Lab Diagnosis of Immune Defects:

Why Blood is thicker than Water

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Program in Infectious Diseases and Immunity in Global Health

Disclosures:

- **CSL Behring Canada**: Advisory board consultant; Speaker; Research grant
- **Astellas Canada**: Research Grant

Objectives:

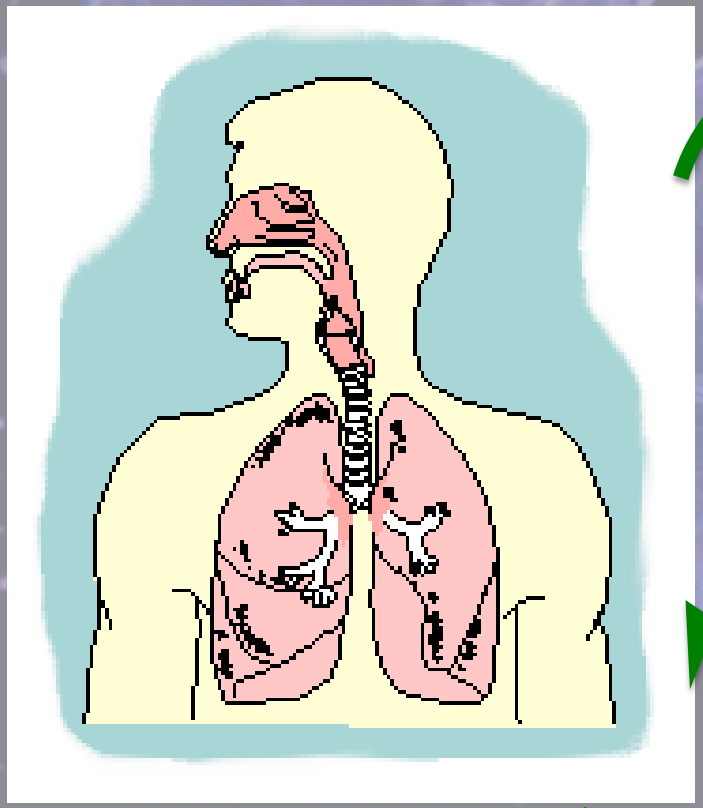
(1) Overview of host defenses and relevant immunological diagnostic tests;

(2) Diagnostic strategies: Tailoring Immunology tests to Infectious disease syndromes;

(3) Genetic testing of the MAcrobe: Targeted vs. Agnostic approaches… and why should we even care

Immunology for the ID/MM: Overview of Host Defenses

How to think about Immunity



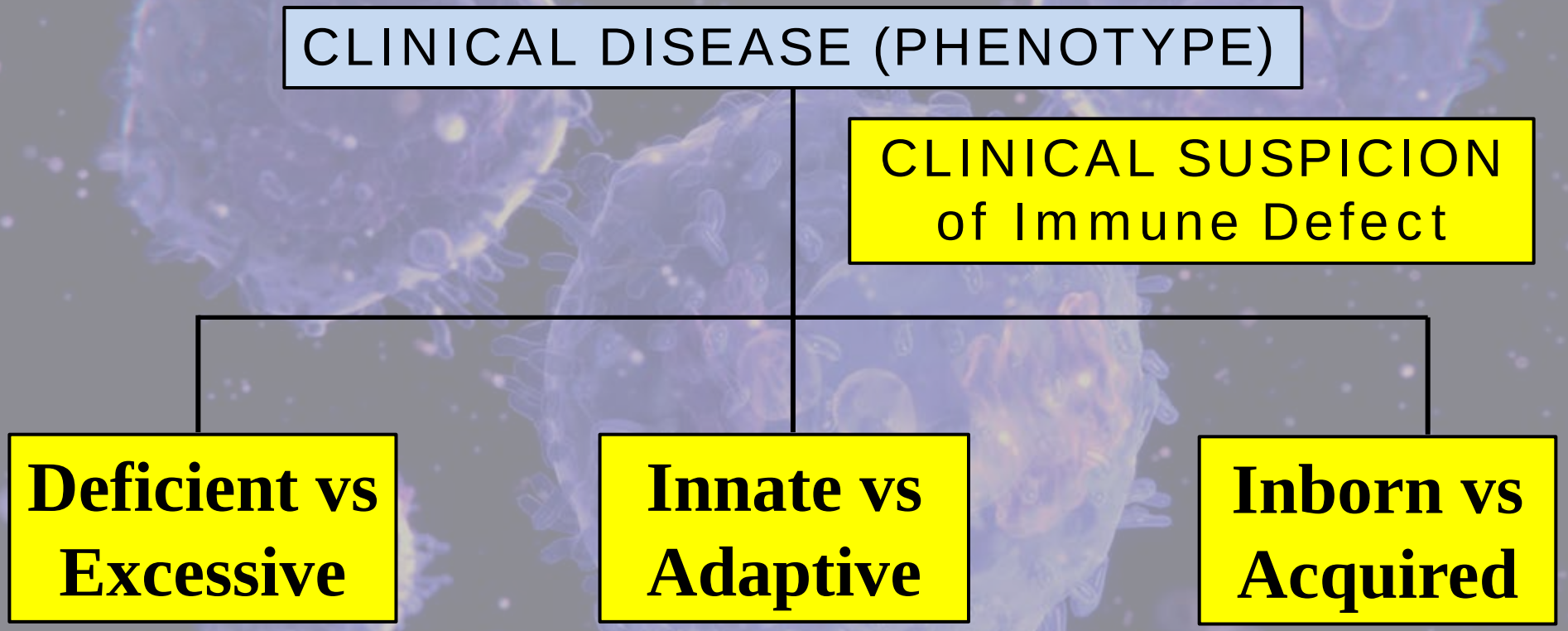
INFECTIOUS DISEASE

MICROBIOLOGY

Anti-Microbial therapy

HOST

How to think about Immunity



Immunity 101: Why aren't you sick?

Immune System

INNATE

- Pre-formed
- Pattern Recognition
- Immediate (seconds-hours)

Effectors:

- Complement
- Neutrophils
- Monocytes
- NK cells

- Epithelium
- Endothelium ?

ADAPTIVE

- Antigen-induced
- Antigen-recognition
- Delayed (days-weeks)

Effectors:

- Antibody
- B & T lymphocytes

Sense & Search
Ignore / Destroy

Immunodeficiency: Inborn vs Acquired

- Primary Immunodeficiency (PID)
- "Primary": disease–causing **genotype**
- PID : inborn error of immunity that results in fatal (or life-threatening) infection
- Expanded phenotype:
  - Atopy
  - Immune Dysregulation (Autoimmunity / Auto-inflammatory)
  - Cell proliferation: Lymphoproliferative, Cancer

Immunodeficiency: Inborn vs Acquired

- Secondary Immunodeficiency (SID)
- "Secondary": not inherent (genetic)

Aging	**Iatrogenic immunosuppression (drugs)**
Malnutrition	**Infections (e.g. HIV, measles)**
Malignancy	**Loss (e.g. Nephrotic; PLE); AutoAbs**

- Defect**S** ; Multifactorial

Microbiology = critical!

PATHOGEN DICTATES IMMUNE DEFECT

VIRUS

RNA:
Picorna
(e.g. Enter
Orthomyxo
Paramyxo

DNA:
Herpes

HPV

PARASITES

minths:
ns

zoa:

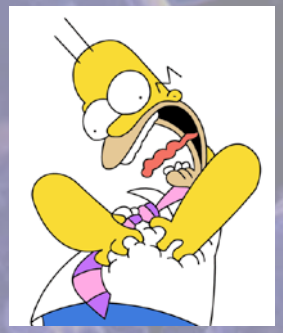
Pneumocystis jiroveci

DIAGNOSTIC STRATEGIES:

Infections, Infections, everywhere

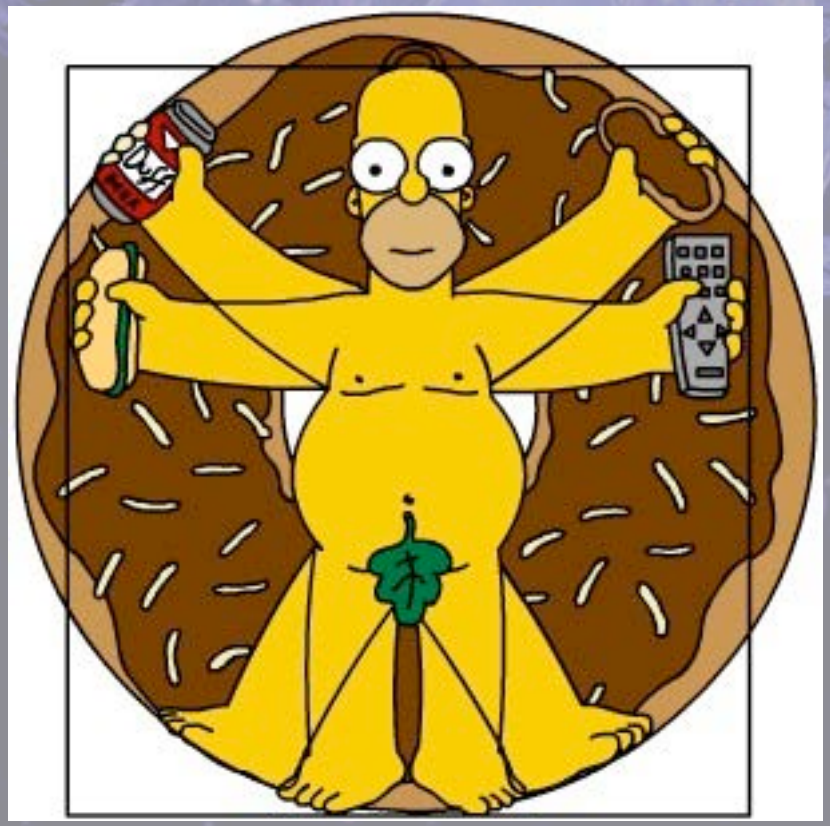
Meningitis/ Encephalitis

Pneumonia



Gastro-enteritis

Cellulitis



Arthritis

UTI

Who's Got a Problem?

