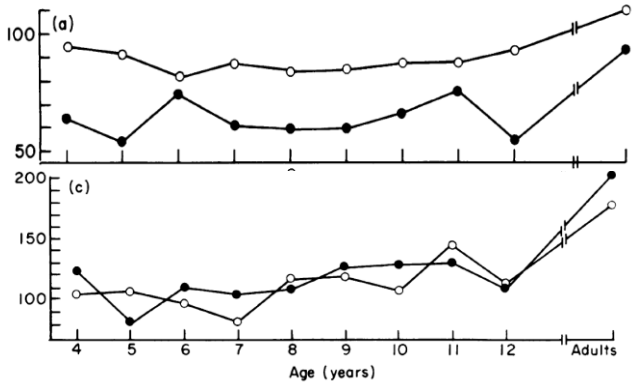






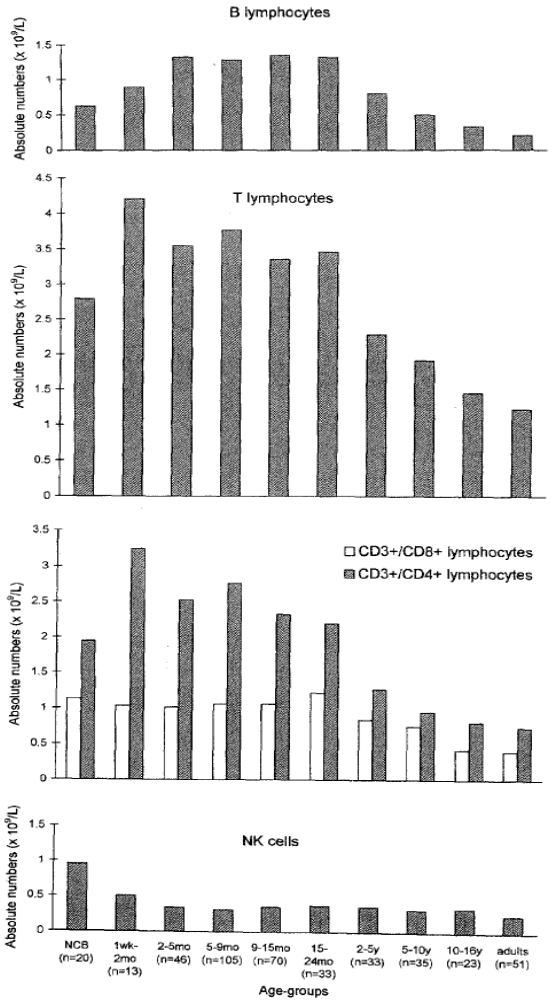
Some general aspects of laboratory evaluation

- Age related differences are significant
- Always use age specific reference intervals
- Absolute count important
- Be aware of gender and ethnic variation



S-IgM

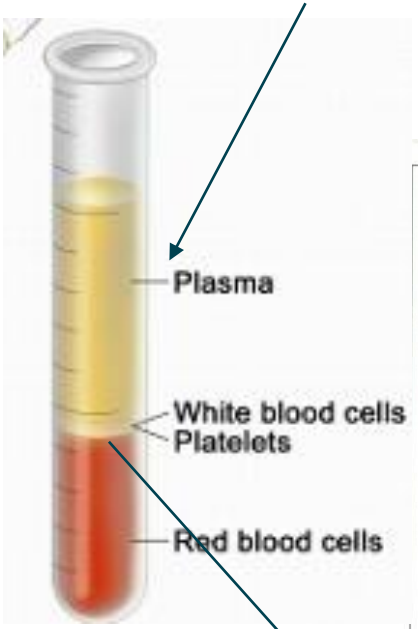
S-IgA

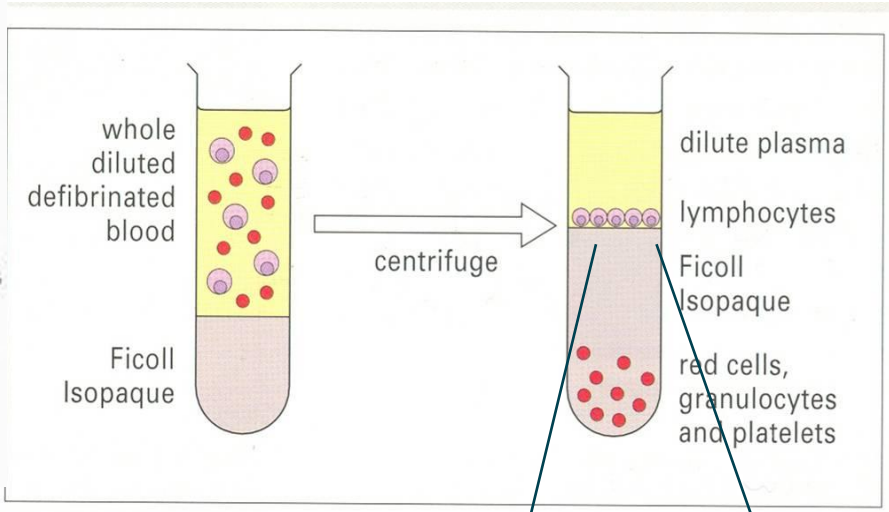


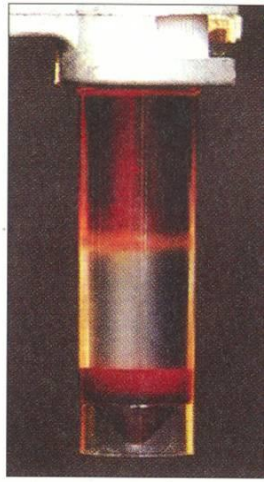
Comans-Bitter et al. 1997
Stoop et al. 1969

Peripheral blood sample → general immune evaluation

Complement, Ig's, antibodies







DNA for genetic testing

Neutrophils for chemotaxis, chemiluminescence and MAIGA

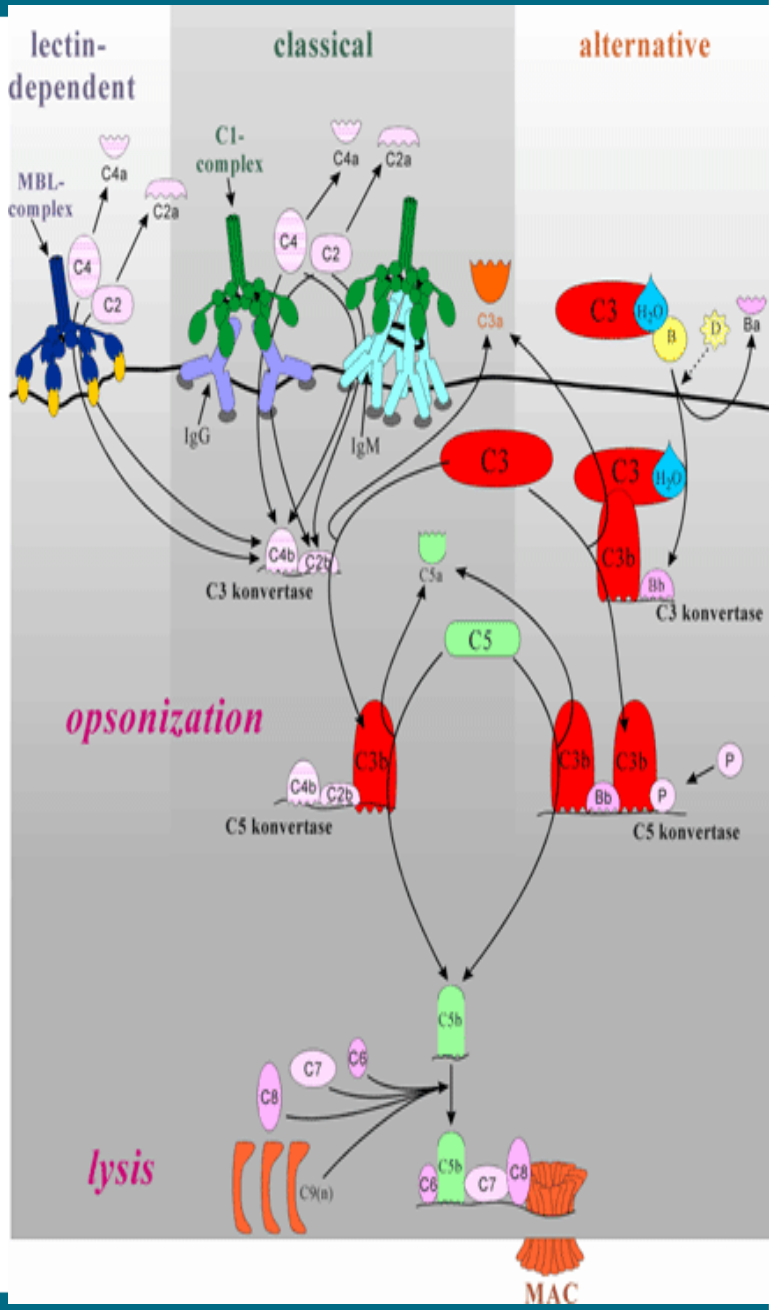
Whole blood for immunophenotyping

PMC for proliferation / stimulations

Complement function

- Defects in classical pathway: CH50 ~ 0%
- Defects in alternative pathway: AH50~ 0%

- Hemolytic assays:
  - Sample incubated with sensitized sheep erythrocytes (CH50) or non-sensitized rabbit erythrocytes (AH50).
  - Erythrocytes disintegrate
  - Lysis measured by decreased optical density
  - Deficient sera can be used to characterize discrete defects



Nilsson B, Ekdahl KN. 2012

Case - I

Twins, pneumococcal sepsis in first months of life

No familial history of immunodeficiency

Normal cell counts

Twin A: CH50: < 10%
Twin B: CH50: <10%

CH50 with deficient sera: C2 < 10%

Patient	C2type 1 defekt
A	homozygot
B	homozygot
Mother	heterozygot
Father	heterozygot

Case - II

- 1 year old child
- Recurrent severe sinopulmonary infections
- Servere hypogamma
- Work up ?

