

## Investigating children with recurrent infections

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JL

- 4 year old boy
- recurrent febrile episodes over the past 18 months
- “Group A Strep” x2 (clinical diagnosis), recurrent AOM and chesty cough
- Fever spiking to 39.5°C to 40°C within a few hours of onset

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## What do you want to know about the fever episodes?

- How many “infections” are OK in 1 year?
- Are the siblings and parents getting ill as well?
- Are the fevers and/or other symptoms real?
  - have the parents kept a diary?
- Is there any pattern?
- How many antibiotic courses are OK?
  - were they really needed? proof?
- Specific pathogens? (fungal, bacterial, viral)
- Growing and thriving?
- Where is the family from?
- Family history of immunodeficiency

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## Family history of immunodeficiency: what do you ask?

- early infant death/ still birth
- death due to infections/unknown cause
- consanguinity
- infections/lung conditions/skin/nail
- repeat FHx (don't always receive all info first time round)
  
- think about global epidemiology of HIV and travel hx

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## Which children with recurrent infections need investigation and/or referral?

- Age is important (eg recurrent sinobacterial infections associated with physiological transient hypogammaglobulinaemia of infancy)
- FHx immunodeficiency
- Single infection with unusual/opportunistic organism
- Single infection that is atypically severe or at an atypical age
- Recurrent minor bacterial infections
- More than one episode of serious bacterial infection
- Recurrent infections associated with severe allergy/autoimmune disorder

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## Could this be a immunodeficiency?

- If yes, is it primary (3-4/100000 in UK) or secondary?
- If yes, severe/moderate/mild?
- If decide needs investigation:
  - HIV (needed or not? **What organisms would suggest HIV or T cell deficiencies?**)
  - full blood count and film
  - T-B-NK flow cytometry
  - IgG, A, M (and E if associated symptoms eg severe eczema) to rule out major severe immunodeficiencies as a non-immunologist

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**Case A:  
Autoimmune Polyglandular  
Endocrinopathy-Candidiasis-Ectodermal  
Dystrophy (APECED)  
– a family history?**

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**Clinical Summary**

- X and Y brothers presenting with recurrent chest infections and oral candidiasis from age 9 months.
  
- Mother Z has long term sequelae of recurrent lower respiratory tract infections and chronic candidiasis
  - Bronchiectasis
  - Oesophageal strictureIn addition Z had thyroid disease

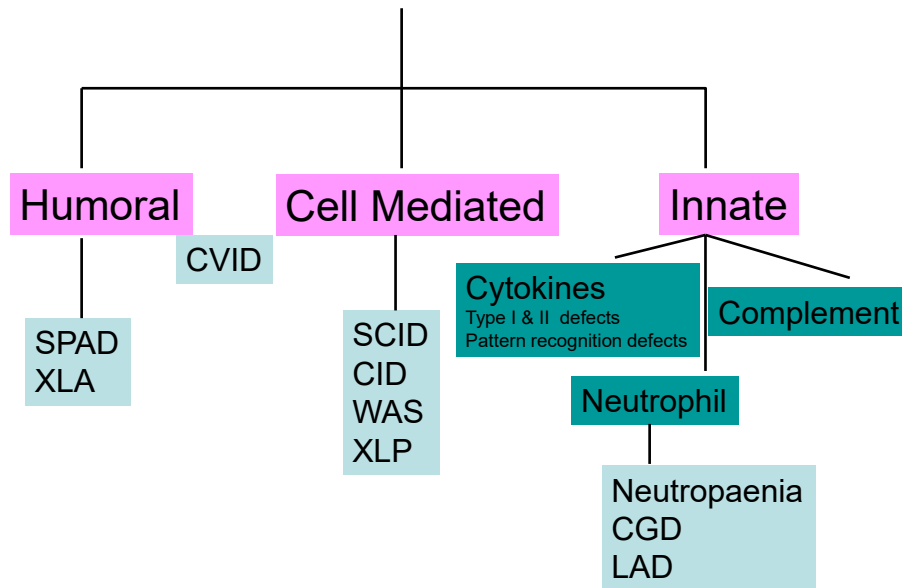
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## Clinical Summary 2

- Suggestive history of inherited immunological deficiency or dysregulation
- Simultaneous referral of Mum from Adult Respiratory Medicine to Clinical Immunology  
and
- Children from Paediatric Endocrinology to Paediatric Infectious Disease and Immunology

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## Primary Immune Deficiency



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## General Investigation

- FBC
    - Hb
    - Total WCC
    - Differential WCC
    - Platelet
      - Number
      - Volume
    - Blood Film
  - Micro
    - Everything!
    - Atypical pathogens
    - Persisting typicals
- X and Y**  
**NORMAL**

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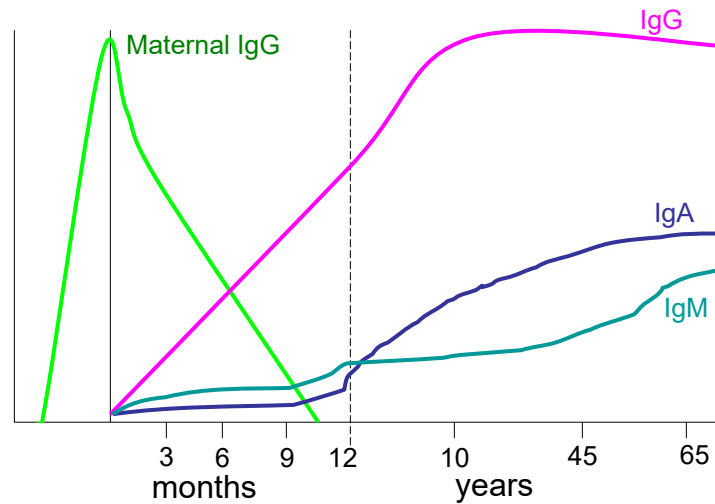
## Immunoglobulins

***Going to transfuse? Store Serum!***

- Ig Levels
    - Ig G, A, M
    - Widely available
    - Age specific normal ranges
    - A and M useful < 6 months if tests sensitive enough
    - IgE if relevant history
  - ~~IgG subclasses~~ **not generally useful**
    - Not if < 2y
    - Not if total IgG low
    - Use functional antibodies instead
- X and Y**  
**NORMAL**

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## Age related Changes in Serum Igs



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## Specific antibody responses

- **PROTEIN**
  - Diphtheria and Tetanus
  - Good immunogens
- **CONJUGATES**
  - Hib
  - Reasonable immunogen
  - Prevenar serotypes
- **POLYSACCHARIDE**
  - Pneumococcal
  - Carbohydrate antigen
  - Poor immunogen if < 2y

X and Y

**NORMAL**

	HH	JH	
HiB	9	1.42	ug/ml
Pneum.	76.1	17.3	IU/ml
Tetanus	0.3	0.12	IU/ml

*N.B. don't check vaccine antibody responses from approx. 6-7 months until 3-4 weeks after the 1 year boosters.*

*Why not?*

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## Specific antibody responses

- **PROTEIN**
  - Diphtheria and **Tetanus**
  - Good immunogens
- **CONJUGATES**
  - **Hib**
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X and Y