10 Warning S of Primary Immunod

Primary Immunodeficiency (PI) causes children and adults to have infections that are unusually hard to cure. 1:500 persons are affected by one of the known Prim If you or someone you know is affected by two or more of the following Warnir physician about the possible presence of an underlying Primary Immunod

1. Four or more new ear infections within one year.
2. Two or more serious sinus infections within one year.
3. a
4. Two or more pneumonias within one year.
5. Failure of an infant to gain weight or grow normally.
6.
7. Persistent thrush in mouth or fungal infection on skin.
8. Need for intravenous antibiotics to clear infections.
9. inf
10. A family history of PI.

Presented as a public service by:

Jeffrey Modell Foundation | Curing PI Worldwide | CDC Funding was made possible in and Prevention (CDC). | National Heart, Lung, and Blood Institute (NHLBI) | National Cancer Institute | PPTA Plasma Protein Therapeutics Association | Baxter BioScience | Biotest | CSL Behring | GRIFOLS

These warning signs were developed by the Jeffrey Modell Foundation Consultation with Primary Immunodeficiency experts is strongly suggest

For information or referrals, contact the Jeffrey Modell Foundation: info

APIQ
Association des Patients Immunodéficients du Québec

10 signes cliniques d'ALERTE d'un déficit immunitaire primaire

Plus de **2 SINUSITES** par an

2 PNEUMONIES par an

2 INFECTIONS SÉVÈRES par an ayant nécessité une hospitalisation

PLUS DE 2 MOIS de traitement aux antibiotiques par an avec peu d'effets

La nécessité d'un traitement aux **ANTIBIOTIQUES** par voie intraveineuse (iv)

Une infection persistante par **CHAMPIGNONS** dans la bouche ou sur la peau

ABCÈS profonds et récurrents sur la peau ou autres organes

Plus de **4 OTITES** par an

RALENTISSEMENT de la **CROISSANCE** chez l'enfant

ANTÉCÉDENTS FAMILIAUX d'un déficit immunitaire primaire

L'un ou l'autre de ces signes cliniques peuvent laisser penser à un déficit immunitaire sans toutefois qu'il en soit systématiquement ainsi.
Veuillez consulter votre médecin pour un diagnostic.

Si vous voulez des renseignements sur l'immunodéficience, consultez notre site Web
www.cipo-apiq.ca

Sans Frais 1 855 561-4563 (Pour laisser un message dans notre boîte vocale)
info@cipo-apiq.ca (Pour nous transmettre vos questions par courriel)

Warning sings Youden index	Warning sings Max. sensitivity
≥8 ear infections within 1 year	
≥2 pneumonias within 1 year	
Failure to grow normally	Failure to grow normally
Failure to thrive	Failure to thrive
	Recurrent deep skin or organ abscesses
	Need for intravenous antibiotics
Lymphopenia	Lymphopenia Hypogammaglobulinemia

Who's Got a Problem?

Table 1
The 6 ESID warning signs for adult primary immunodeficiency diseases

1. Four or more infections requiring antibiotics within 1 year (otitis, bronchitis, sinusitis, pneumonia)
2. Recurring infections or infection requiring prolonged antibiotic therapy
3. Two or more severe bacterial infections (osteomyelitis, meningitis, septicemia, cellulitis)
4. Two or more radiologically proven pneumonia within 3 years
5. Infection with unusual localization or unusual pathogen
6. PID in the family

Abbreviations: ESID, European Society of Immunodeficiency; PID, primary immunodeficiency disease.

Who's Got a Problem?

- **Abnormal frequency of infection**
  - Recurrent Respiratory Tract Infections (RRTIs)
  - Recurrent/recalcitrant warts
- **Abnormal presentation of infections**
  - Fungal CNS infection
- **Specific unusual infections**
  - *Pneumocystis jirovecii* pneumonia
  - *Burkholderia cepacia* complex lymphadenitis
  - Infection with a bug that ID/MM cannot pronounce

The "Immunocompromised" Host

Immunosuppressive Diseases or Conditions	• Hematologic malignancies (leukemias, lymphomas) • Transplantation (bone marrow, solid organ) • Acquired immunodeficiency syndrome • Neutropenia associated with diseases or drugs • Collagen vascular diseases (when treated with immunosuppressive drugs) • ICU patients who have had gastrointestinal surgery or perforation, central venous catheter, total parenteral nutrition, broad-spectrum antibiotics, burns, multisystem organ failure, or are neonates • Diabetes mellitus with ketoacidosis • Iron chelation therapy with deferoxamine
Immunosuppresive Therapy	• Corticosteroids • Cancer chemotherapeutic agents • Cyclosporine, tacrolimus

Table 1

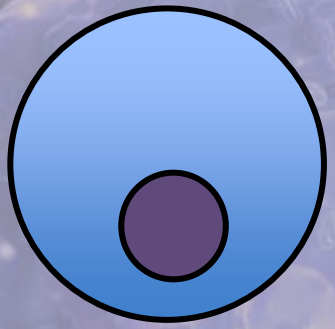
USUALLY: Discernable by History, Physical Exam, some basic lab tests

The "Not-Obviously Immunocompromised" Host

CLINICAL PHENOTYPE

MICRO-BIOLOGICAL PHENOTYPE

CELLULAR / IMMUNO PHENOTYPE



TESTING

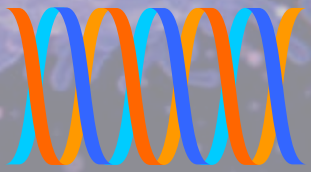
QUANTITATIVE

- **Too high, too low (consistently so)**
- **Commonly available**
- **Fast TAT**

QUALITATIVE

- **Protein detection (flow cytometry; Western blot)**
- **Functional assays**
- **Specialized/Research labs**
- **Slow TAT**