Flowcytometric immunophenotyping

T-cells (CD3,CD4,CD8), B-cells (CD19,CD20), NK-cells (CD16,CD56)

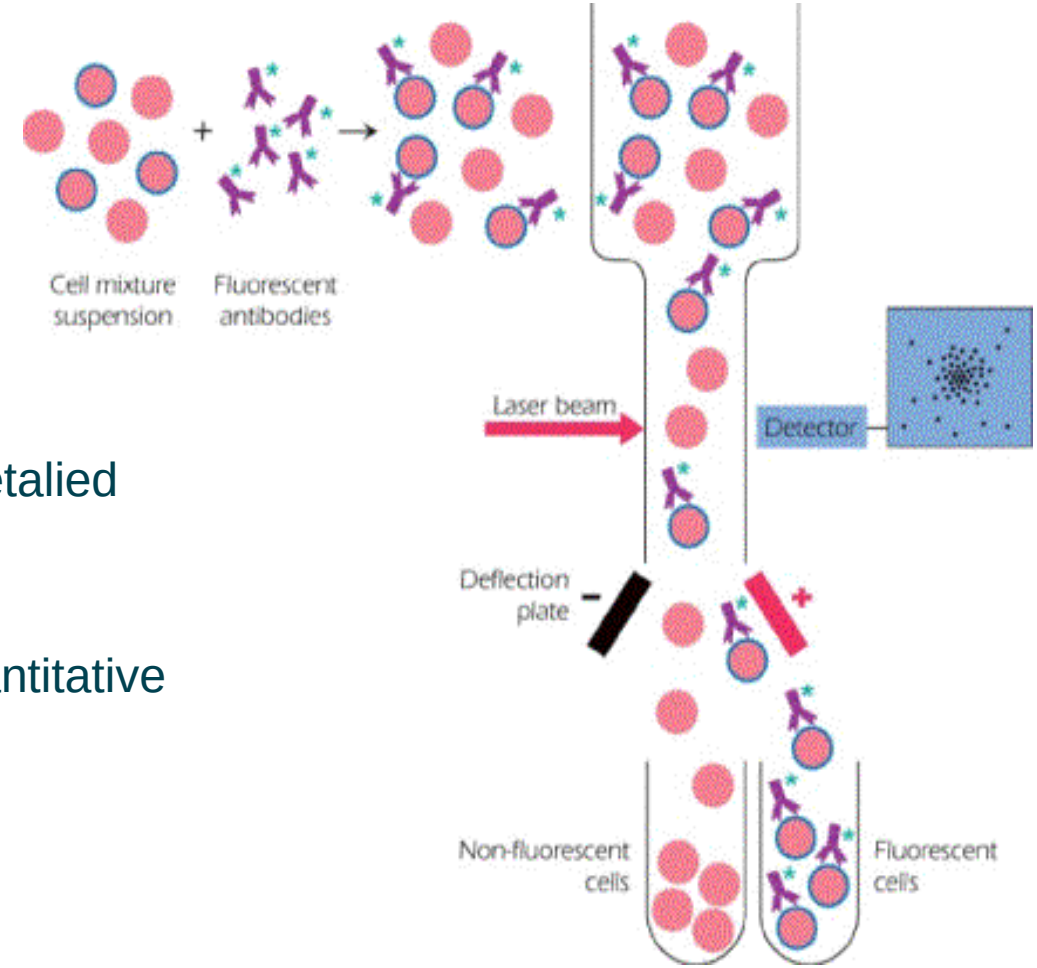
- Evaluation for a specific cell proteins (CD18, CD40/CD154, IFNγR, IL-12R)
- Intracellular proteins: (BTK, FOXP3, STAT-1, STAT-4)

Assessment for biologic effect

- Memory/naive T cells
- Memory B cell - isotype switch (CD27, IgM, IgD)
- Regulatory T- and B-cells

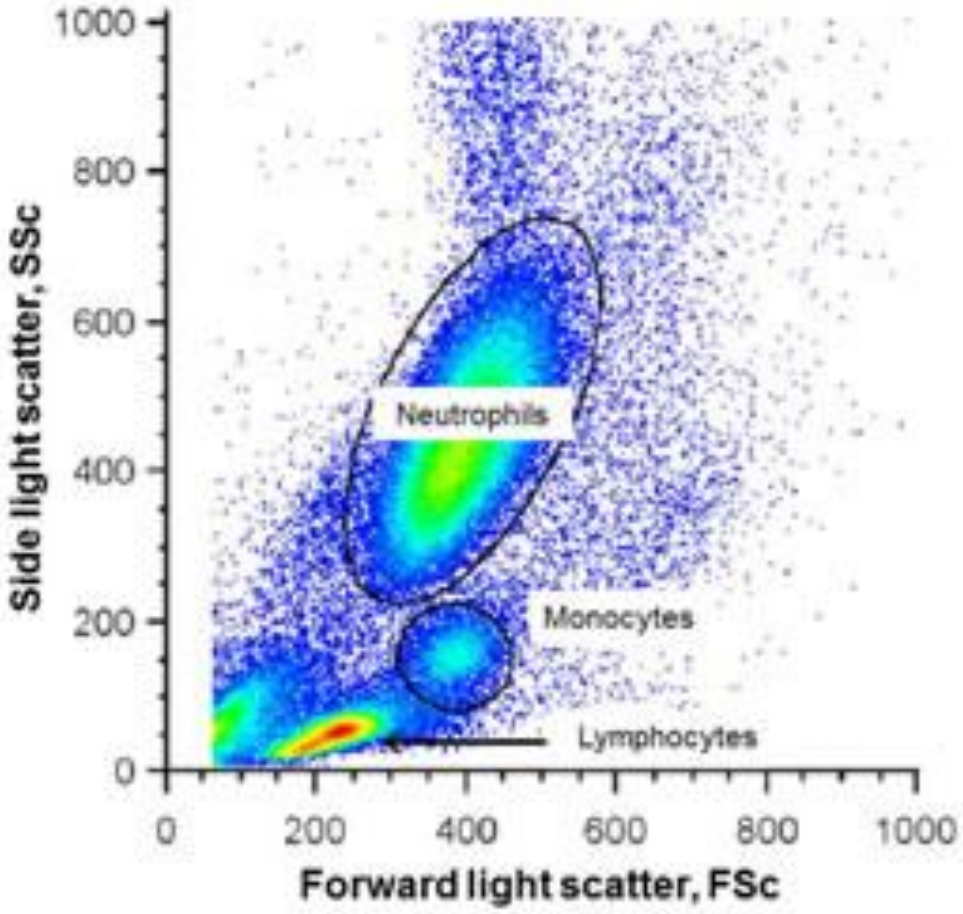
Flowcytometry

- ”Advanced microscopy”
- FAST! – 10.000 cells/sec
- Multicolor cytometry allows for detalied charactization of cells
- Qualitative, semiquantitative, quantitative