**NORMAL**

	X	Y	
HiB	9	1.42	ug/ml
Pneum.	76.1	17.3	IU/ml
Tetanus	0.3	0.12	IU/ml

**2018 *Salmonella typhi* vaccine (in specialist clinic only)**

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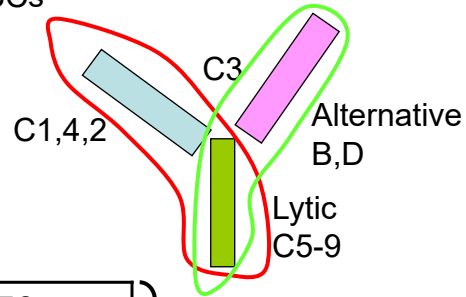
## Complement Tests

- Quantitative
  - C3, C4
  - MBL (not very clinically useful)
  - Do not reflect activity
- Functional
  - Assess integrity of complete pathway
    - Classical - CH50 / CH100 / THC (C1-C9)
    - Alternate - AP50 (Factor B/H and P)

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## CH50/AP50

- Ability of complement in the patients serum to lyse animal red cells
  - CH50 sensitised sheep RBCs
  - AP50 Rabbit RBCs
- Must be a fresh sample



<i>Defect</i>	CH50	AP50
Classical	low	normal
Alternative	normal	low
Terminal	low	low

} then measure individual components

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## Complement Test Results

- C3, C4
  - CH50
- } X and Y  
NORMAL

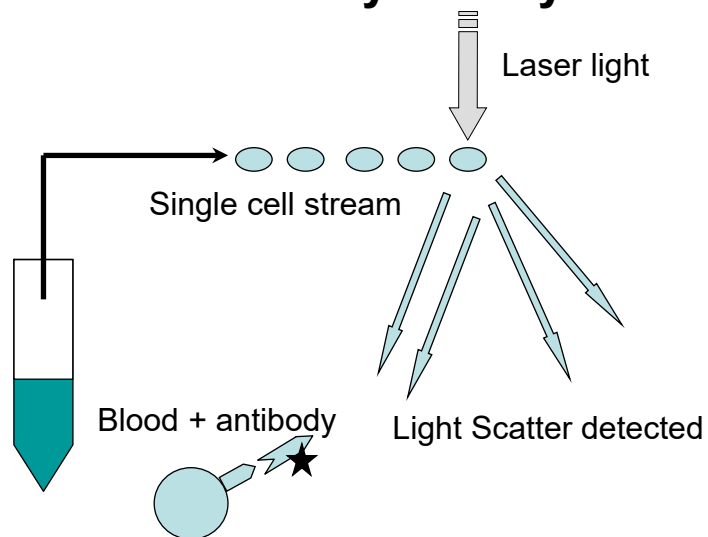
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## Cells

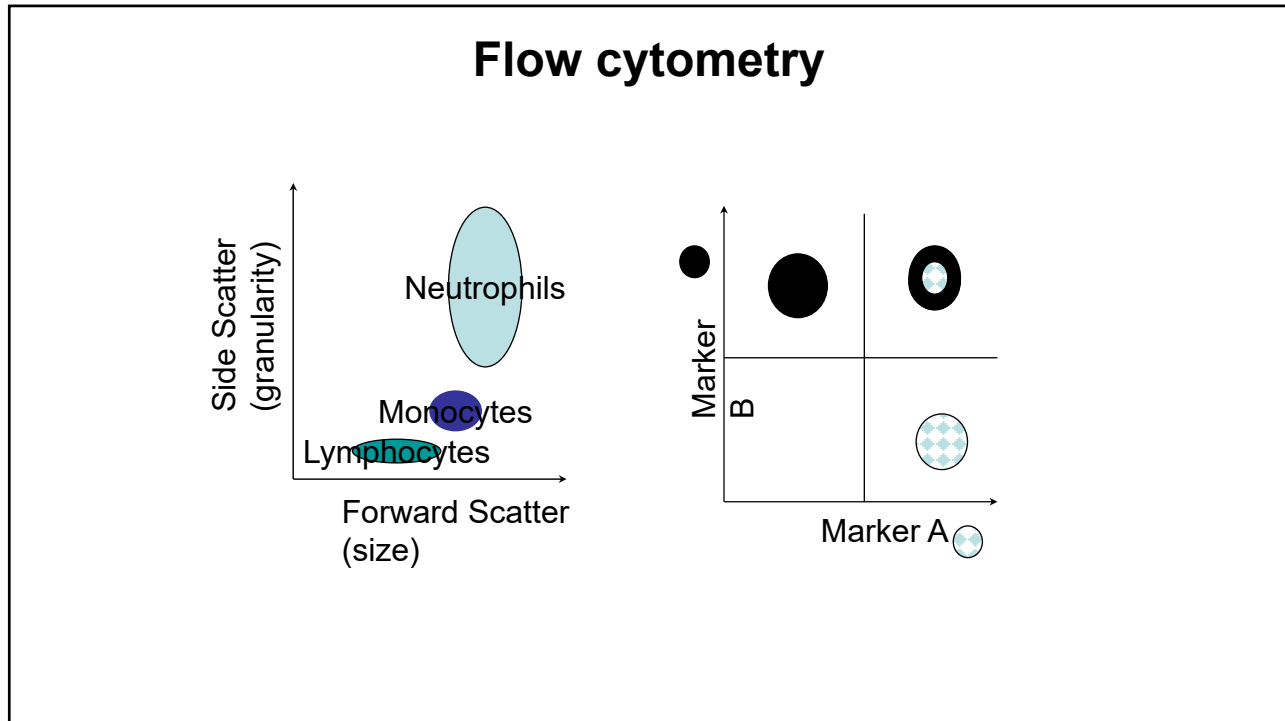
- Neutrophils
- Lymphocytes

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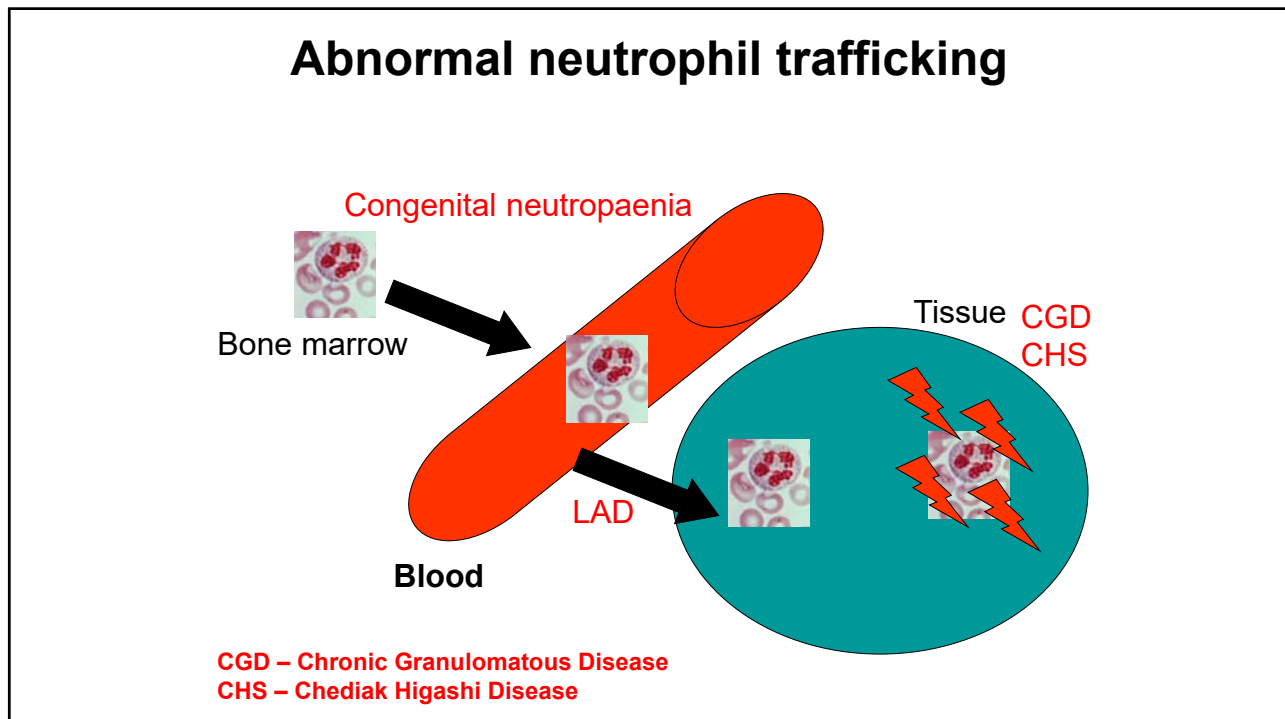
## How cells are looked at in the lab - Flow Cytometry