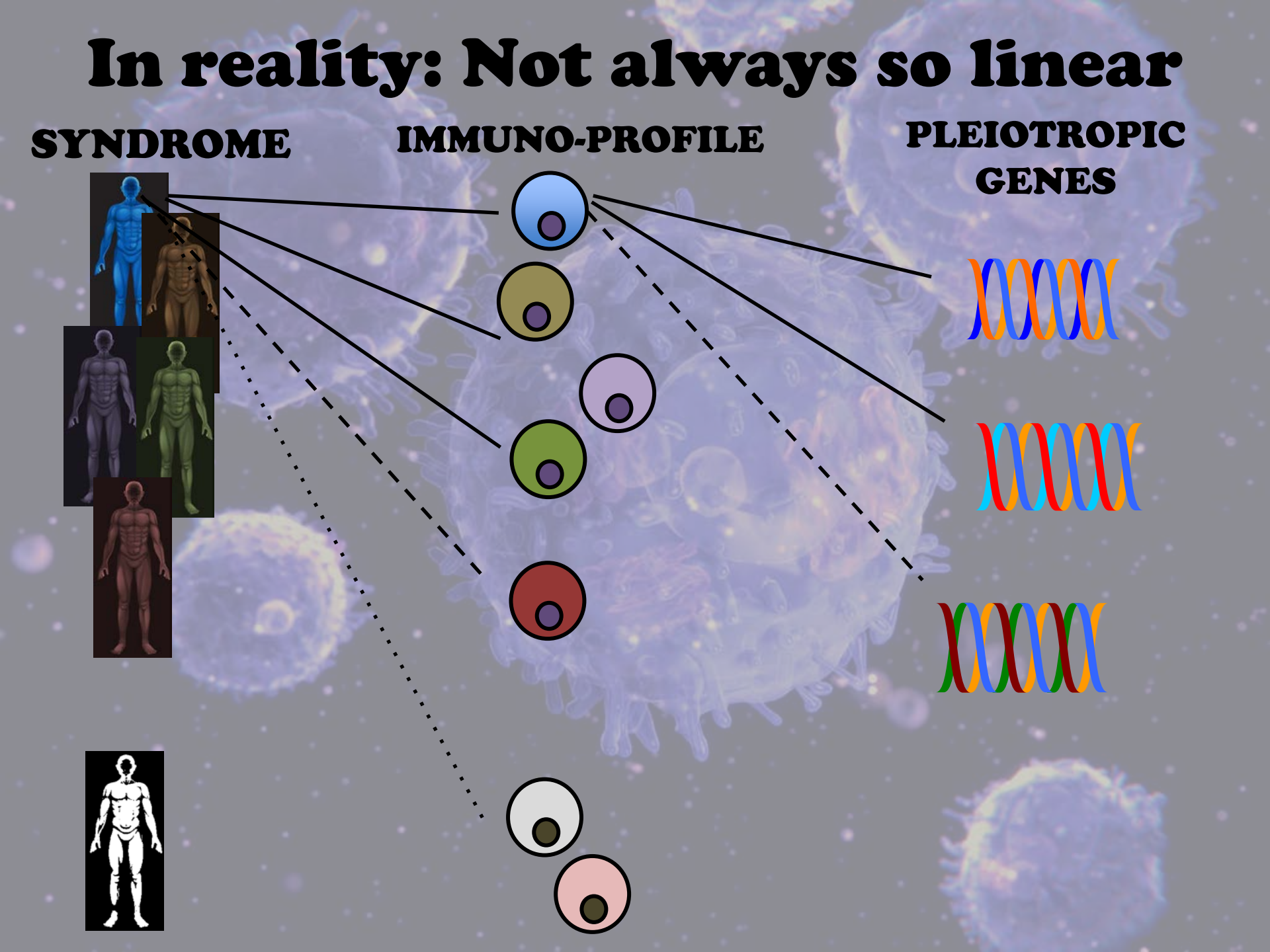
GENOTYPE

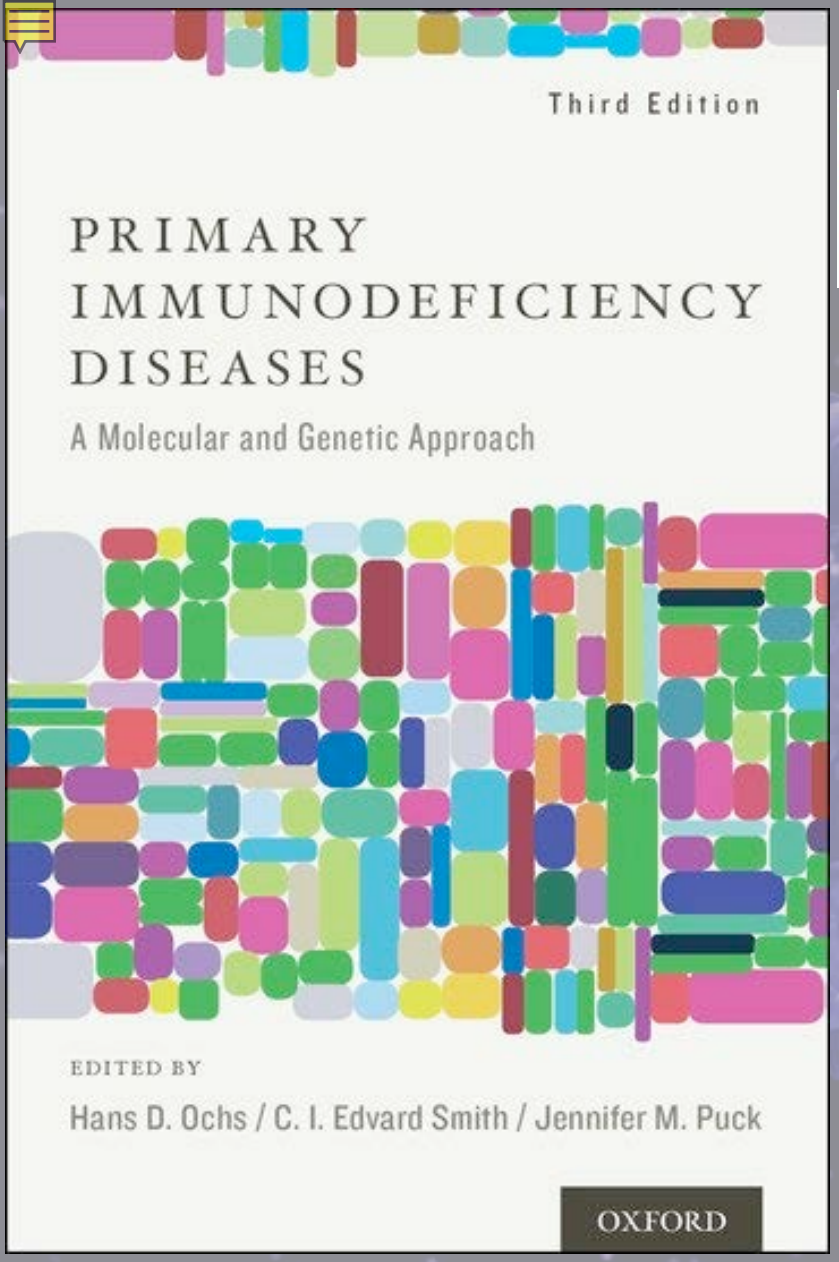


TESTING

- targeted
- gene panels
- agnostic

In reality: Not always so linear





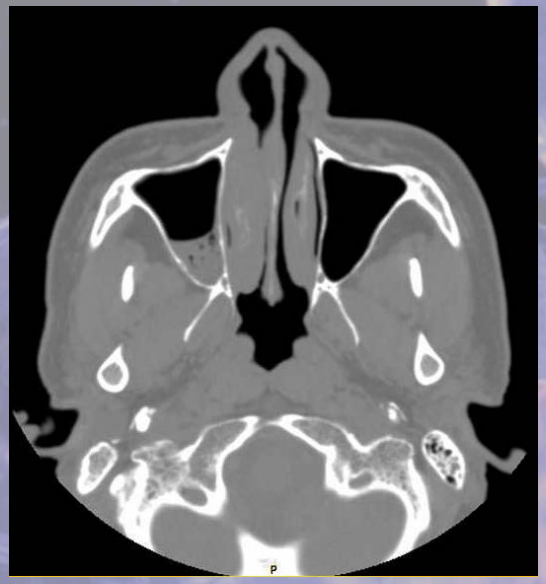
Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency

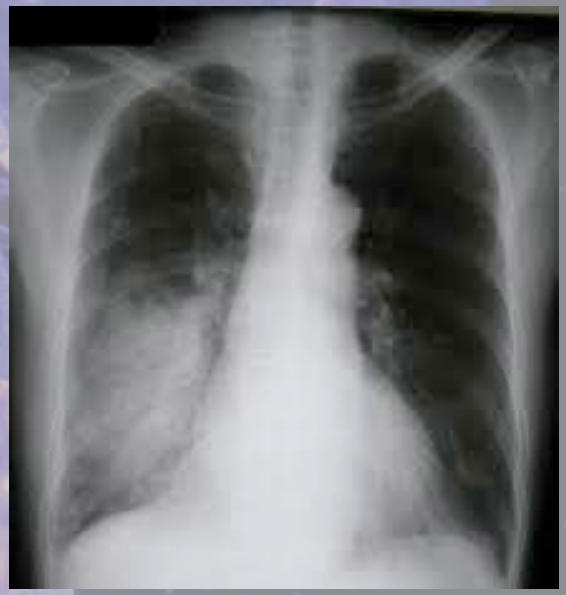
2015: >260 PID

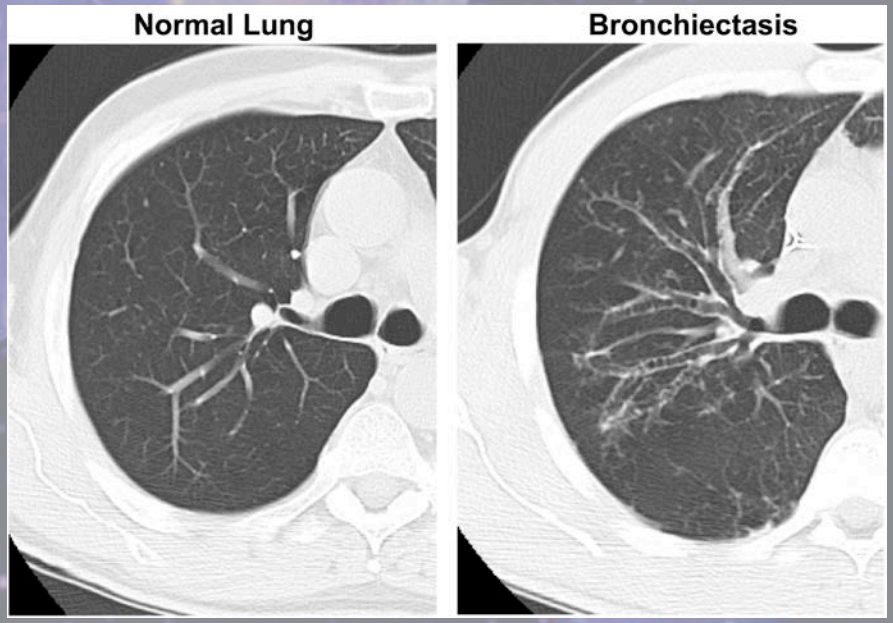
(increase of 30 since 2011)