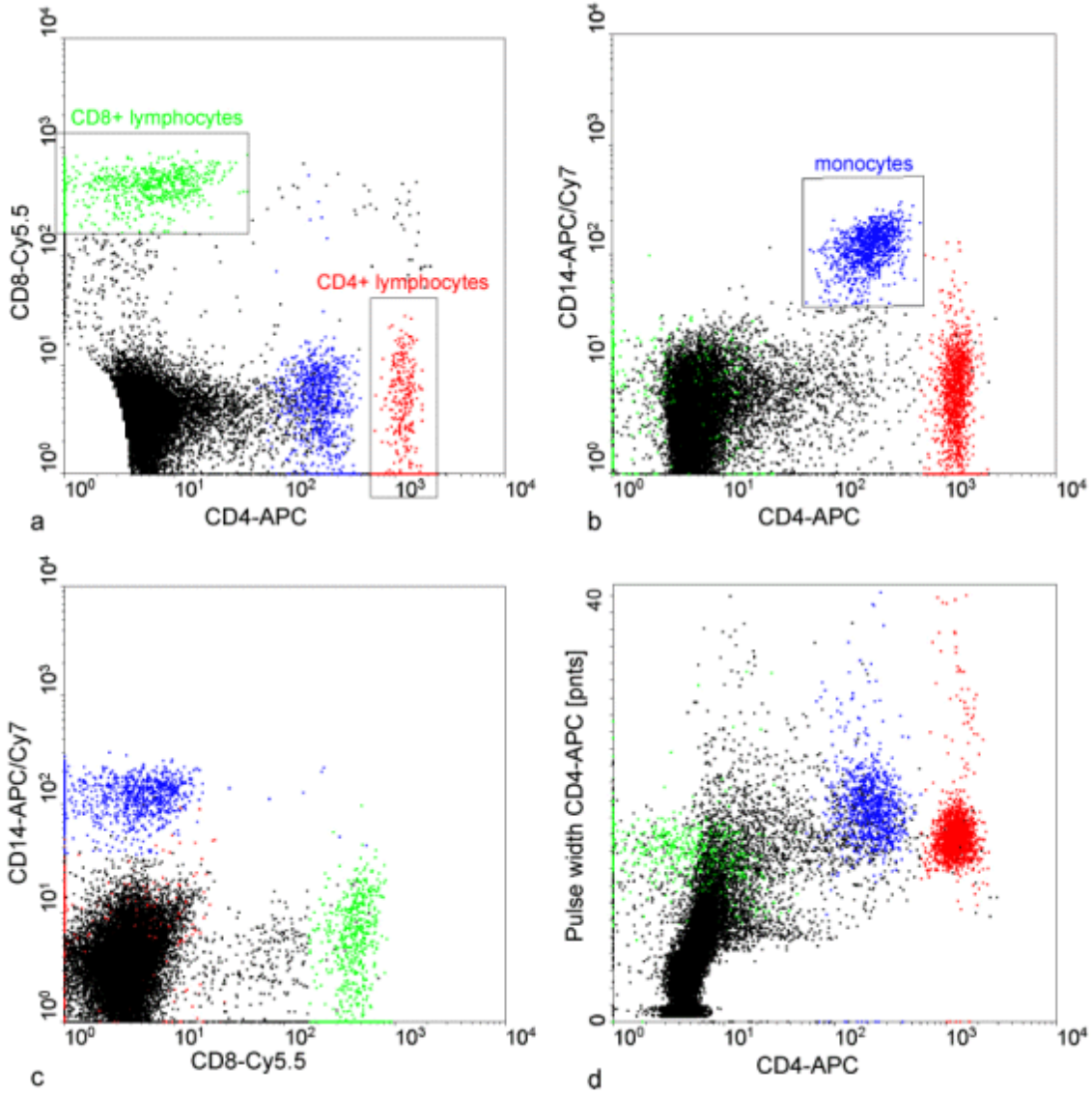


Ormerod MG. Flowcytometry 2. edition

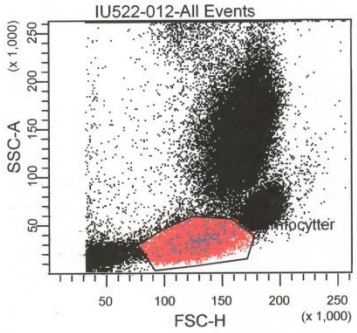
Size and granularity



Subpopulations

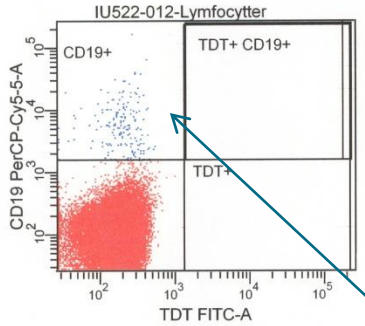


Case - II



Tube: IU522-012

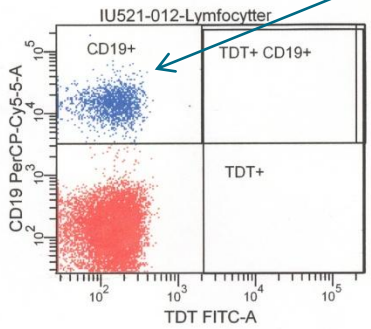
Population	#Events	%Parent	%Total
All Events	112,763		100.0
Lymfocytter	64,558	57.3	57.3
TDT+	0	0.0	0.0
CD19+	179	0.3	0.2
TDT+ CD19+	0	0.0	0.0



PATIENT

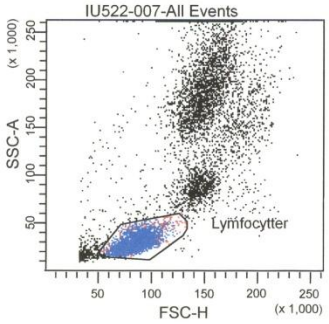
Population	#Events	%Parent	TDT FITC-A Mean	CD19 Per... Mean
Lymfocytter	64,558	57.3	177	108
TDT+	0	0.0	####	####
CD19+	179	0.3	209	8,967
TDT+ CD19+	0	0.0	####	####

B-cells



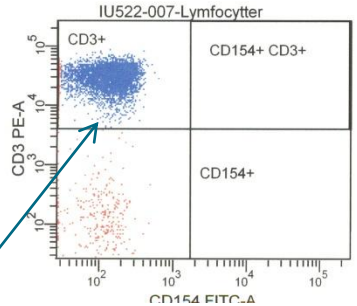
Controle

Population	#Events	%Parent	TDT FITC-A Mean	CD19 Per... Mean
Lymfocytter	17,068	6.7	140	1,631
TDT+	0	0.0	####	####
CD19+	1,613	9.5	150	16,118
TDT+ CD19+	0	0.0	####	####



Tube: IU522-007

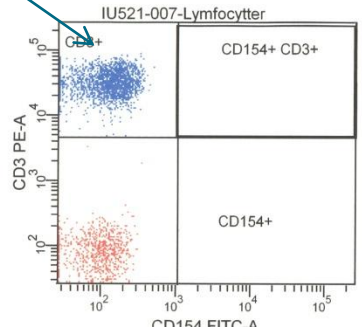
Population	#Events	%Parent	%Total
All Events	10,884		100.0
Lymfocytter	6,313	58.0	58.0
CD154+	0	0.0	0.0
CD3+	5,482	86.8	50.4
CD154+ CD3+	0	0.0	0.0



PATIENT

Population	#Events	%Parent	CD154 FI... Mean	CD3 PE-A Mean
Lymfocytter	6,313	58.0	137	30,705
CD154+	0	0.0	####	####
CD3+	5,482	86.8	150	32,600
CD154+ CD3+	0	0.0	####	####

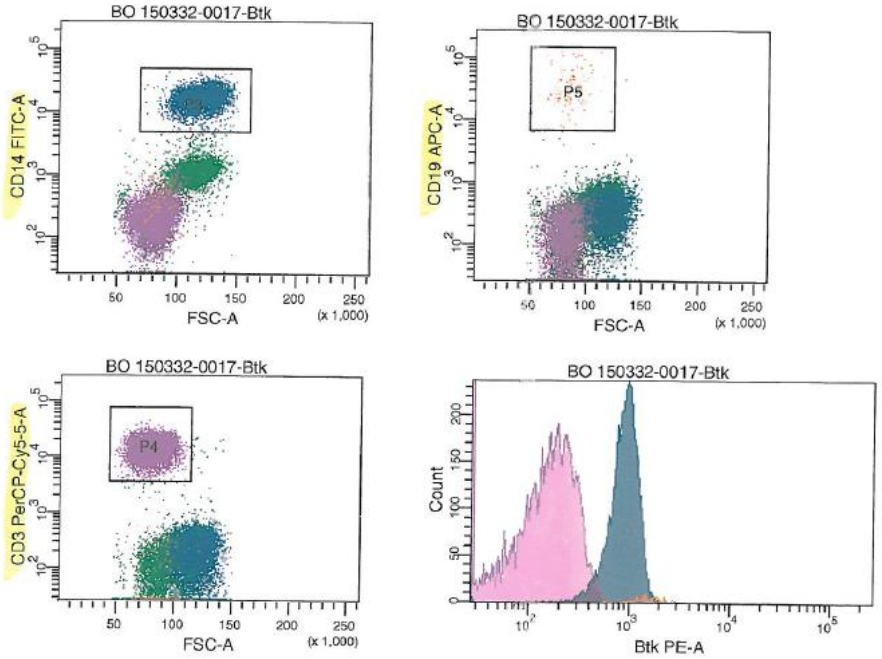
T-cells



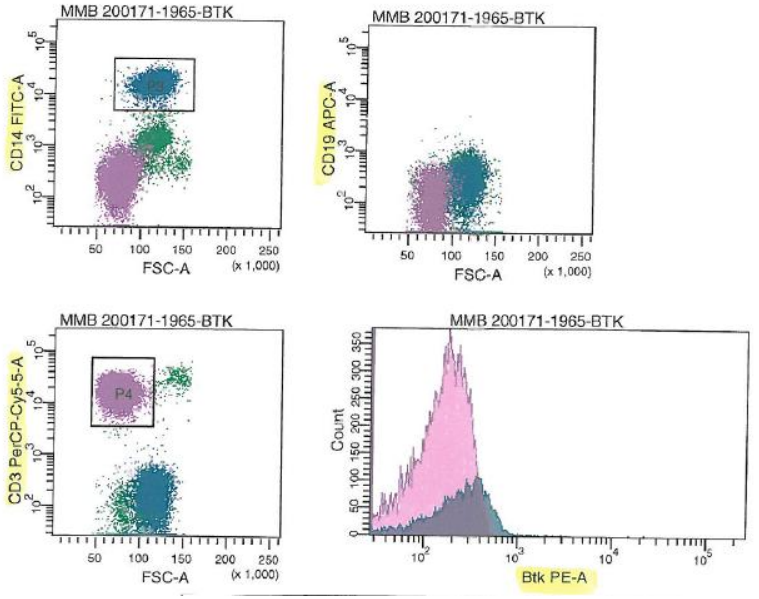
Controle

Population	#Events	%Parent	CD154 FI... Mean	CD3 PE-A Mean
Lymfocytter	3,310	31.5	121	21,444
CD154+	1	0.0	1,069	173
CD3+	2,064	62.4	133	34,355
CD154+ CD3+	0	0.0	####	####