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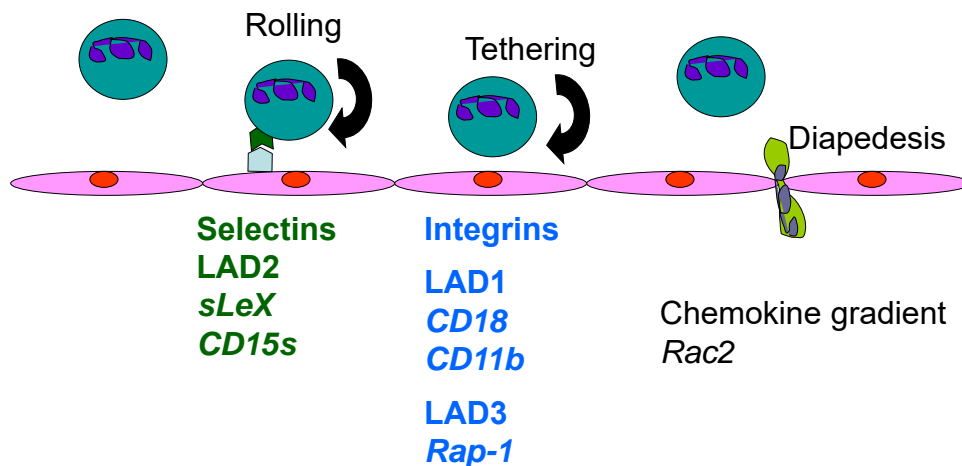
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## Can you name any organisms that might make you think about CGD?

- Bacteria (particularly those that are catalase-positive)
  - *Staphylococcus aureus*
  - *Serratia marcescens*
  - *Listeria* species
  - *E. coli*
  - *Klebsiella* species
  - *Pseudomonas cepacia* (*Burkholderia cepacian*).
  - *Nocardia*.
- Fungi
  - *Aspergillus* species.
  - *Candida* species.

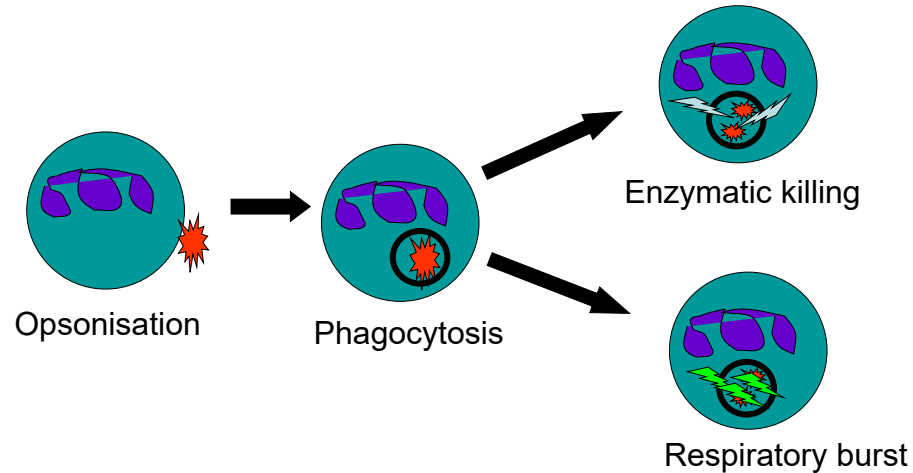
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## Neutrophil migration defect Leukocyte Adhesion Defects (LAD)



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## Neutrophil Killing



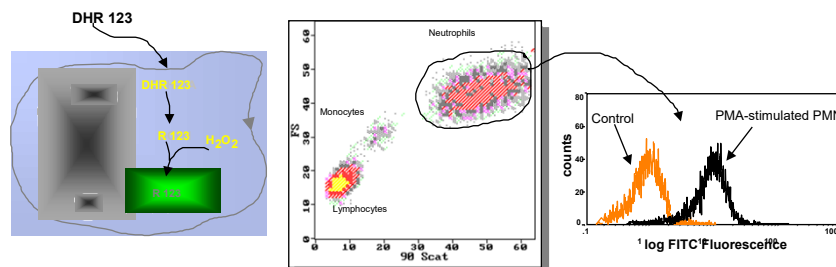
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## Oxidative burst - NBT

- Cells are stimulated
- Determination of intracellular oxidative capacity of cell

### **How the assay works:**

- Cells are stimulated with PBS/FMLP/E.coli/PMA
- A non-fluorescent compound is added
- Oxidation causes the compound to fluoresce
- Fluorescence represents cells undergoing an oxidative reaction



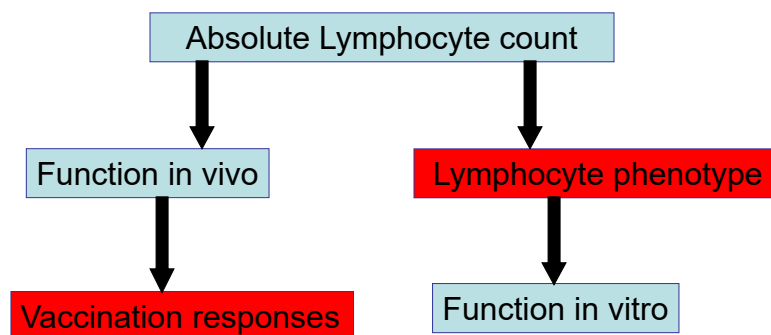
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## Lymphocytes

- Total lymphocyte count
- Lymphocyte subpopulations
  - Immunophenotyping (**easy to do**)
- Functional assays (**hard to do, specialist clinic only**)
  - Mitogen (PHA)
  - CD3
  - Recall antigens (Candida, PPD)