Recurrent Respiratory Tract Infections (RRTIs)

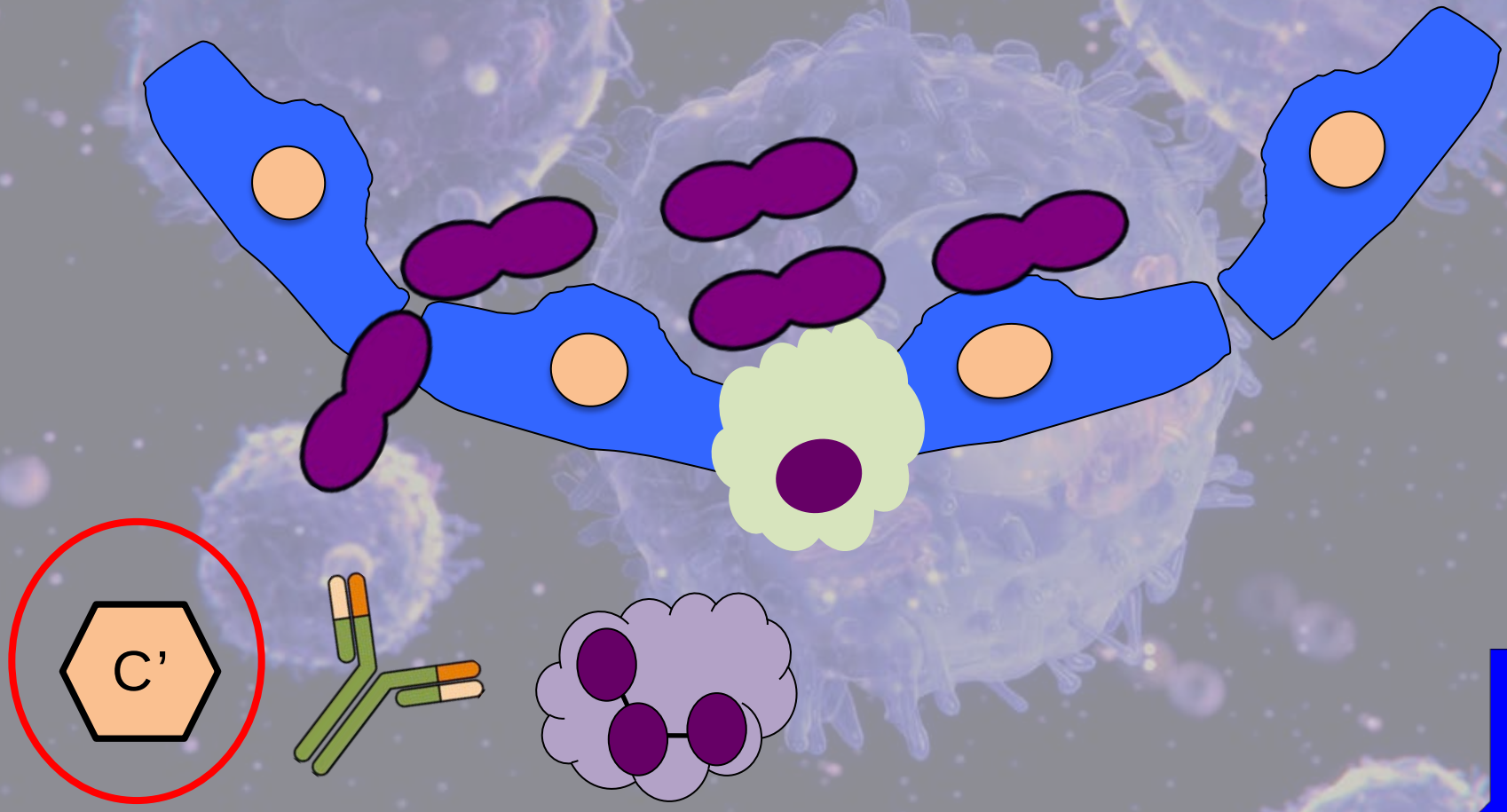
- Recurrent:
- Respiratory:
  - Upper
  - Lower
- Bacterial
- Radiologically documented/clearance
- Invasive disease







Who let the Pneumococcus out?



Complement:

Ag – Ab

CLASSICAL

C1q; C1r; C1s

C4; C2

Microbe surface

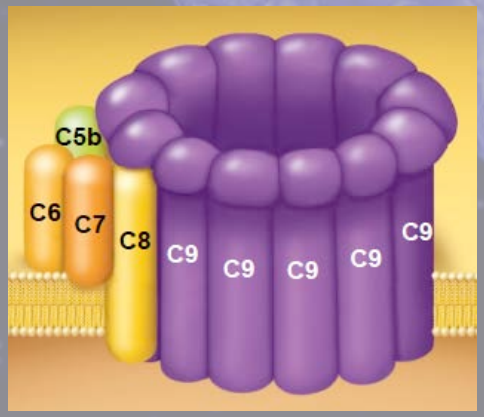
ALTERNATIVE

C3b;
Factor D;
Factor B;
Properdin

Factor H;
Factor I

LECTIN

C3



Testing of C':

(1) C3 ; C4

(2) Classical C' + Alternative C'

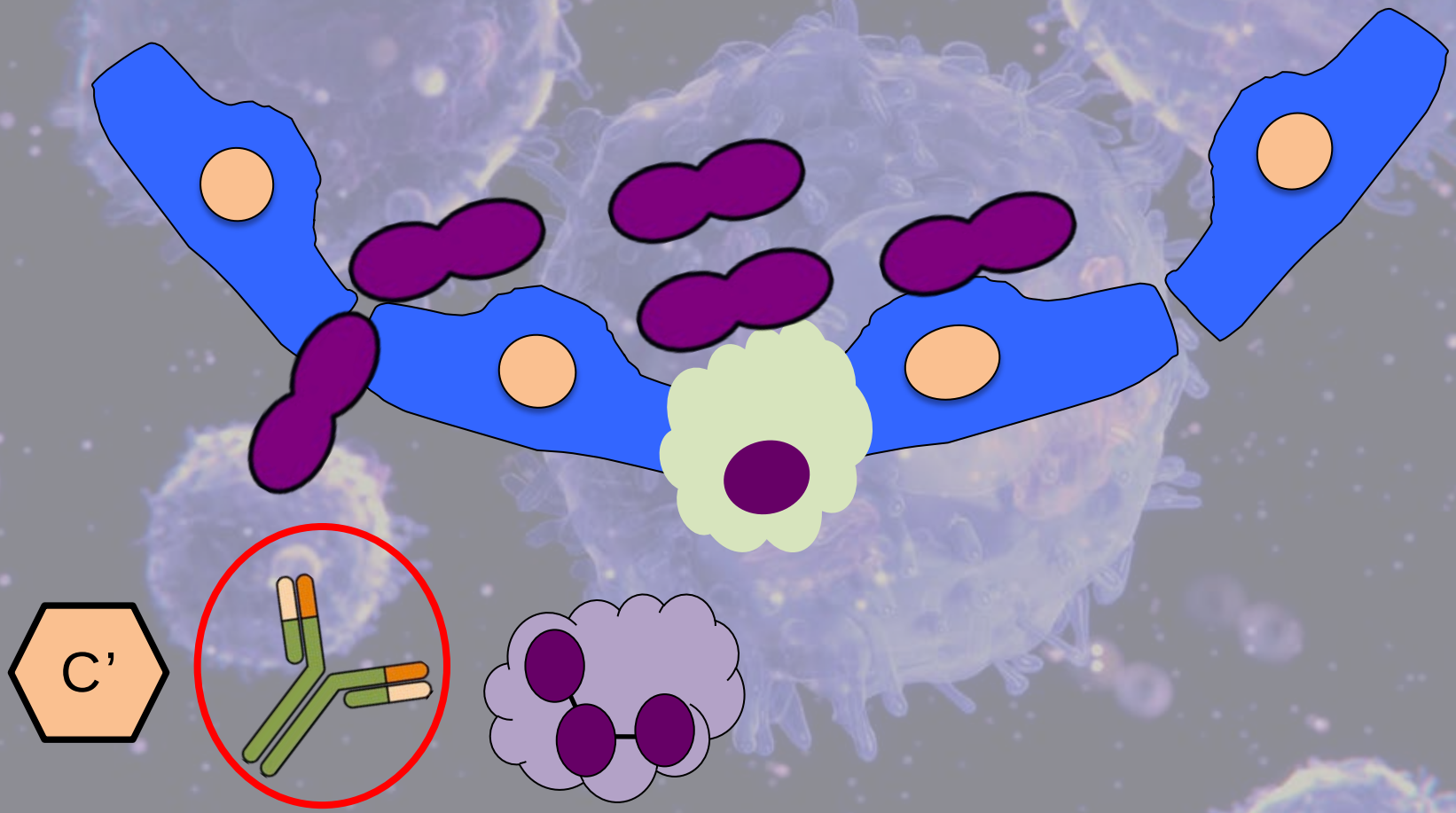
(3) Repeat if ab(n)

[R/O in vitro consumption]

(+/-) Individual C' components

(+/-) ? Cause

Who let the Pneumococcus out?