BTK staining

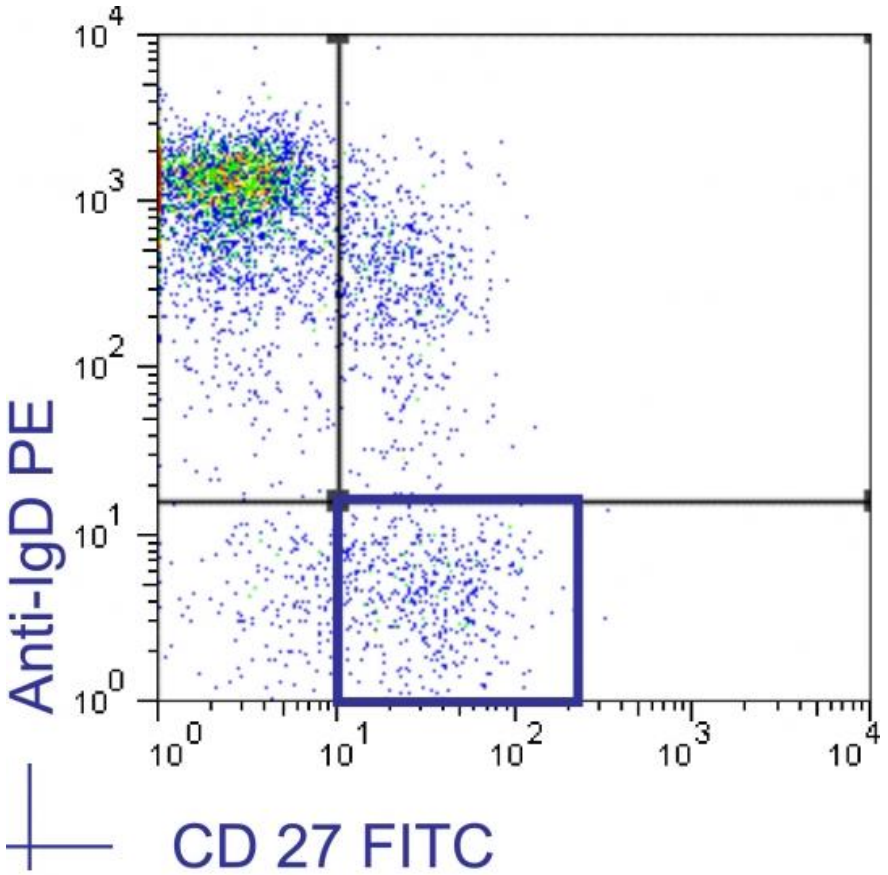


XLA patient

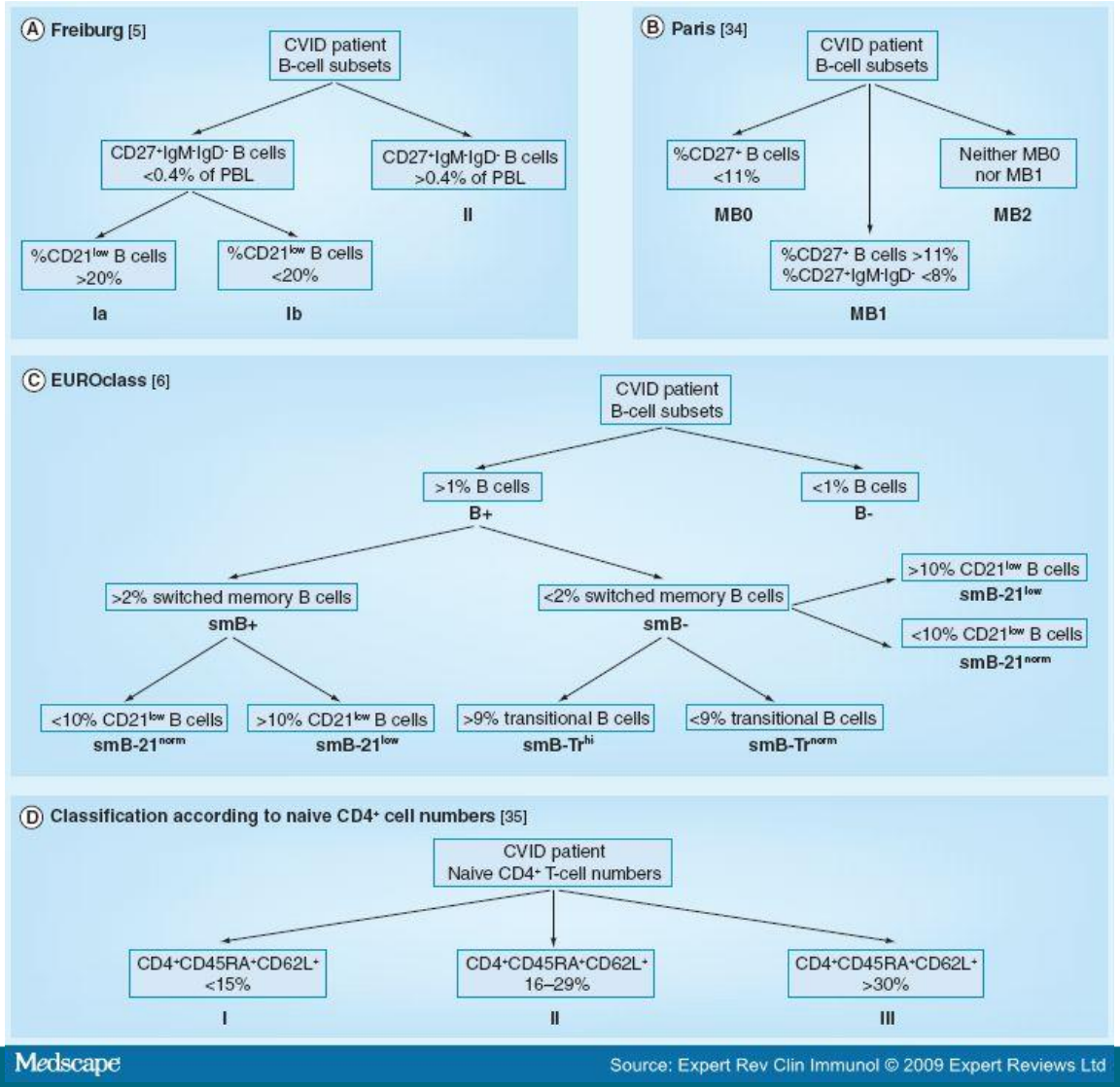


Characteristics of discrete subpopulations

- Class switched memory B-cells
- CVID



Classification of CVID – more data needed



Medscape

Source: Expert Rev Clin Immunol © 2009 Expert Reviews Ltd

Bergbreiter et al. 2009

Case - III
Recurrent infections with atypical *mycobacteria*

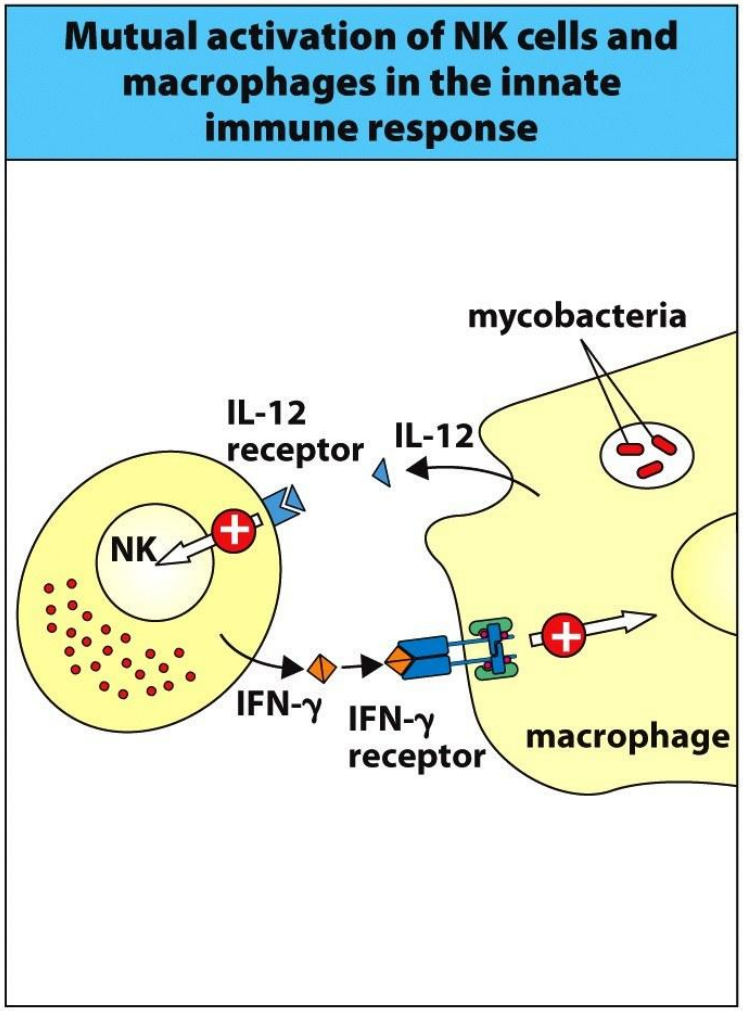


Figure 11.16 The Immune System, 3ed. (© Garland Science 2009)

Case - III
Recurrent infections with atypical *mycobacteria*

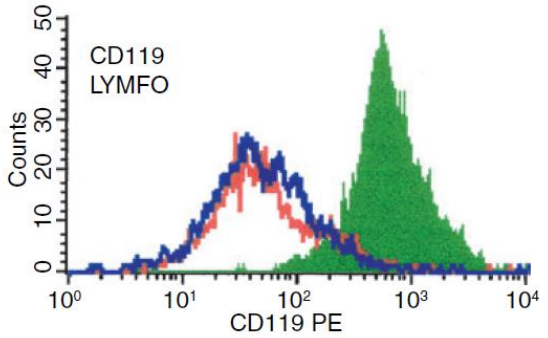


Figure 1 Interferon-γ receptor expression on unstimulated lymphocytes (CD119). Blue and red curves, two normal controls. Green curve, patient.

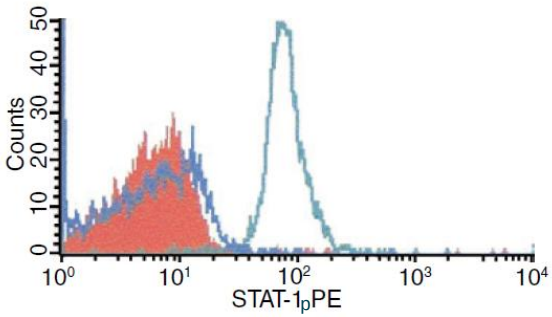
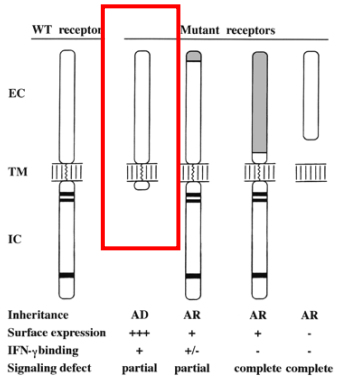


Figure 2 STAT1p production in interferon-γ (IFN-γ)-stimulated mononuclear cells (cytometric bead assay). Blue, unstimulated control. Green, control stimulated with IFN-γ (10 IU/ml). Red, patient stimulated with IFN-γ (10 IU/ml).