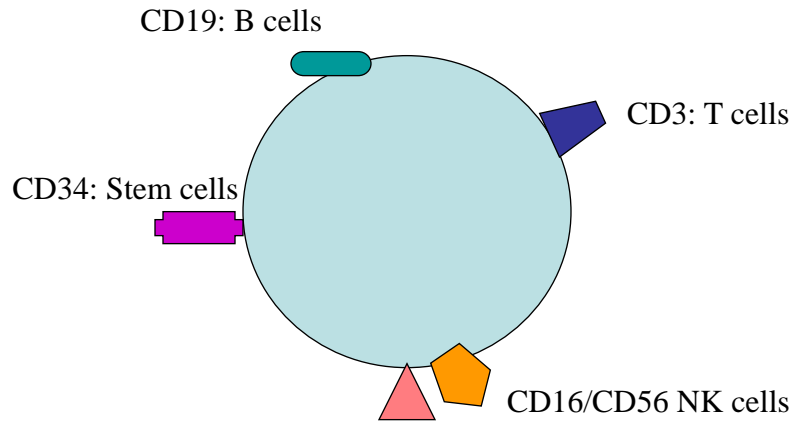
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## Lymphocyte investigations



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## Immunophenotyping: definition of cells



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## Cell markers

CD3	All T cells
CD4	Helper T cells
CD8	Cytotoxic T cells
CD19	B cells
CD16/56	Natural killer cells
DR	MHC II, normally expressed on B cells. A marker of activated T cells
CD45	Exists as RA and RO. Distinguishes T cells that have not met antigen from those which have
Gamma delta	An uncommon type of T cell, usually very low numbers

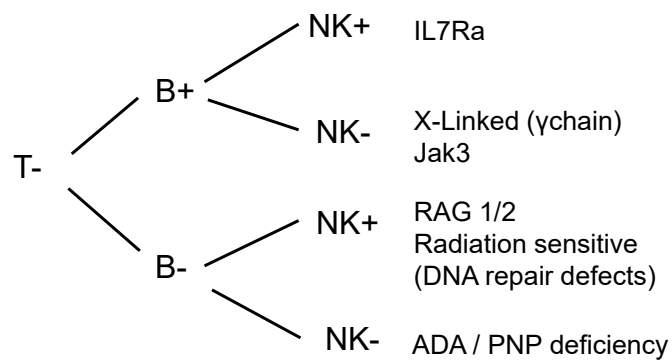
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## Interpretation

- Need FBC (dual platform)
- Technically valid
  - $T+B+NK = 100\%$
- Normal numbers
- Normal distribution

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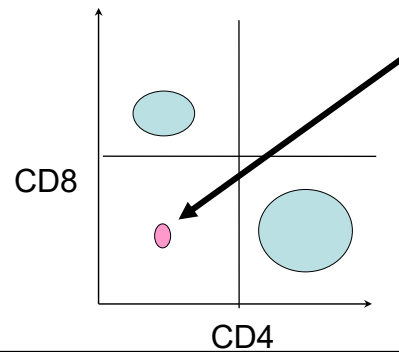
## State of the art **2005 (before cheap molecular tests):** Using Lymphocyte Phenotype to define type of SCID



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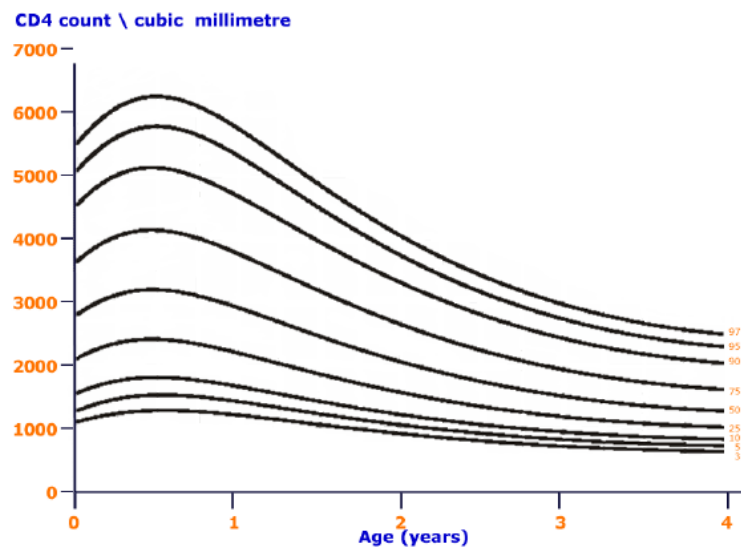
## Autoimmune lymphoproliferative syndrome (ALPS)

- Lymphoproliferation
  - Autoimmune disorders (haemolytic anaemia and thrombocytopenia)
  - Tiredness/pallor
  - +/- recurrent infections
- 
- Apoptotic defects – most commonly FAS gene error
  - Most have Increased numbers of Alpha Beta T cells in the circulation LACKING CD4 or CD8
    - “double negative T cells”



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## Young children have high absolute lymphocyte counts!



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## Young children have high absolute lymphocyte counts!

J ALLERGY CLIN IMMUNOL  
VOLUME 112, NUMBER 5

**TABLE III.** Cell-subset counts of peripheral blood lymphocytes in healthy children: Distribution

Subset	N	0-3 mo	3-6 mo	6-12 mo	1-2 yr	2-6 yr
WBC	800	10.60 (7.20-18.00)	9.20 (6.70-14.00)	9.10 (6.40-13.00)	8.80 (6.40-12.00)	7.10 (5.20-11.00)
Lymphocytes	800	5.40 (3.40-7.60)	6.30 (3.90-9.00)	5.90 (3.40-9.00)	5.50 (3.60-8.90)	3.60 (2.30-5.40)
3	699	3.68 (2.50-5.50)	3.93 (2.50-5.60)	3.93 (1.90-5.90)	3.55 (2.10-6.20)	2.39 (1.40-3.70)
19	699	0.75 (0.30-2.00)	1.35 (0.43-3.00)	1.52 (0.61-2.60)	1.51 (0.72-2.60)	0.75 (0.39-1.40)
16/56	770	0.42 (0.17-1.10)	0.42 (0.17-0.83)	0.40 (0.16-0.95)	0.36 (0.18-0.92)	0.30 (0.13-0.72)
4	699	2.61 (1.60-4.00)	2.85 (1.80-4.00)	2.67 (1.40-4.30)	2.16 (1.30-3.40)	1.38 (0.70-2.20)
8	699	0.98	1.05	1.04	1.04	0.84

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## Lymphocyte panel

	X	Y	
<b>CD19 (B cells)</b>	<b>21%</b>	<b>25%</b>	} <b>X and Y NORMAL</b>
CD19+ve absolute nos.	380	1080	
<b>CD3+ve (T cells)</b>	<b>61%</b>	<b>68%</b>	
CD3+ve CD4+ve	30%	41%	
CD3+ve CD4+ve absolute nos.	540	1760	
CD3+ve CD8+ve	24%	24%	
CD3+ve CD8+ve absolute nos.	430	1030	
CD3+ve absolute nos	1100	2920	
<b>CD3-CD16+CD56 (NK)</b>	<b>16%</b>	<b>4%</b>	
Eosinophils	0.1	1.0	
<b>Lymphocytes</b>	<b>1.8</b>	<b>4.3</b>	
Monocytes	0.5	0.7	
NK cells Abs nos.	290	170	
Neutrophils	3.4	6.1	
White cell count	5.9	12.1	
T-helper-suppressor ratio	1.25	1.71	