HypoGammaGlobulinemia (HGG)

	IgG	IgA	IgM	IgE
Property	Major serum Ig (75%) **Major extra-vascular Ig** Crosses placenta	Mucosal secretions (breast milk, saliva, tears, mucus)	First type of Ab produced after T cells find an antigen	Usually low levels
t½	21 d	6 d	5 – 10 d	2 d
structure	Monomer	Dimer (secretions)	Pentamer	Monomer
Function	Fixes complement Opsonization	Mucosal defences	Fixes complement Agglutination	Bound to mast cells / basophils

IgG subclasses:

- **IgG1**
- **IgG2**
- **IgG3**
- **IgG4**

QUALITY:

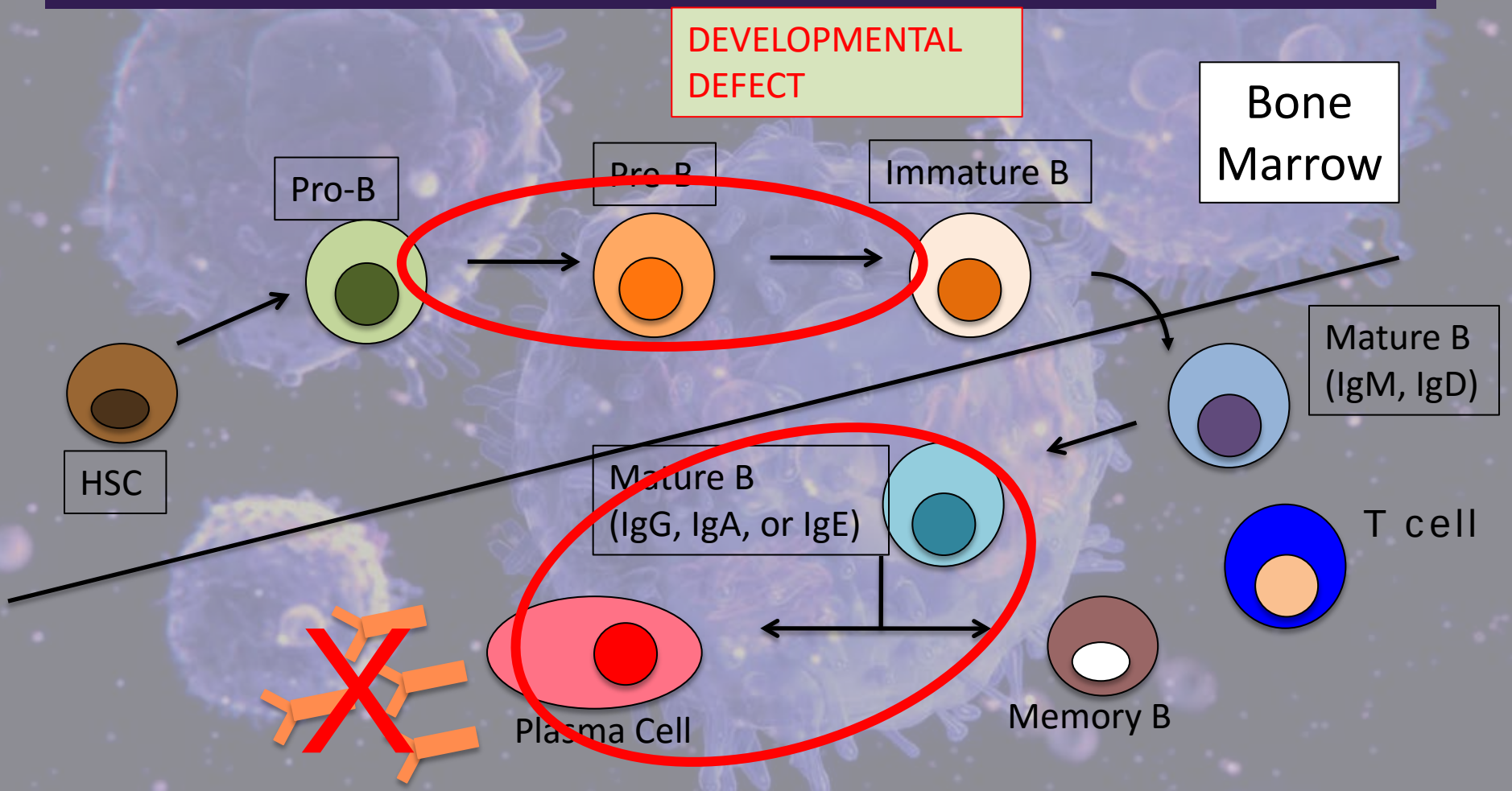