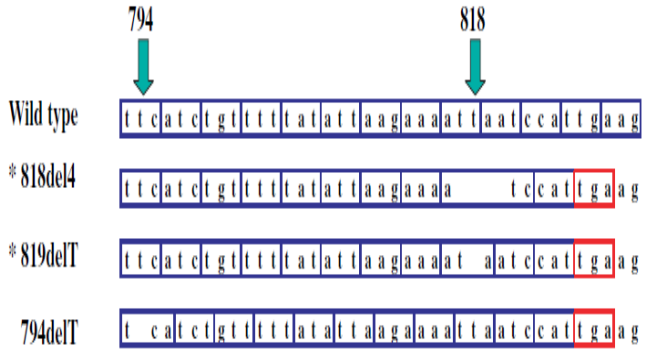


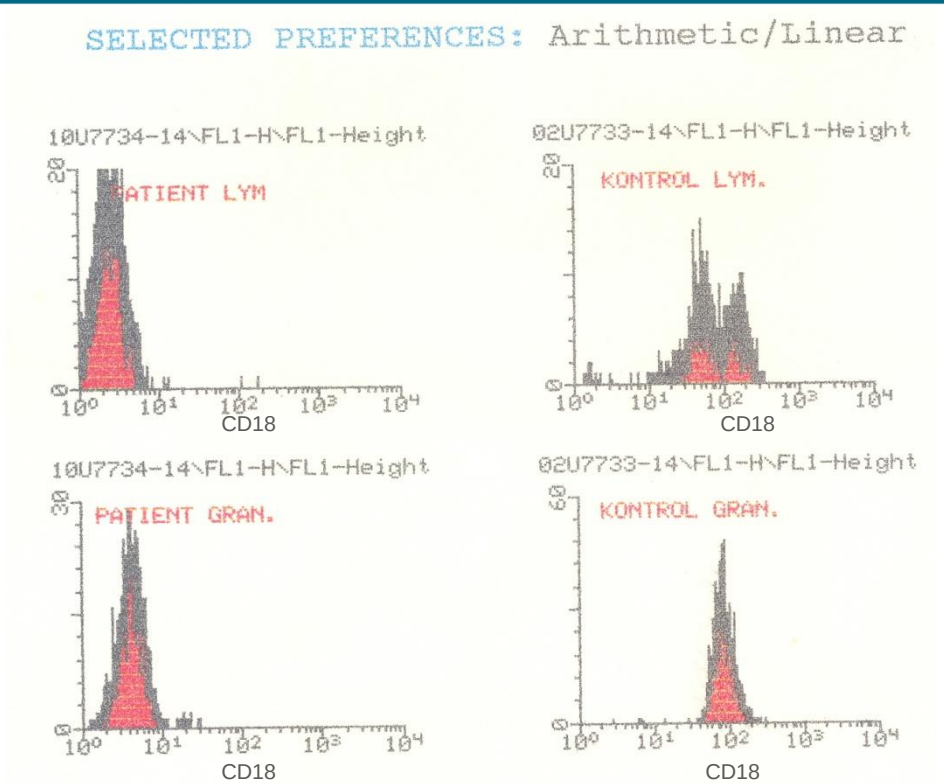
Figure 3 Dominant interferon-γ-receptor alpha-1 (IFNGR1) mutations in exon 6. Red box, a stop codon. *Deletion hotspot described by Jouanguy et al. [6].

Storgaard M, Varming K, Herlin T. 2006

CASE - IV

- 10 months old girl
- Omphalitis at birth
- Conjunctivitis
- Nail infections
- Pneumonias
- Deep skin infections

- Normal Igs + complement
- Normal subpopulations
- Severe neutrocytosis



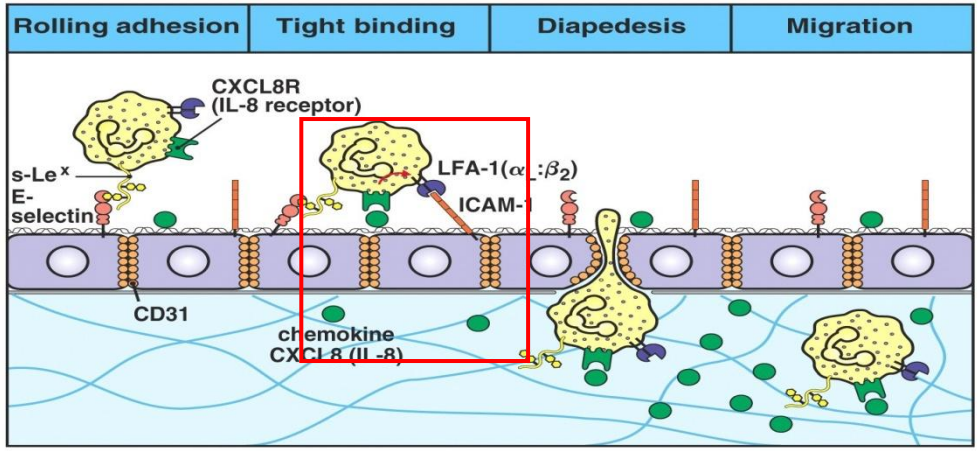
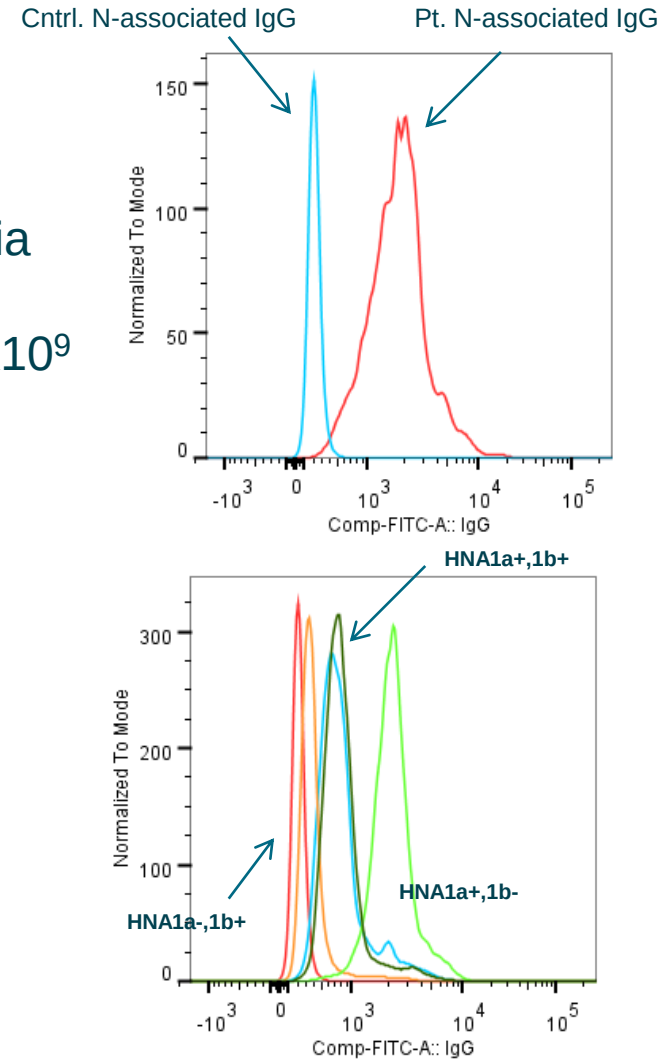


Figure 2-44 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)

CASE - V

6 months old girl
Servere pneumonia

Neutropenia $0.11x10^9$



Autoimmune neutropenia
Flow + MAIGA: anti-HNA-1a

Genotype	HNA-1a	HNA-1b	HNA-1c
Pt	Pos	Neg	Neg
Mater	Pos	Pos	Neg

Commercial Luminex based HNA-antibody kit	Anti-HNA-1a	Anti-HNA-1b	Anti-HNA-1c
Pt.	Neg	Neg	Neg
Mater	Neg	Neg	Neg

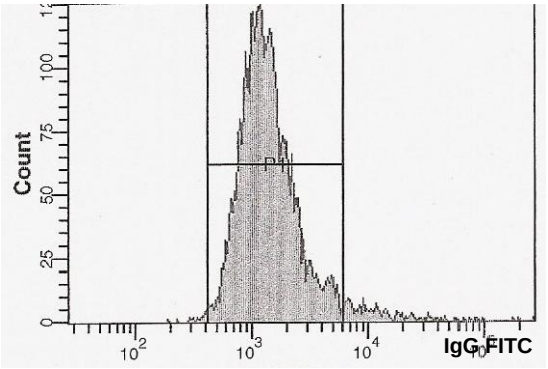
Nielsen KR. et al. 2011, 2012

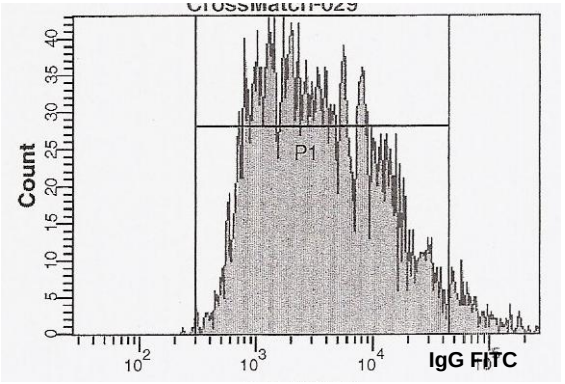
CASE - VI

- 4 week old boy
- Admittet with servere bacterial airway infection
- Neutropenia $0.3x10^9/L$
- Neutrophil count 1.1 at control 3 weeks after

- Neonatal alloimmune neutropenia

Granulocyte crossmatch





Kølbæk M, Nielsen KR. 2010

Evaluation of cell function

- Lymphocyte proliferation (mitogens, antigens, allogenic cells
- NK-cell degranulation
- Granulocyte superoxide (Flowcytometry, chemiluminescens)
- Granulocyte chemotaxis
- Inflammatory mediator production (TLR signalling)

Case - VII

18 months old boy
Deep skin infections, sinopulmonary infections, salmonella osteomyelitis