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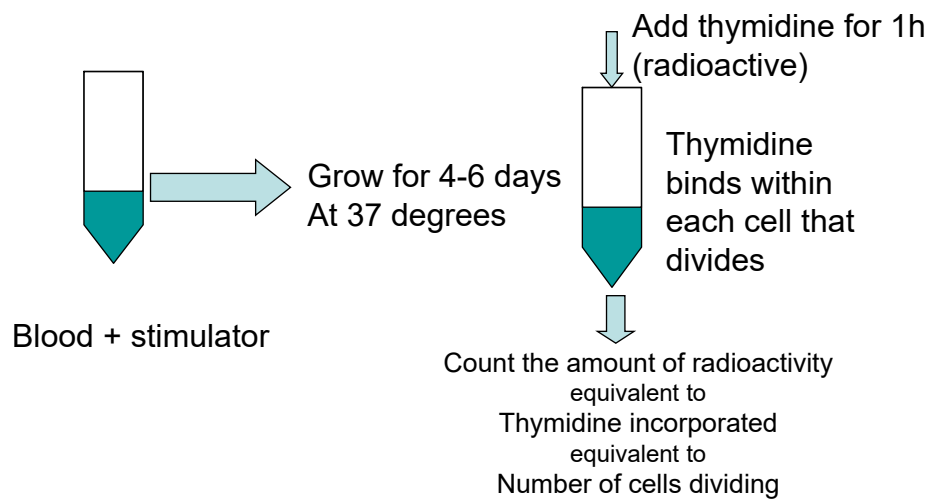
## Proliferation assays

- Functional assay
  - large within/between assay variation
- No External Quality Assurance (EQA)
- Interpretation
  - local knowledge
  - normal ranges?
  - semiquantitative
- Clinical
  - didn't I tell you he was on steroids?

**CLINICAL DETAILS ARE ESSENTIAL FOR CORRECT TEST CHOICE AND INTERPRETATION**  
**Specialist clinic only**

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## Principles of lymphocyte proliferation assays

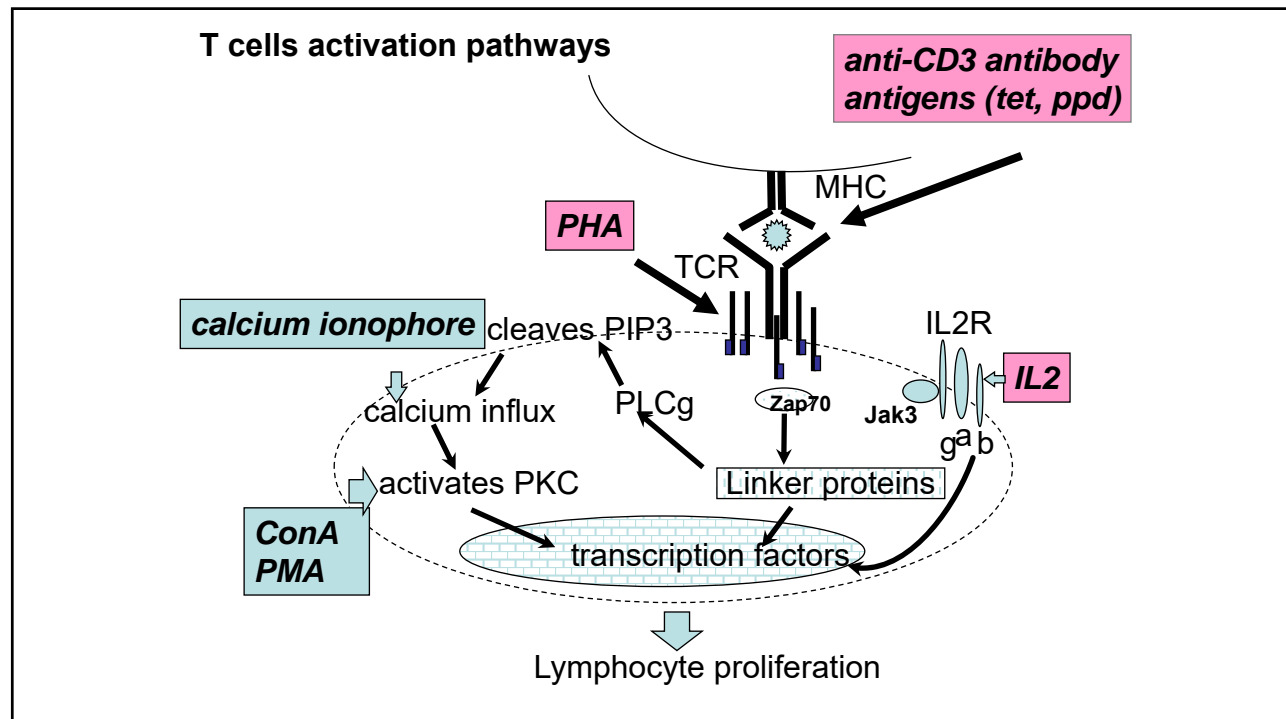


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## Lymphocyte proliferation assays

- **Mitogen**
  - Phytohaemagglutinin (PHA)
    - Plant extract
    - Kicks cells
- **Recall antigen**
  - Candida, PPD, tetanus, measles
- **Third party cells**
  - Mixed lymphocyte culture
- **antiCD3 antibody**
  - Stimulate the T cell receptor directly
- **IL2**
  - A messenger molecule produced by T cells that stimulates their proliferation

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## Proliferation assays

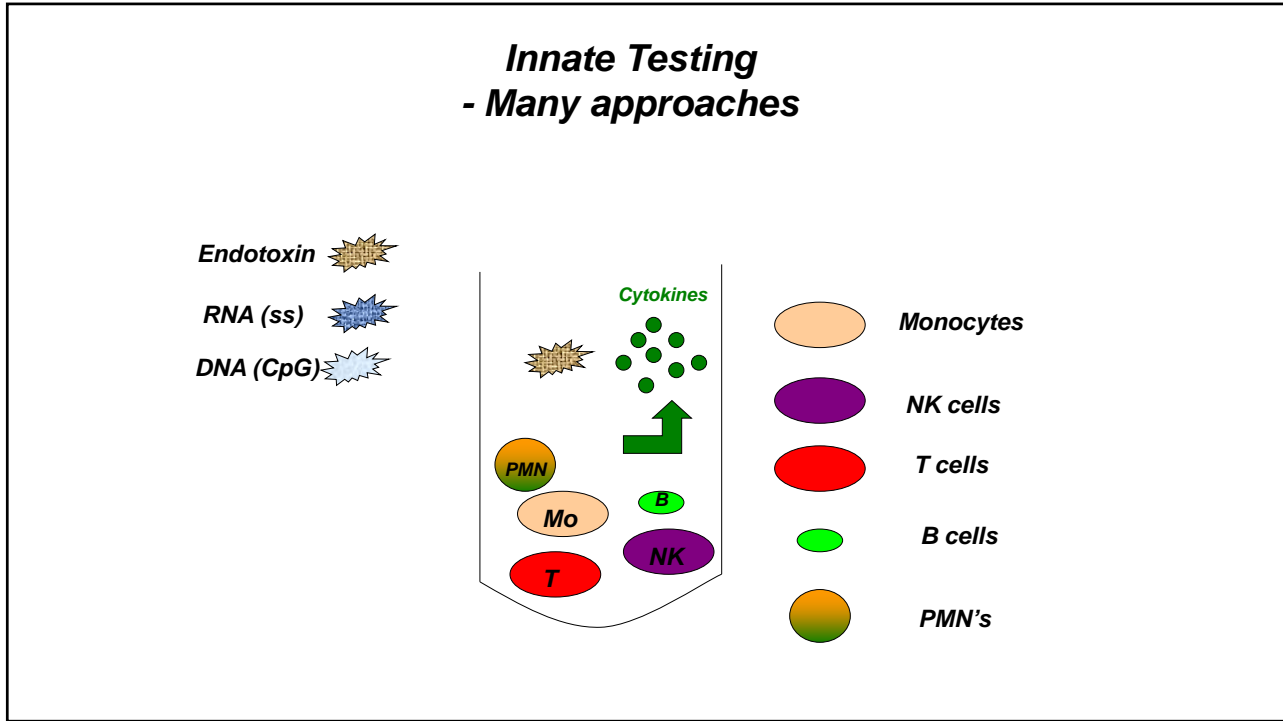
	Whole Blood	Seperated Cells
PHA	x	x
CD3	x	
Candida		x
PPD		x
PHA/IL2		x
MLC		x