(1) SPECIFIC Abs

- **Vaccines: Tetanus, Hib**
- **Natural infections: VZV**

(2) DIVERSITY: SPEP, UPEP, FLC

RETENTION/LOSS: ALBUMIN

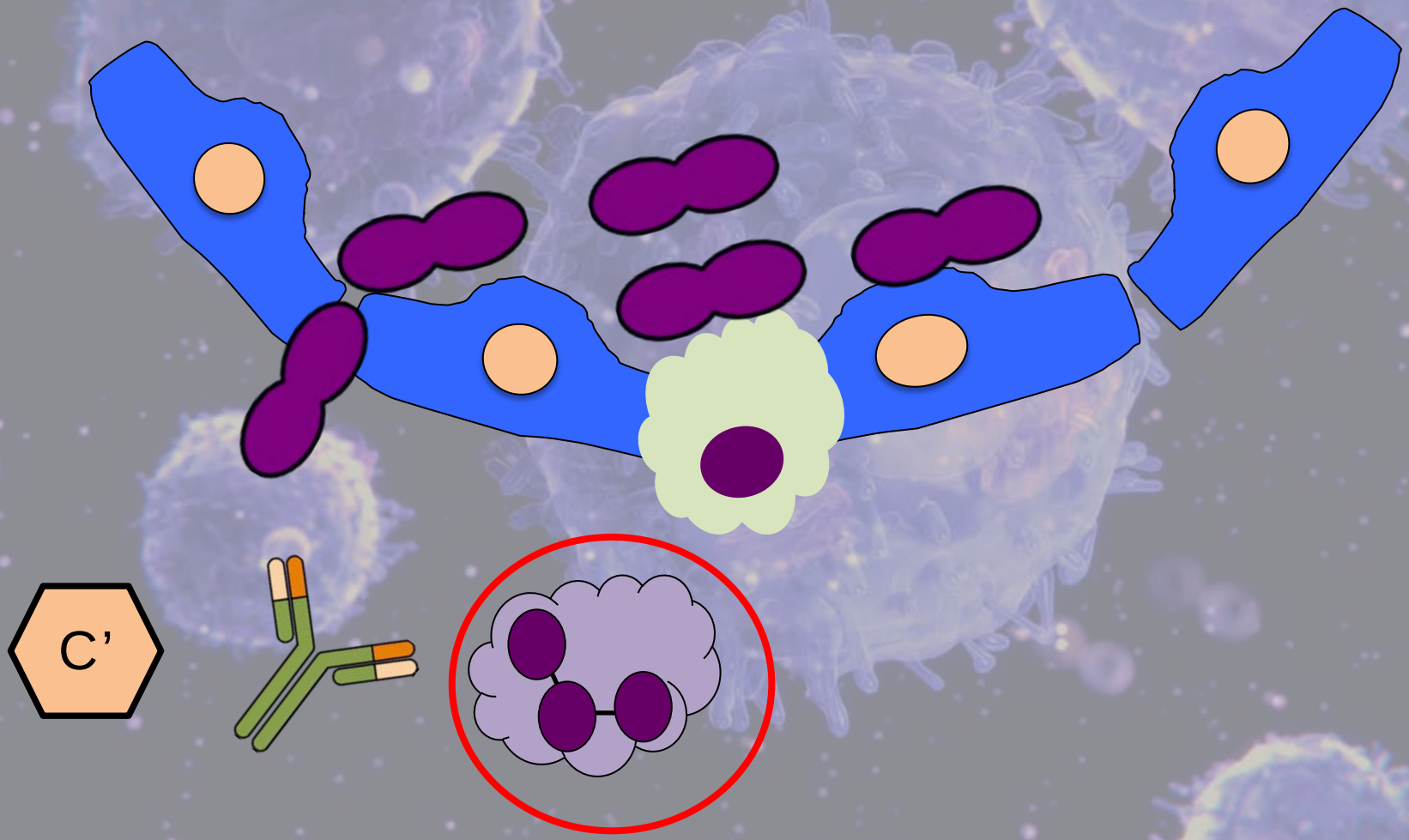
B cell Development



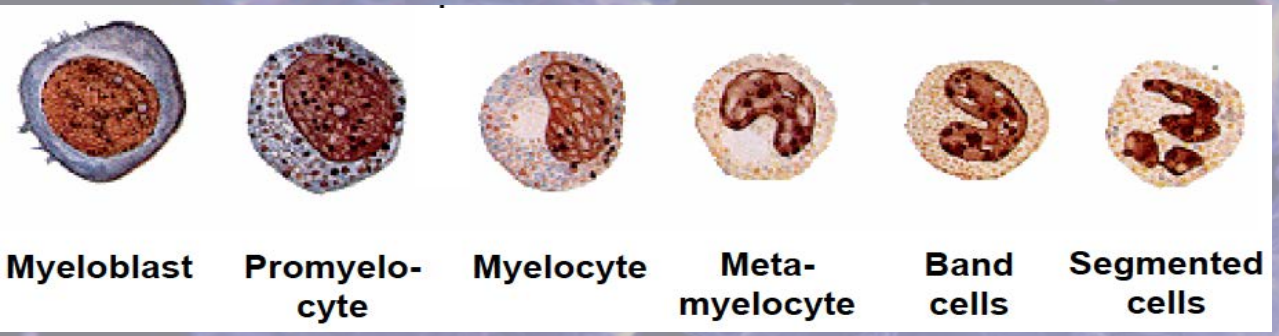
TESTING:

- **B cells (CD19/CD20)**
- **T cells (CD3; CD4; CD8)**

Who let the Pneumococcus out?



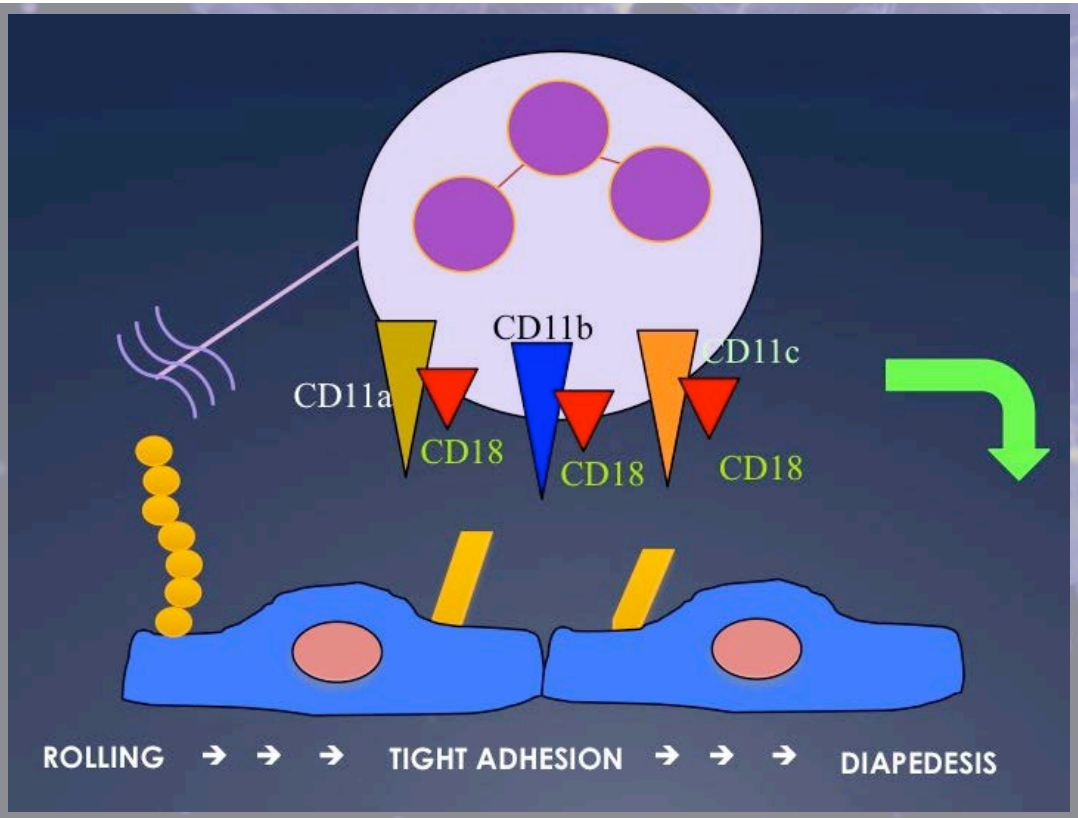
NEUTROPHILS:



NEUTROPENIA:

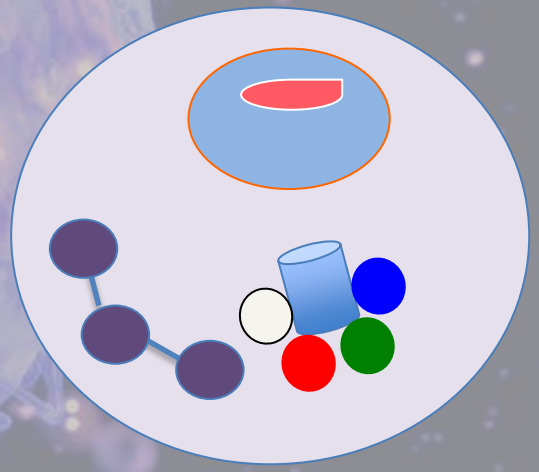
- SEVERE CONGENITAL
- CYCLIC
- many others

NEUTROPHIL COUNT (absolute; manual)