Normal cell count, Igs, complement and immunophenotype

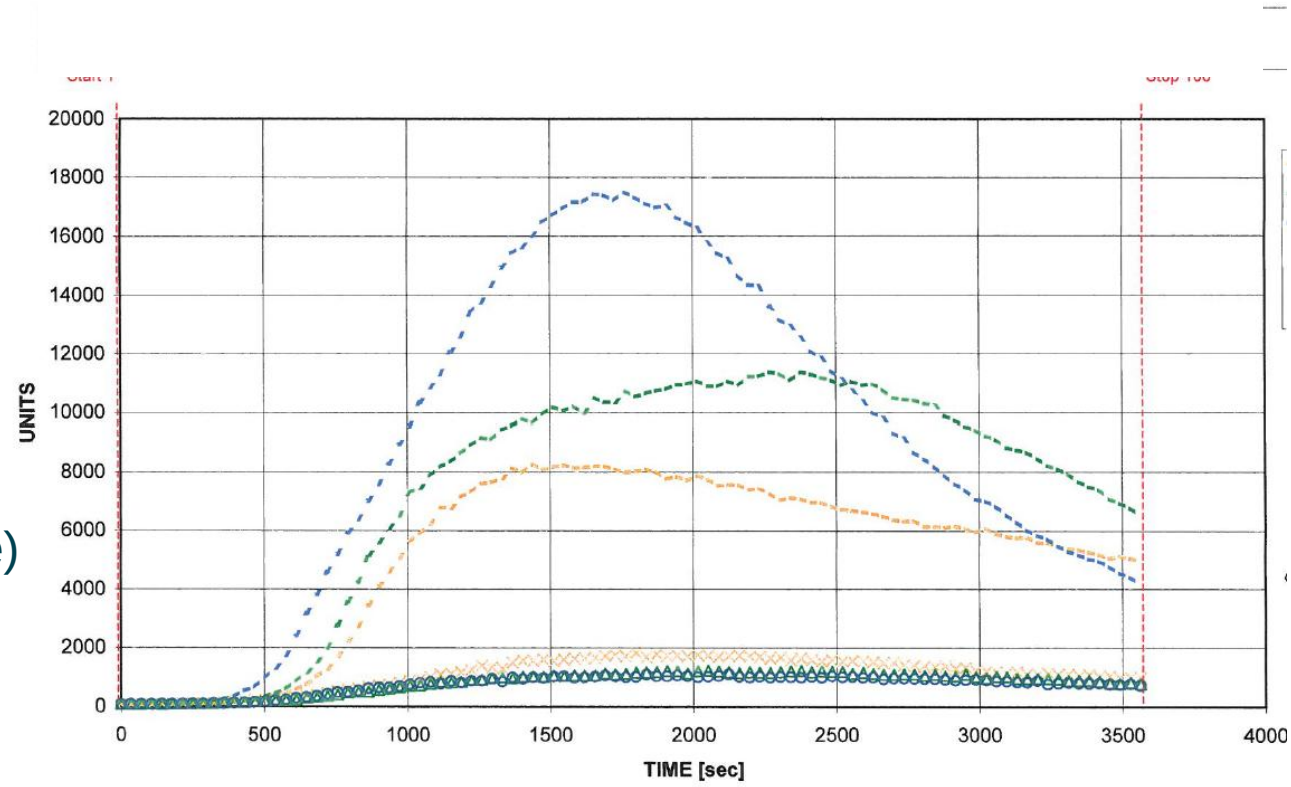
Chemilumiscens assay

Activation of neutrophils

Luminol (CGD and MPO)

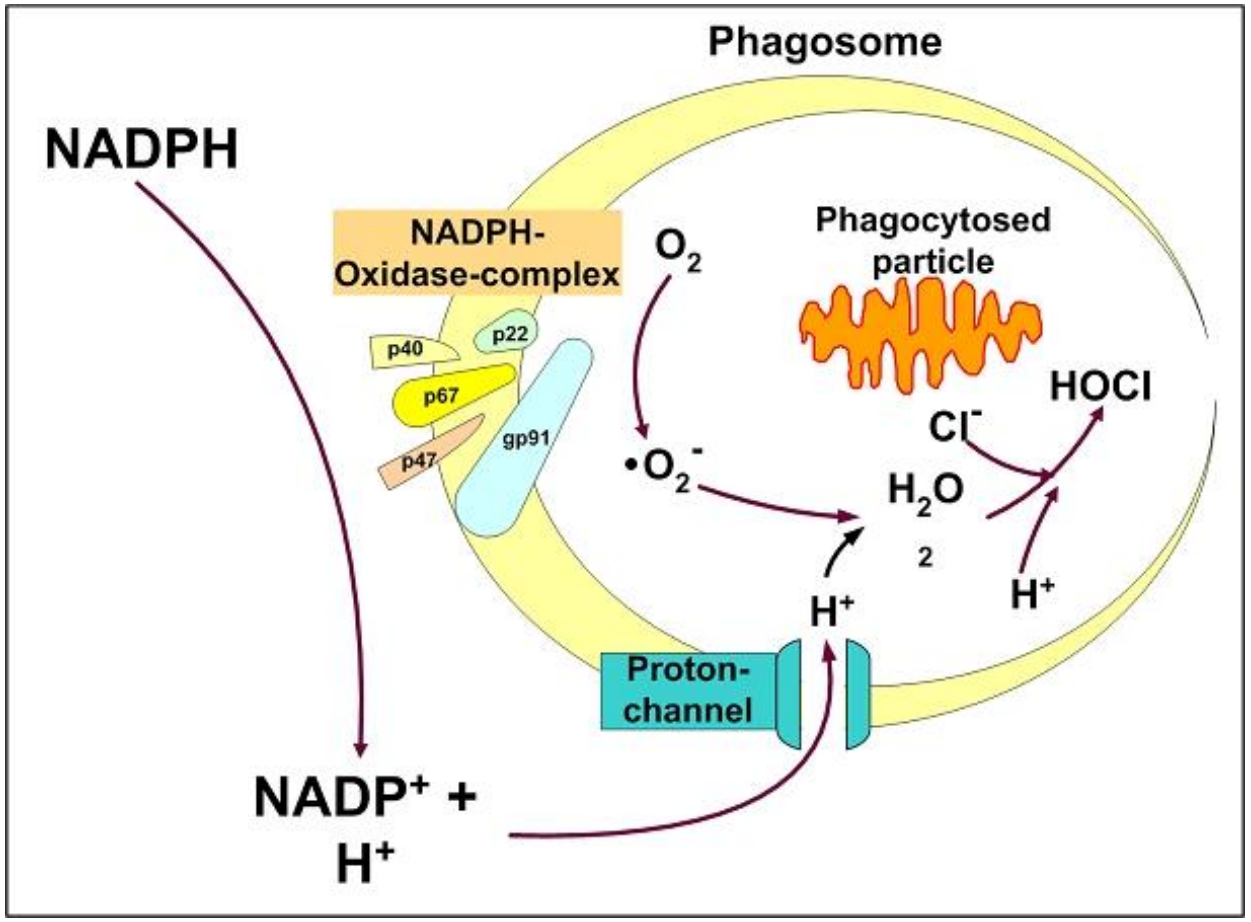
Lucigenin (MPO sensitive)