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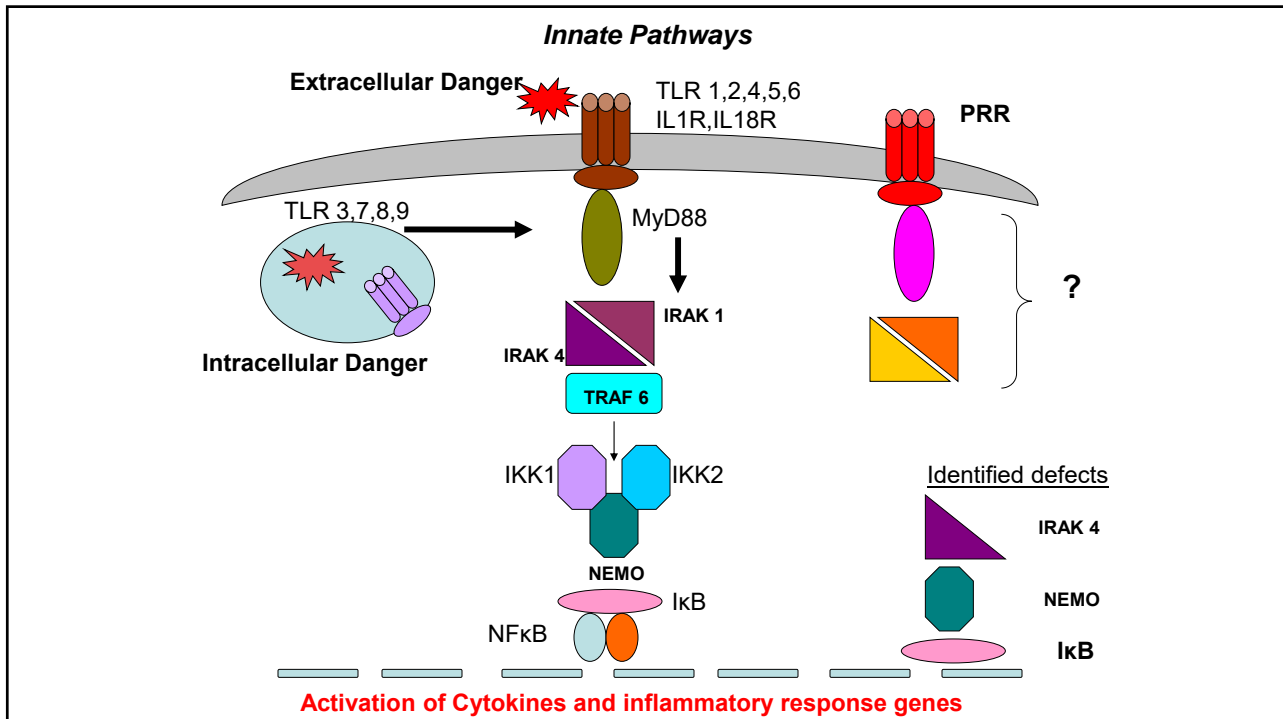
## Alternatives for lymphocyte function

- Cytokine production (sent from specialist clinics)
  - Eg Interferon gamma by ELISA
  - Eg Expression of activation markers
  - Results completely dependant on local assay method
- Molecular genetics (sent from specialist clinics)
  - Targeted gene panels
  - Exome sequencing
  - Whole genome sequencing

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## X, Y and Z

Early onset recurrent upper/lower respiratory infections and bronchiectasis + chronic mucocutaneous candidiasis

*Innate Screen 1*

Whole Blood	LPS/IL12	BCG/IL12	IL18/IL12	Mitogen	LPS/IFN $\gamma$
IFN $\gamma$					
IL12 (p70)					

*Innate Screen 2*

Whole Blood	Pam <sub>3</sub> CSK <sub>4</sub> (TLR 1/2)	Pam <sub>2</sub> CSK <sub>4</sub> (TLR 2/6)	LTA (TLR 2)	LPS (TLR 4)	Imiquimod (TLR 7/8)	TNF- $\alpha$	Zymosan	Candida
IL-6								
TNF- $\alpha$								

## Interpretation

- 1) Normal Innate screen 1 for IFN $\gamma$  and IL12
- 2) Normal innate screen 2 apart from specific candida defect

Working diagnosis

Pathogen specific defect - ? Defect in Dectin/Card9/Bc110 pathway

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## X, Y and Z: Laboratory Summary

- Selective disorder for Candida sensing consistent with clinical presentation
- Contrasting phenotype to other families with repeated bacterial sepsis and TOLL pathway abnormalities
- Distinctive pathogen sensing pathway of innate immunity
  - Candidate genes
  - Functional microarray

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## **Aim of clinical management?**