LEUKOCYTE ADHESION DEFICIENCY

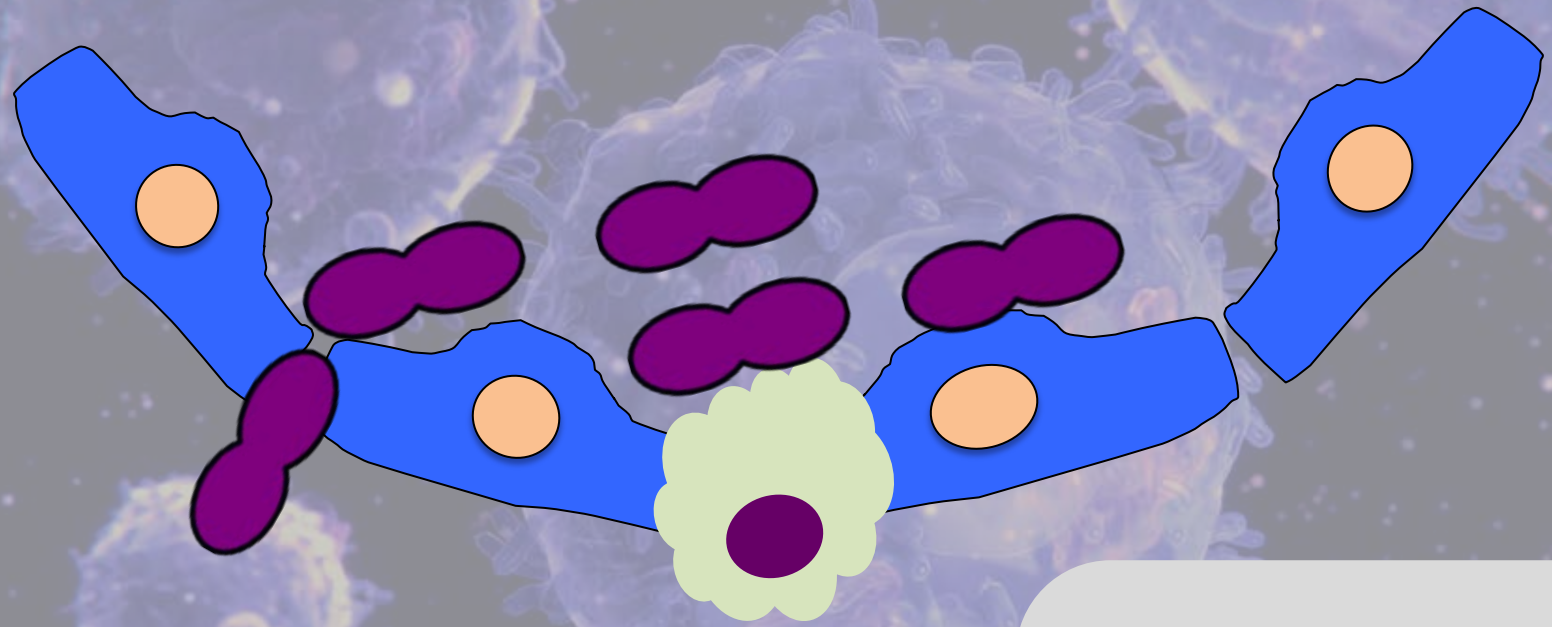
CD11b/CD18

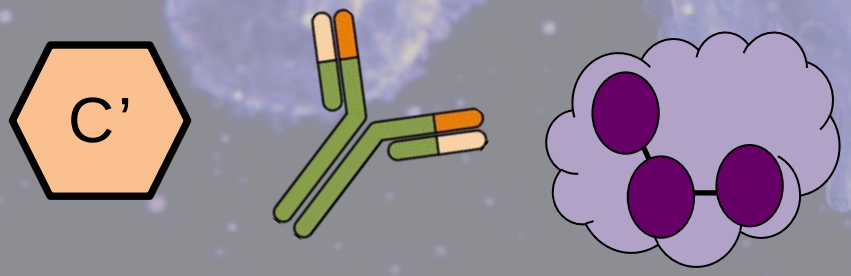


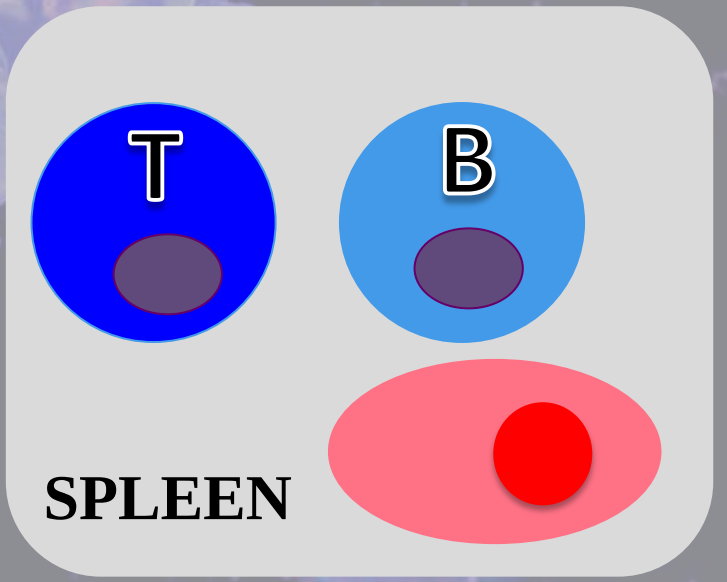
CHRONIC GRANULOMATOUS DISEASE

DHR-123

Who let the Pneumococcus out?





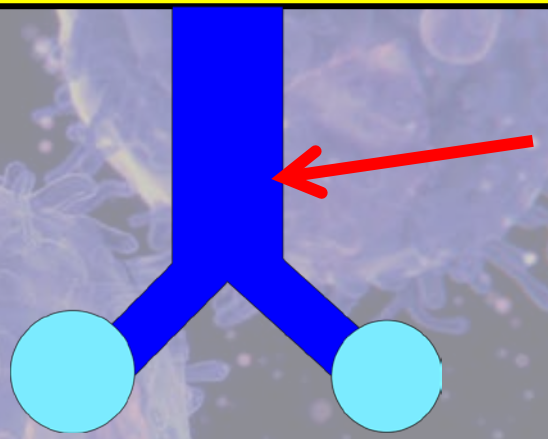


Immunologic w/u for RRTIs:

1) **Complement:**
   - **C3; C4**
   - **CLASSICAL & ALTERNATIVE C' Pathways**
   - **Individual C' components**
2) **Immunoglobulins:**
   - **IgG / A / M / E**
   - **IgG subclasses**
   - **Albumin**
3) **Enumeration of B & T cells**

And...

4) **Spleen (RRTI + invasive disease)**
5) **Neutrophils (RRTI + other infections)**
   - **Quantitative: Abs Neutrophil count**
   - **Functional: DHR; CD11b/CD18**



- **PULMONARY / ENT causes:**
- Anatomic
- Allergic
- Alpha1-anti-trypsin
- Primary ciliary dyskinesia (nasal NO)
- Cystic Fibrosis
- ENaC (Epithelial Na^+ channel disorders):
  - ab(n) Na/Cl in serum;
  - ab(n) sweat Cl^-

Relevance of Immunologic w/u:

1) **Complement:**
   - C3; C4
   - CLASSICAL & ALTERNATIVE C' Pathways
   - Individual C' components
2) **Immunoglobulins:**
   - IgG / A / M / E
   - IgG subclasses
   - Albumin
3) **Enumeration of B & T cells**

And...

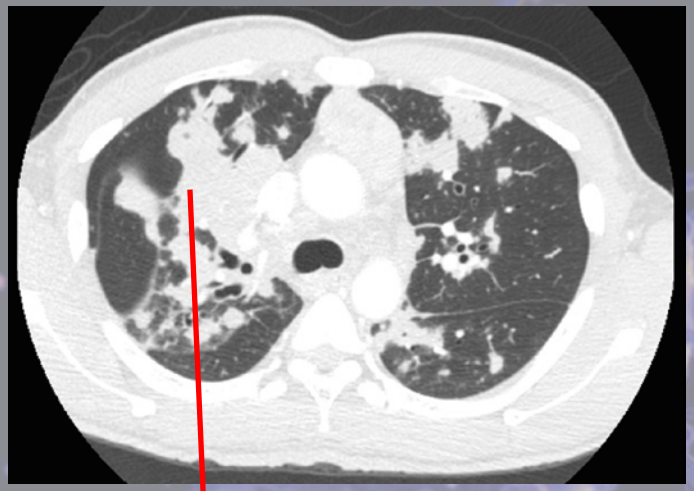
4) **Spleen**
5) **Neutrophils**
   - ANC
   - Functional: DHR; CD11b/CD18

→ **VACCINATION against "ENCAPSULATED" organisms**

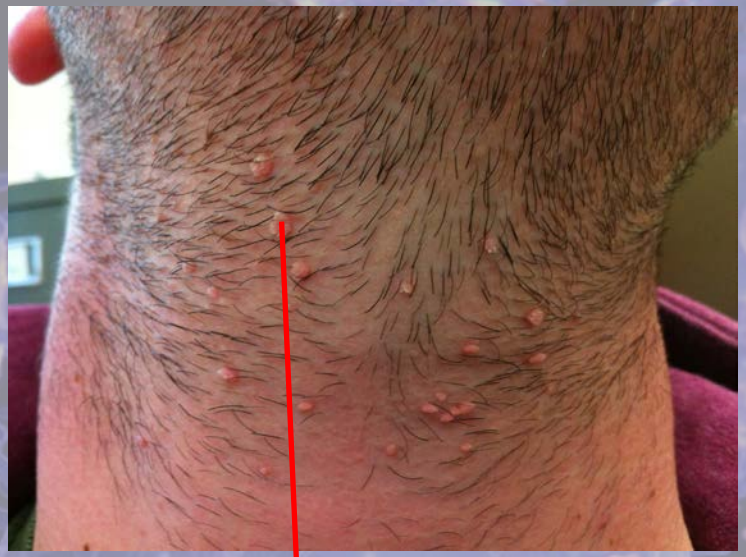
→ **ImmunoGlobulin replacement**
IVIG or SCIG
Target IgG: 8-10 g/L