X-linked CGD



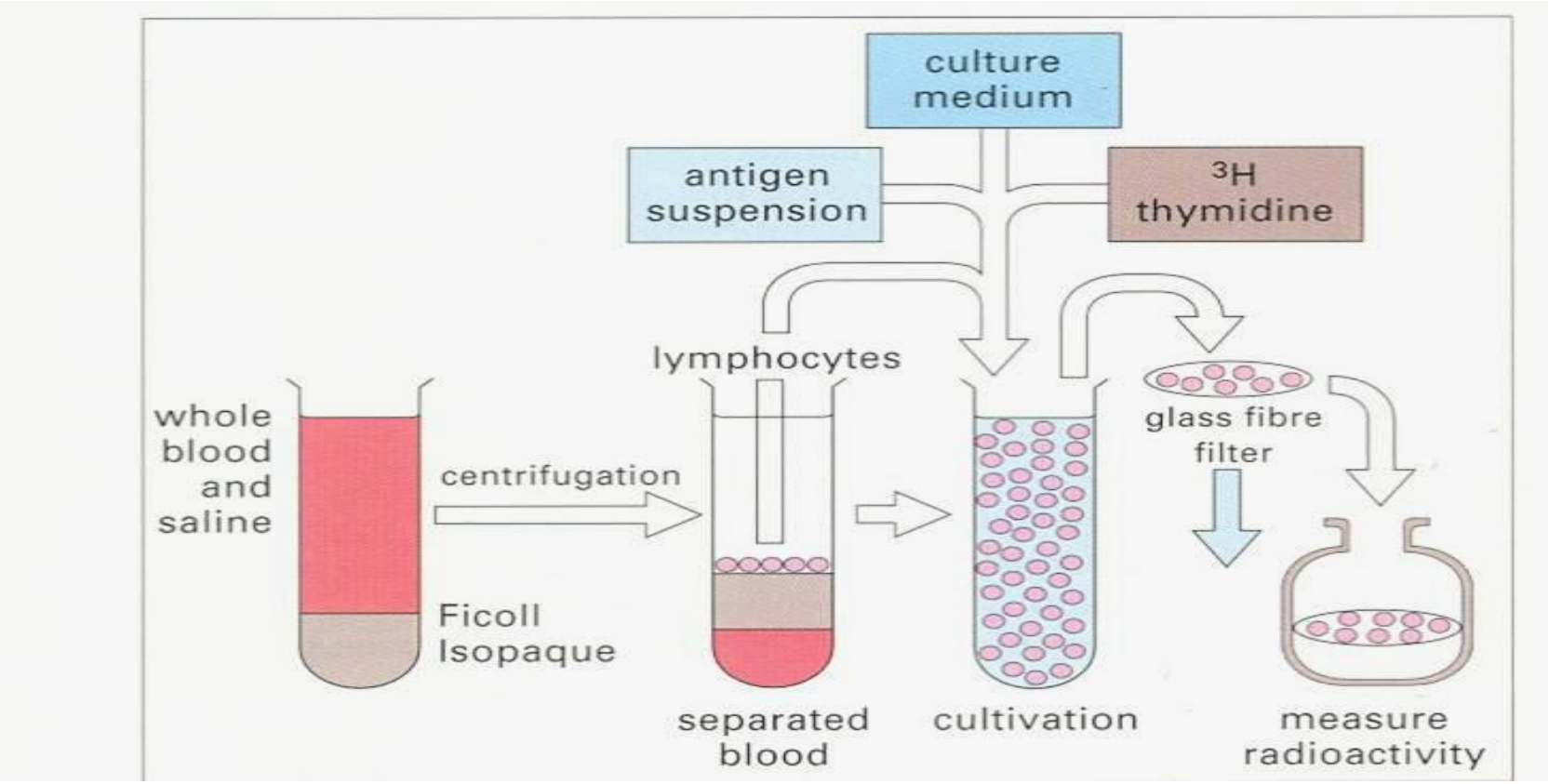
Sørensen NB, Varming K. 2013

CGD - variants

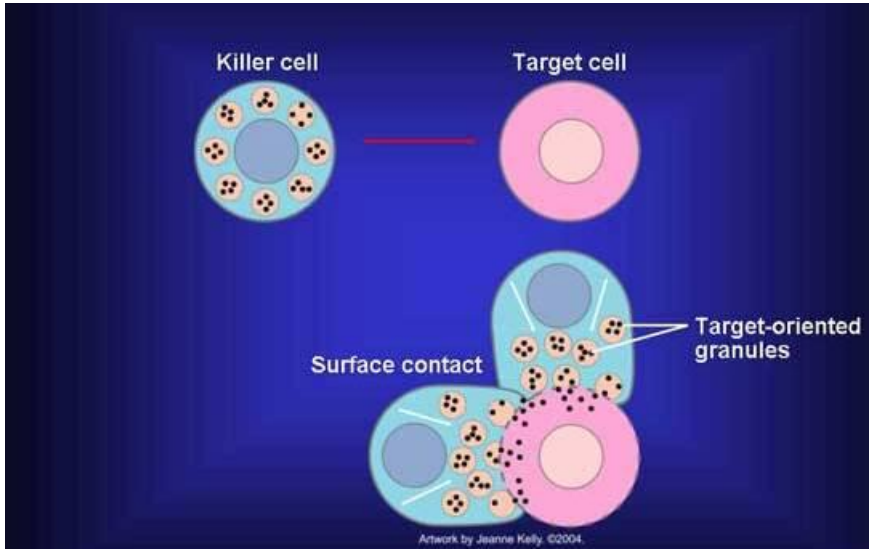


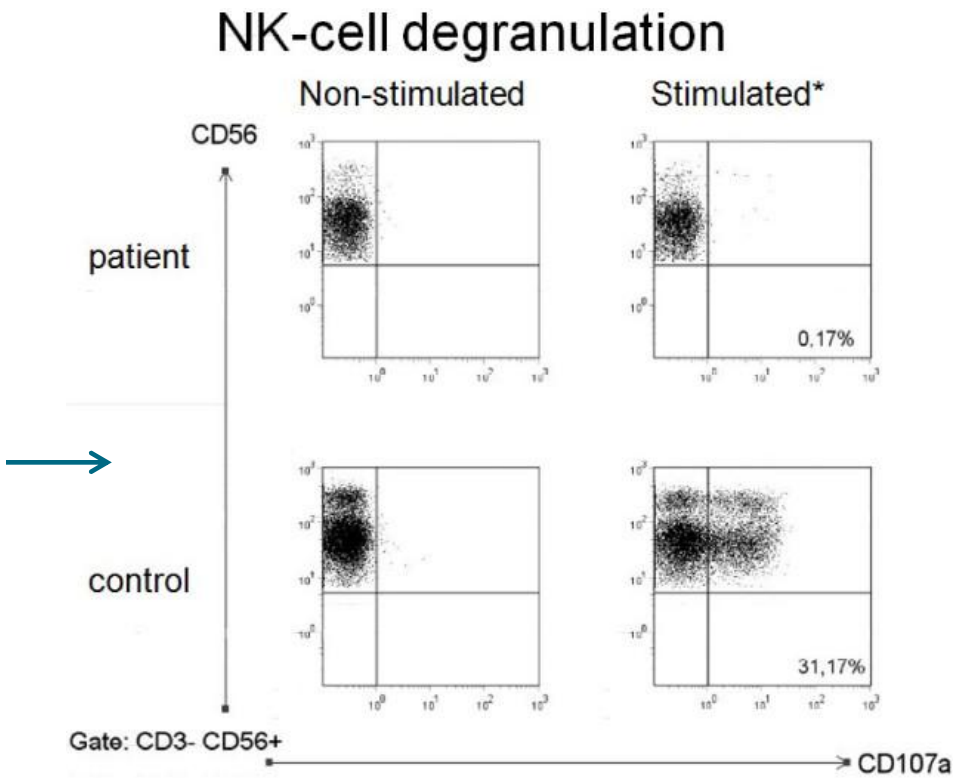
Lymphocyte proliferation (T-cell proliferation)

SCID ~ 0%
DiGeorge, CVID, others: Variate degress of impairment

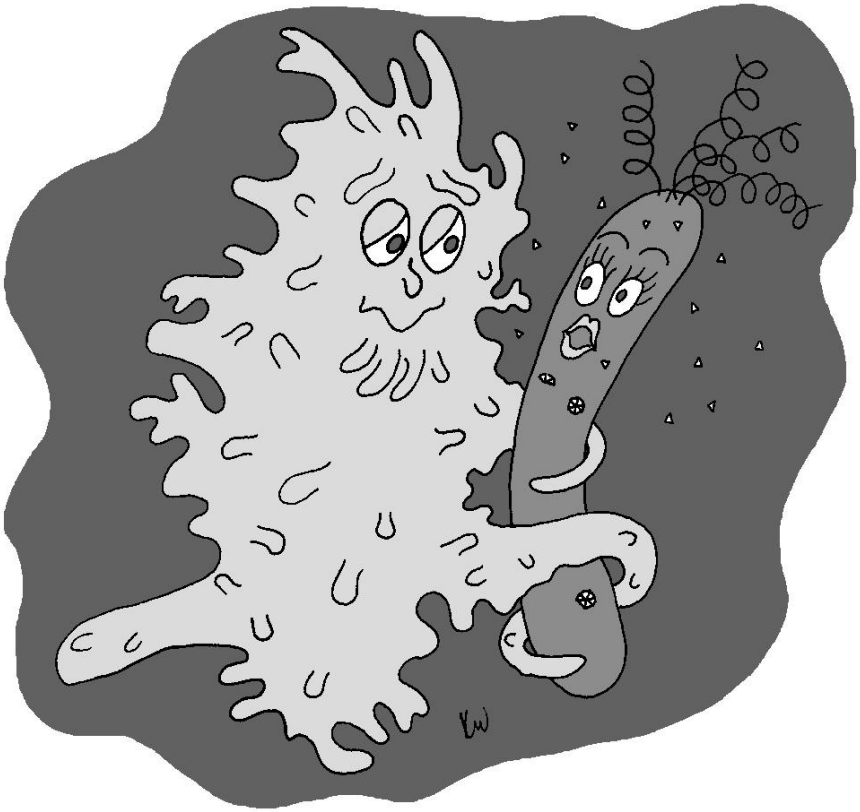


NK-cell degranulation



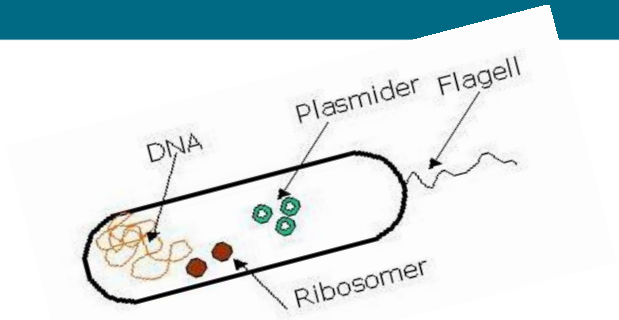


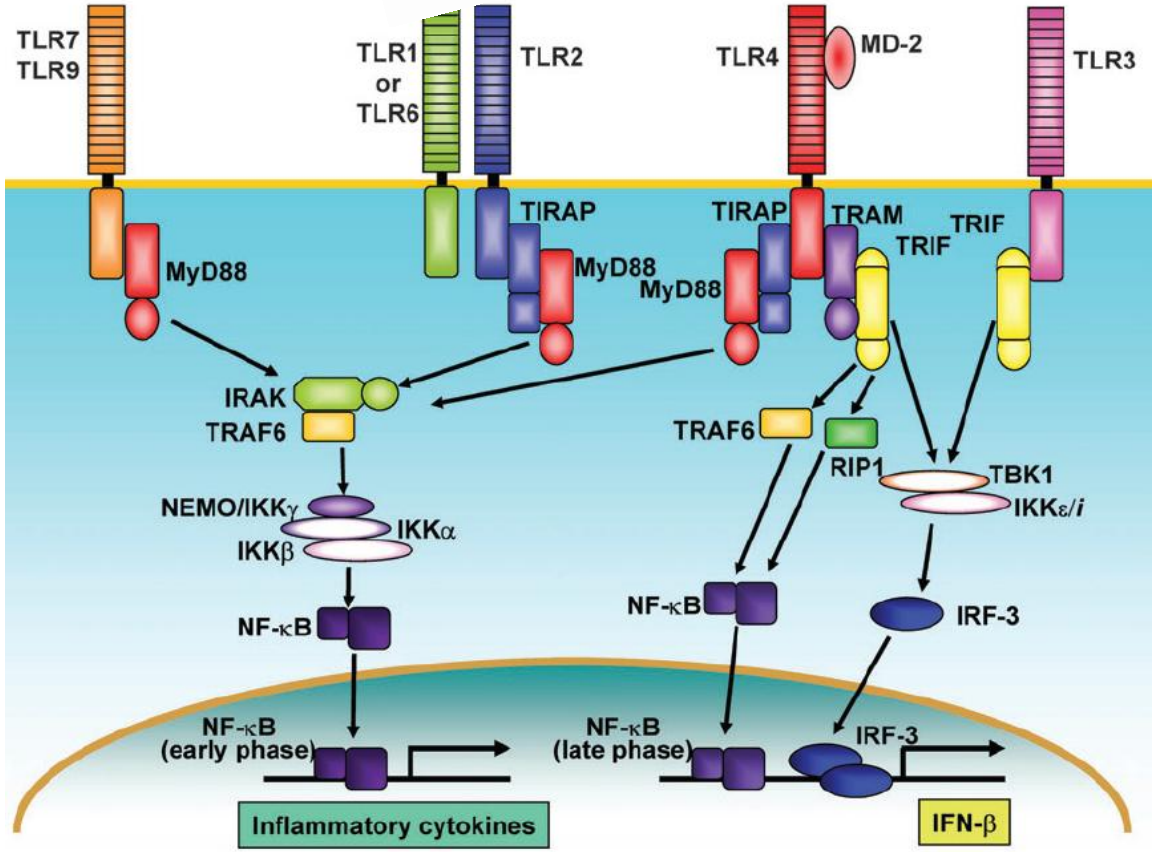
TLR signalling



"My perfume? Why it's activated complement proteins. Do you like it?"