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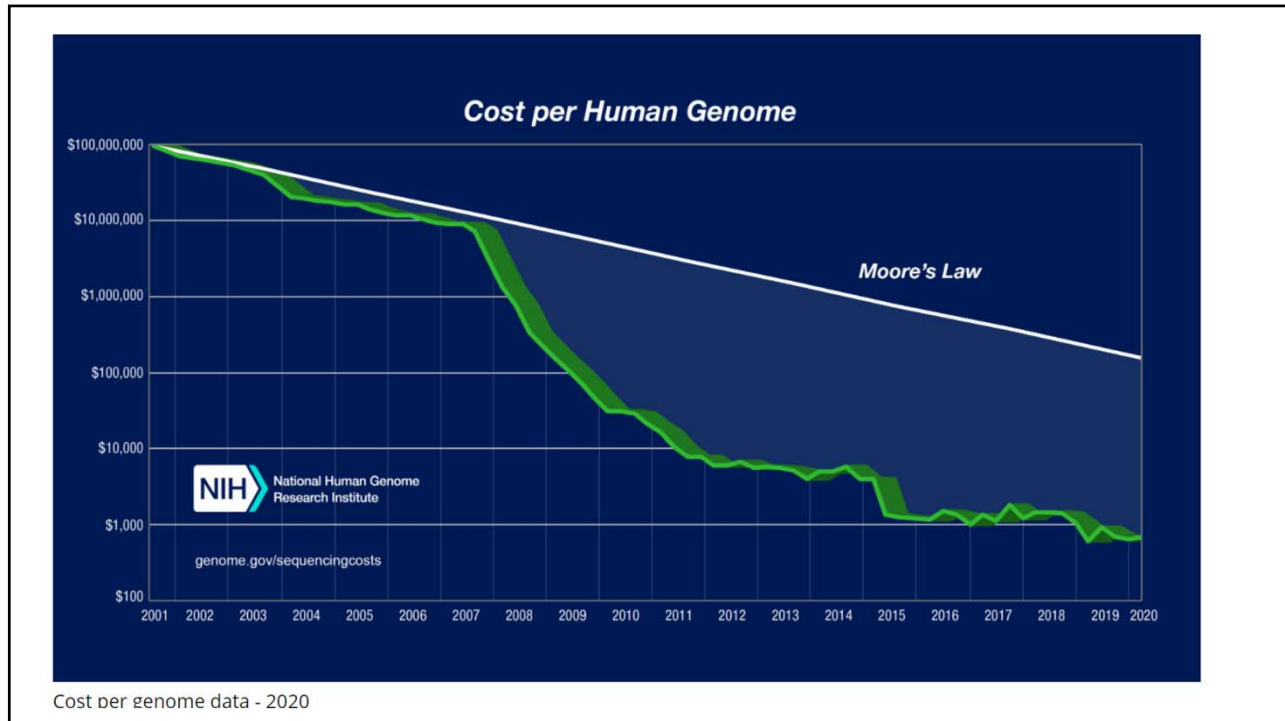
## **Clinical Summary – X and Y**

Clinical aim:

Prevent consequences of recurrent LRTI and candidiasis

- Early “aggressive” antibiotic therapy
  - regular clinical review
  - oral fluconazole if on any antibiotics or at first sign or oral candidiasis
- Low threshold for inpatient therapy

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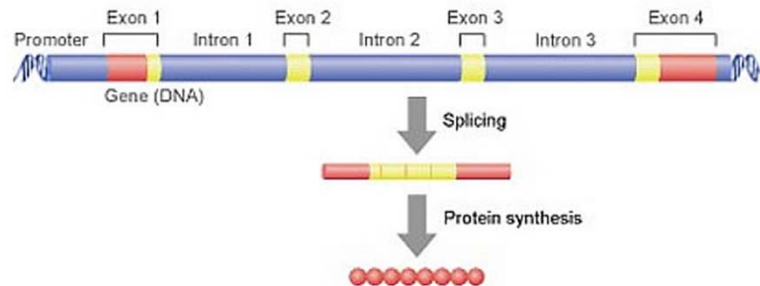
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## Genome versus Exome

<ul style="list-style-type: none"> <li>~ 3,200 Mb (almost) entire genome</li> <li>More expensive             <ul style="list-style-type: none"> <li>~100 x more sequencing required</li> <li>File sizes ~100x larger</li> </ul> </li> <li>Interpretation</li> <li>Will become routine</li> </ul>	<ul style="list-style-type: none"> <li>~50 Mb targeted to coding regions (varies by kit)</li> <li>~85 % of disease causing variants.</li> <li>Requires additional sample preparation steps</li> <li>Cheaper, quicker</li> </ul>
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## Exome Sequencing



- *Exome represents ~1% of human genome*
- *Fast, cost effective (but now sequencing of entire genomes is more affordable)*

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## Exome result and ongoing management – X and Y

- STAT1 gain of function mutation (T821A / R274q)
- Developed highly resistant *Tinea tonsurans* and required long term itraconazole
- Generally grown out of respiratory infections but low threshold for antibiotics

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## Diagnosing a primary immunodeficiency (PID)

- Pattern recognition: large group of rare disorders!



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## Primary Immune Deficiency Disorders

### **Combined T and B-cell immunodeficiencies**

*Simpler papers (click to open):*

### **Well defined syndromes with immunodeficiency**

DNA repair defects

Primitive thrombocytopenia

Immune-osseous dysplasias

Hyper-IgE syndromes

Dyskeratosis congenital

### **Antibody deficiencies**

### **Diseases of immune dysregulation**

Familial Hemophagocytic Lymphohistiocytosis Syndrome

*Full classification papers (click to open):*

Syndromes with autoimmunity

Immuno-deficiency with hypopigmentation

X-linked lymphoproliferative syndrome

### **Defects of phagocyte number and function**

Defects in innate immunity

Auto-inflammatory disorders

Complement deficiencies

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Classification and examples	Clinical presentation
<b>Disorders of adaptive immunity</b>	
<i>T-cell (cellular) immunodeficiency</i>	
• IFN- $\gamma$ /IL-12	Atypical mycobacterial and salmonella infections
• AIRE mutations	Mucocutaneous candidiasis (thrush) and autoimmune endocrinopathy
<i>B-cell (antibody-mediated) immunodeficiency</i>	
• XLA	
• CVID	
• Selective IgA deficiency	Recurrent sinopulmonary infections with encapsulated bacteria
• Specific antibody deficiency	Autoimmune disease and increased risk of malignancy in CVID
• IgG subclass deficiency	
<b>CID</b>	
• Wiskott-Aldrich syndrome	Thrombocytopenia with bleeding and bruising; eczema; recurrent bacterial and viral infections; autoimmune disease
• Ataxia telangiectasia	Chronic sinopulmonary disease; cerebellar ataxia (difficulty with control of movement); small, dilated blood vessels of the eyes and skin; malignancy
• DiGeorge syndrome	Hypoparathyroidism; seizures; cardiac abnormalities; abnormal facies; infection
• Hyper IgE syndrome	Chronic dermatitis; recurrent, severe lung infections; skin infections; bone fragility; failure to shed primary teeth
• SCID	
<b>T<sup>-</sup>, B<sup>+</sup></b>	
• $\gamma$ c deficiency	
• JAK3 deficiency	Severe, recurrent opportunistic infections; failure to thrive; diarrhea; rash
<b>T<sup>-</sup>, B<sup>-</sup></b>	
• ADA deficiency	
• RAG 1/2 deficiency	