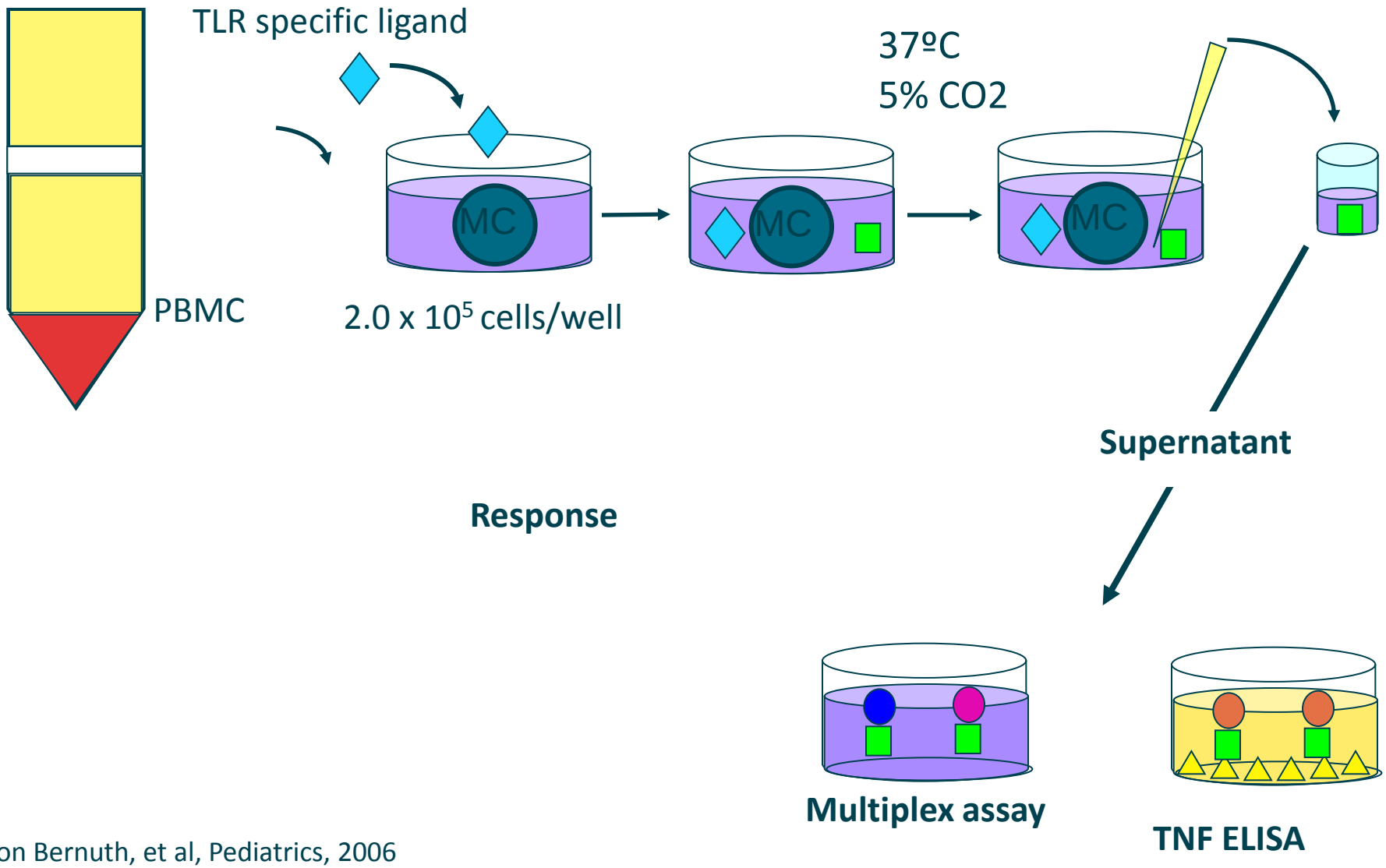
TLR signalling





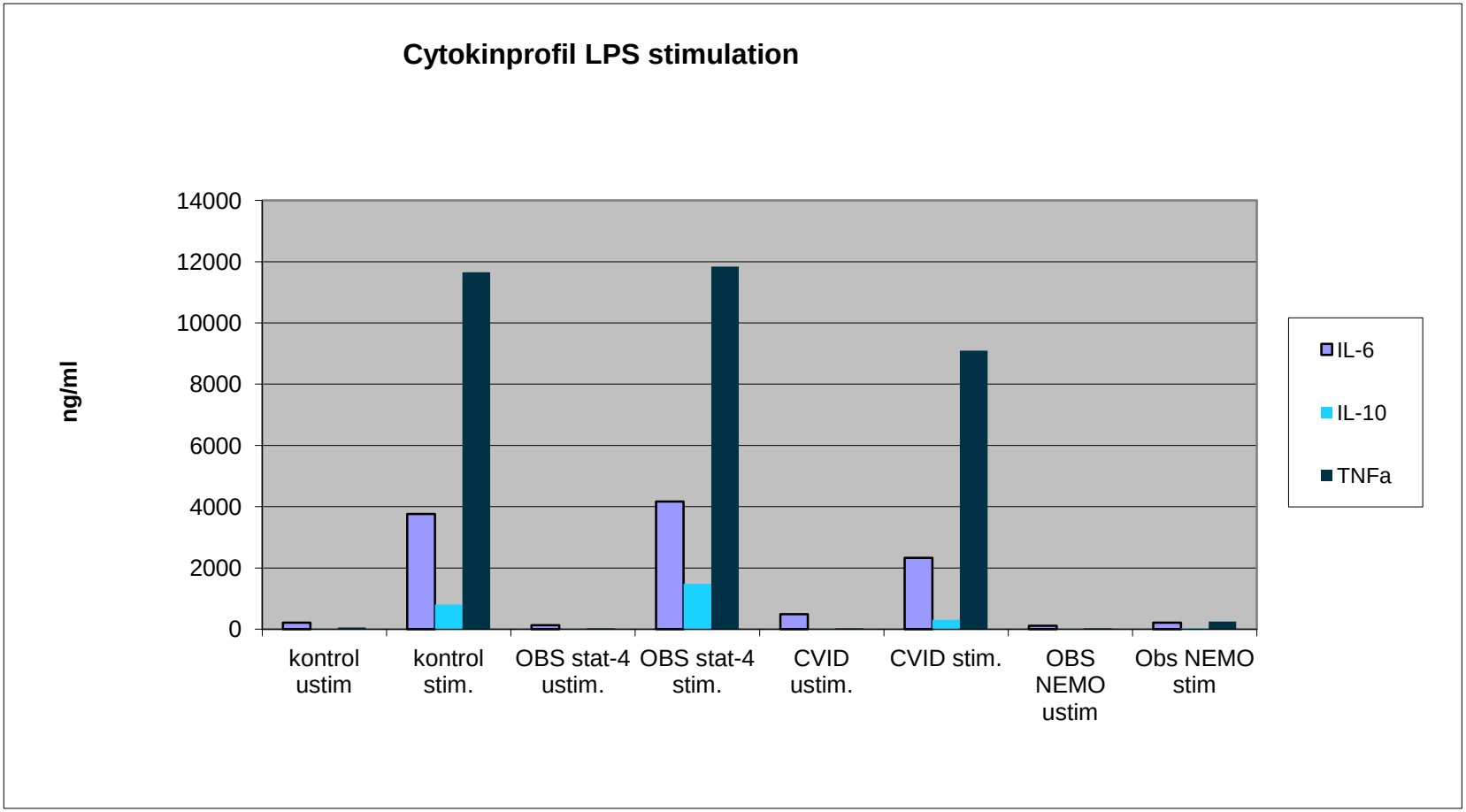
Takeishi et al. 2009

Evaluating TLR Response



Von Bernuth, et al, Pediatrics, 2006

Inflammatory pathway function



Genetic testing

- Genetic testing is emerging as an important diagnostic test in resolving possible PID
  - Human genome 3.3x109 bases
  - Frederick Sanger developed DNA sequencing methods in 1977
  - Cost of Sanger sequencing :$2400 / 10*6 bases
  - Next generation sequencing 0.1-1$ /10x6 bases

- Test of prognostic and diagnostic value often used:
  - TACI in CVID, 22q11.2 deletion, CGD :*CYBB, CYBA*, , *NCF1*, *NCF2,* Somatic hypermutation in B-cells
  - Mutations in SCID, Autoinflammatory syndroms –TNFRSF1A

- Genotype vs. phenotype
- Difficult to address the importance of a new mutation found
- All individuals have a SNPs at 1/1000 bases
- Multigene diseases and gene - gene interactions – epistasis,

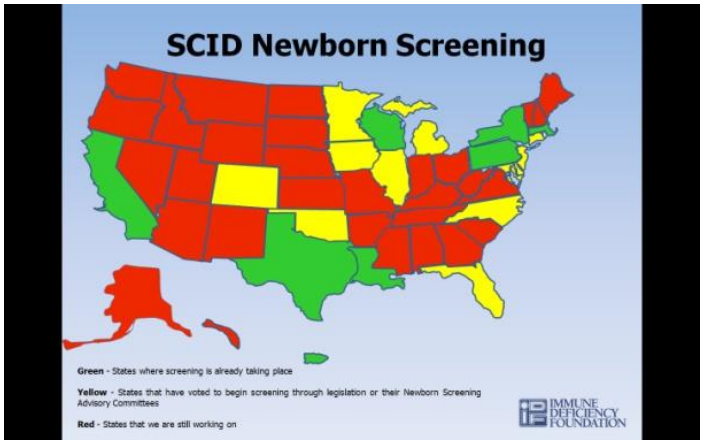
Sequencing for the Diagnosis of Mendelian Disorders. Yang et al. NEJM 2013

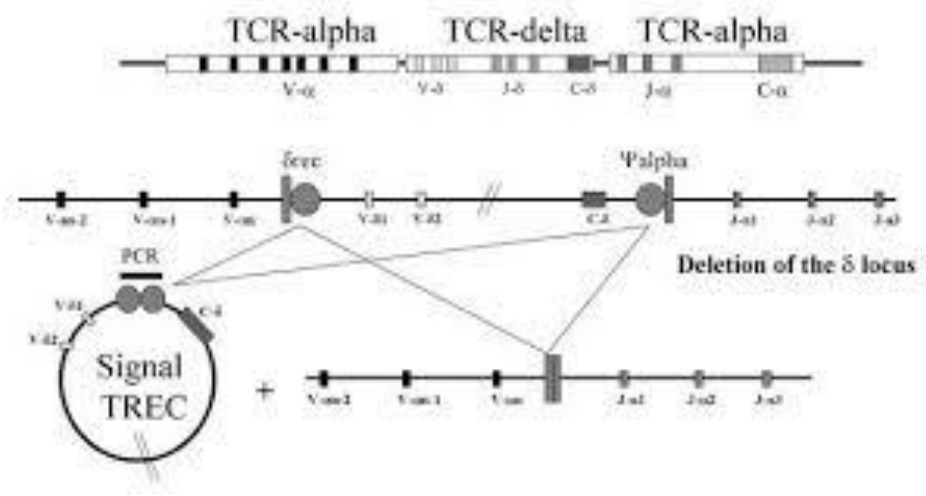
Genetic testing – genetic screening ?

SCID screening from PKU cards

Established f:1/60.000 newborn

False positives ?





www.biomedcentral.com/.../1472-6750-3-18-1-l.jpg

Thank you !