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6157160/>

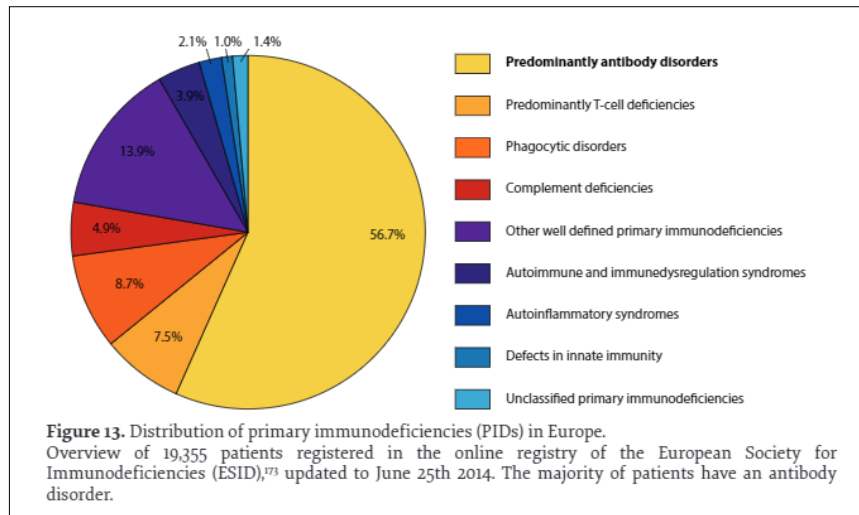
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<b>Disorders of innate immunity</b>	
<i>Phagocyte defects</i>	
• Chronic granulomatous disease	Severe infection; abscesses with granuloma formation
• Leukocyte adhesion deficiency	Recurrent, severe bacterial infections; poor wound healing; delayed separation of the umbilical cord
<i>Complement defects</i>	
• Deficiency in early complement pathway components (C1q, C1r, C2, C4)	SLE-like syndrome, rheumatoid disease, multiple autoimmune diseases, infections
• Deficiency in late complement pathway components (C5, C6, C7, C8, C9)	Neisserial infections, SLE-like syndrome
• C3 and regulatory components	Recurrent infections with encapsulated bacteria
<b>Disorders of immune dysregulation</b>	
• HLH	Fever, splenomegaly, cytopenia, rash
• ALPS	Splenomegaly, adenopathy
• IPEX	Autoimmune enteritis, early onset diabetes, thyroiditis, hemolytic anemia, thrombocytopenia, eczema
• APECED	Autoimmunity affecting parathyroid, adrenal, other endocrine organs; candidiasis; dental enamel hypoplasia

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6157160/>

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## ESID registry: majority antibody disorders



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## Routine laboratory tests

- FBC, Film
- Microbiology
- Immunoglobulins
- Functional antibody responses
- Complement
- T, B, NK Phenotyping
- T and B Cell Function
- Neutrophil Function

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## 10 Warning Signs of Primary Immunodeficiency

<b>1</b>	Eight or more new ear infections within 1 year.	<b>6</b>	Recurrent, deep skin or organ abscesses.
<b>2</b>	Two or more serious sinus infections within 1 year.	<b>7</b>	Persistent thrush in mouth or elsewhere on skin, after age 1.
<b>3</b>	Two or more months on antibiotics with little effect.	<b>8</b>	Need for intravenous antibiotics to clear infections.
<b>4</b>	Two or more pneumonias within 1 year.	<b>9</b>	Two or more deep-seated infections.
<b>5</b>	Failure of an infant to gain weight or grow normally.	<b>10</b>	A family history of Primary Immunodeficiency.

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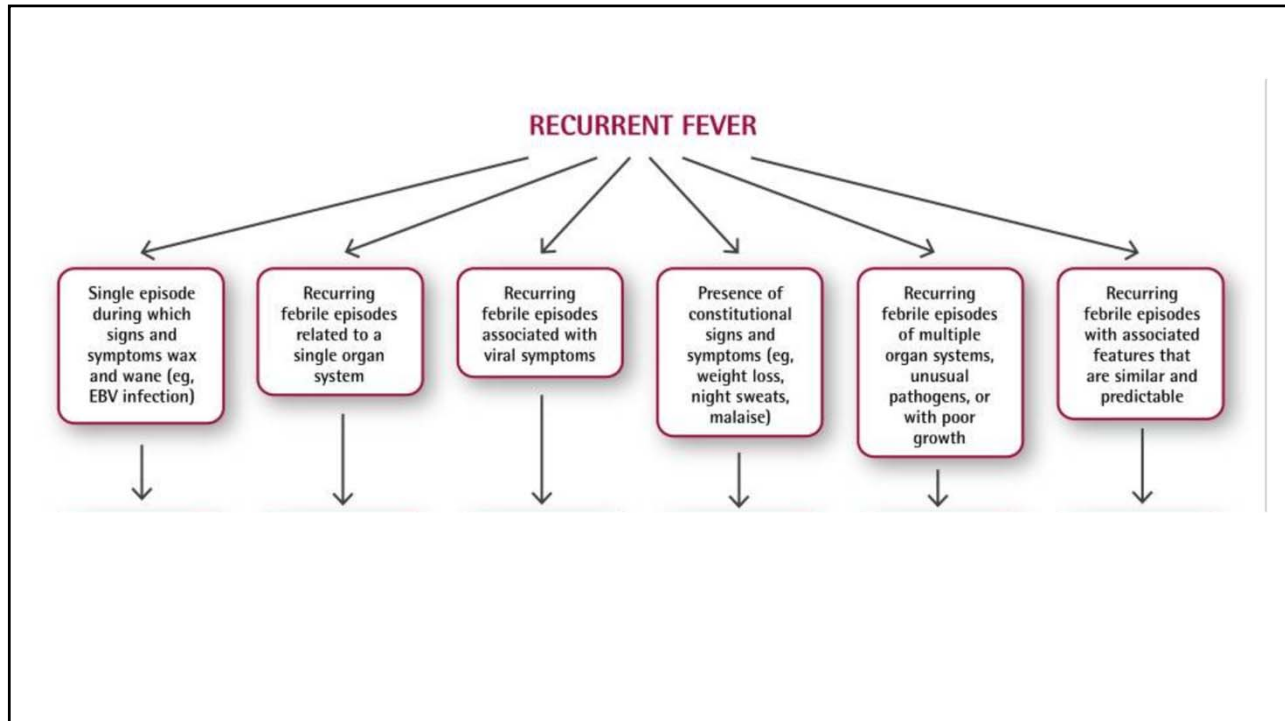
JL

- 4 year old boy
- recurrent febrile episodes over the past 18 months
- Fever spiking to 39.5°C to 40°C within a few hours of onset.
- Episodes recur every 3 to 4 weeks, last 3-5 days
- associated with sore throat, tender cervical lymphadenopathy, occasional oral ulcers
- no rash, eye, respiratory, or musculoskeletal symptoms

- During episode:  
WBC 10.6x10<sup>9</sup>/L, Hb 112 g/L, plts 364x10<sup>9</sup>/L, CRP 90

Referred by ENT

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**What features make you think about a periodic fever syndrome?**

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## Periodic fevers

- Recurring episodes (with either strict periodicity or irregular intervals)
- Lasting days to weeks
- Fever is the cardinal feature and other associated features are similar and predictable
- Intervening intervals of weeks to months, characterized by complete well-being

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## PFAPA

- Onset of disease in early childhood, generally before the age of 5 yrs
- Regularly recurring abrupt episodes of fever lasting approximately 5 days associated with constitutional symptoms and both of the following:
  - aphthous ulceration or pharyngitis (with or without cervical adenitis) in the absence of other signs of respiratory tract infection
  - acute inflammatory markers such as leukocytosis or elevated CRP
- Completely asymptomatic interval periods, benign long-term course, normal growth parameters, and the distinct absence of sequelae
- **Exclusion of cyclic neutropenia**, other episodic syndromes (FMF, hyper-IgD syndrome, TRAPS, Behçet syndrome) by family history and the absence of typical clinical features and laboratory markers
- Absence of clinical and laboratory evidence for immunodeficiency, autoimmune disease, or chronic infection

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## Summary

- Could it be immunodeficiency?
  - History
  - Basic investigations including microbiology
  
- Recurrent fever approach
  - Pattern recognition
  - Think about what tests are needed and when
  - Don't forget fabricated symptoms should always be at the back of your mind