→ **VACCINATION against "ENCAPSULATED" organisms & Early/Self-initiated Abx**

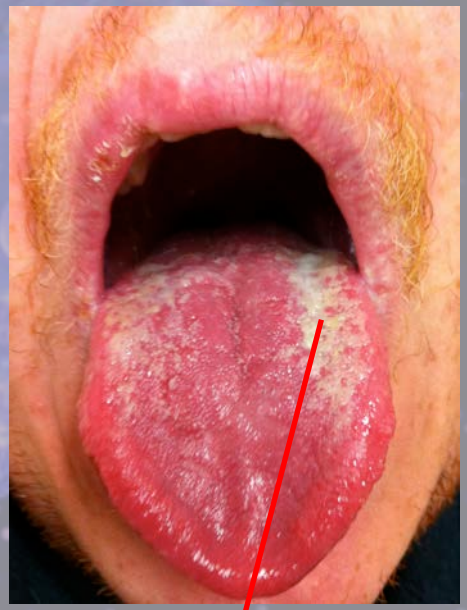
→ **G-CSF**



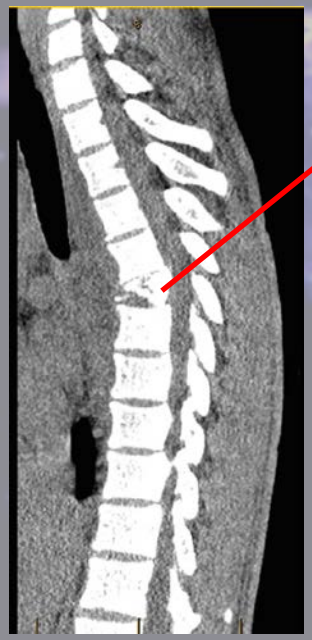
BAL showed *Pneumocystis*



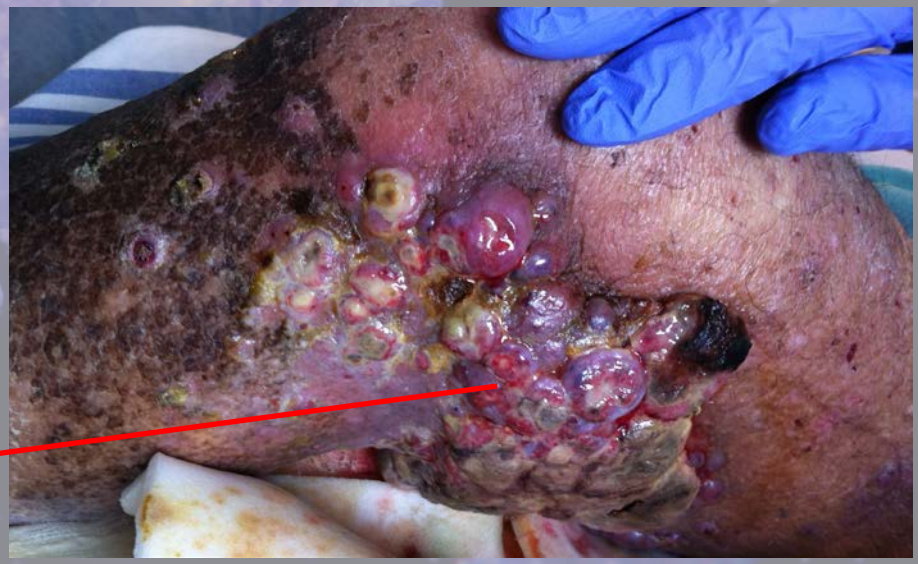
Snip excision revealed HPV-6



Culture yielded *Candida albicans*

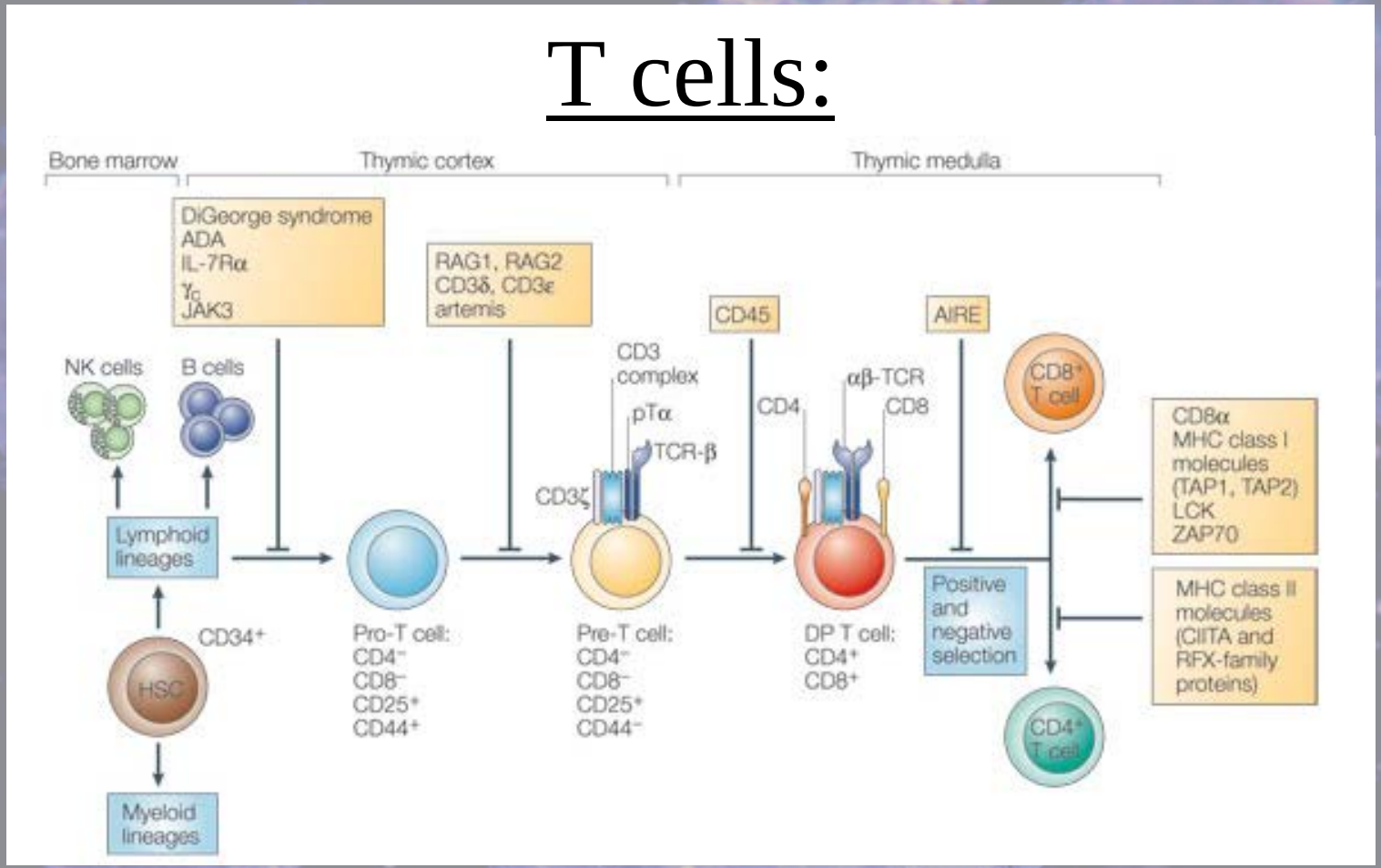


Pott’s (TB spine)

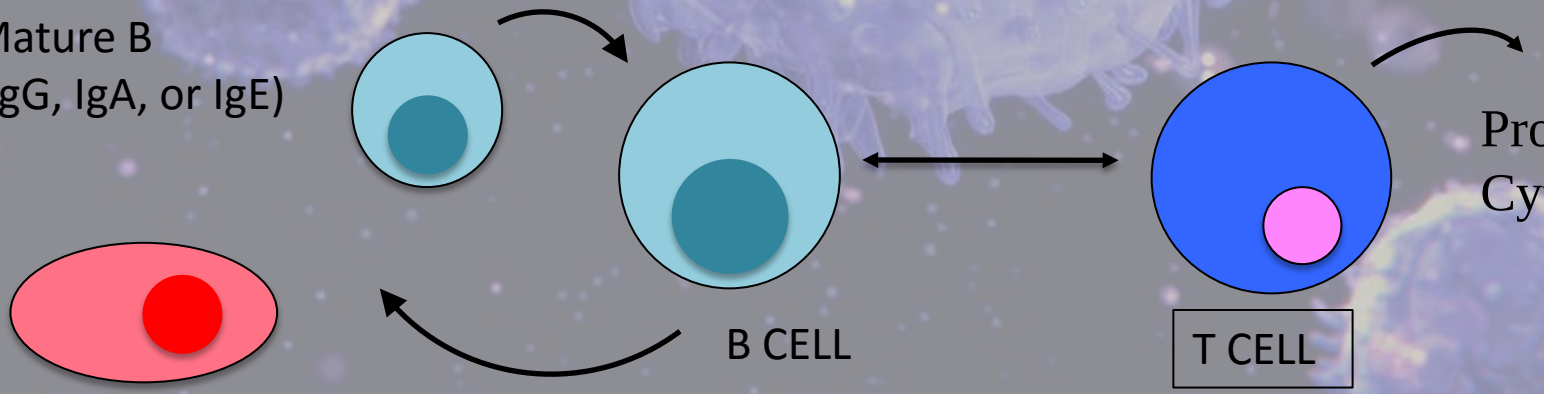


Kaposi’s sarcoma

T cells:



Mature B
(IgG, IgA, or IgE)



Proliferation
Cytokines

Cunningham-Rundles, Nat Imm 2005

Adaptive Immunity: T cells

NO T cells

LOW/ Dysfunctional T cells

Table 1 | **Aetiologies of severe combined immunodeficiency**

Type of SCID	Chromosomal location	Reference
T⁻B⁺NK⁺		
Interleukin-7 receptor α-chain deficiency	5p13	
CD3 δ-chain deficiency	11q23	
CD3 ε-chain deficiency	11q23	4
T⁻B⁺NK⁻		
d recessive SCID (γ_c defici	Xq1	
deficiency	1q31–1q32	6
eficiency	19p13.1	7
T⁻B⁻NK⁺		
Artemis gene-product deficiency	10p13	8
RAG1 and RAG2 deficiency		
T⁻B⁻NK⁻		
Adenosine-deaminase deficiency	20q13.11	10

γ_c, common cytokine-receptor γ-chain; JAK3, Janus kinase 3; NK, natural killer; RAG, recombination-activating gene; SCID, severe combined immunodeficiency.

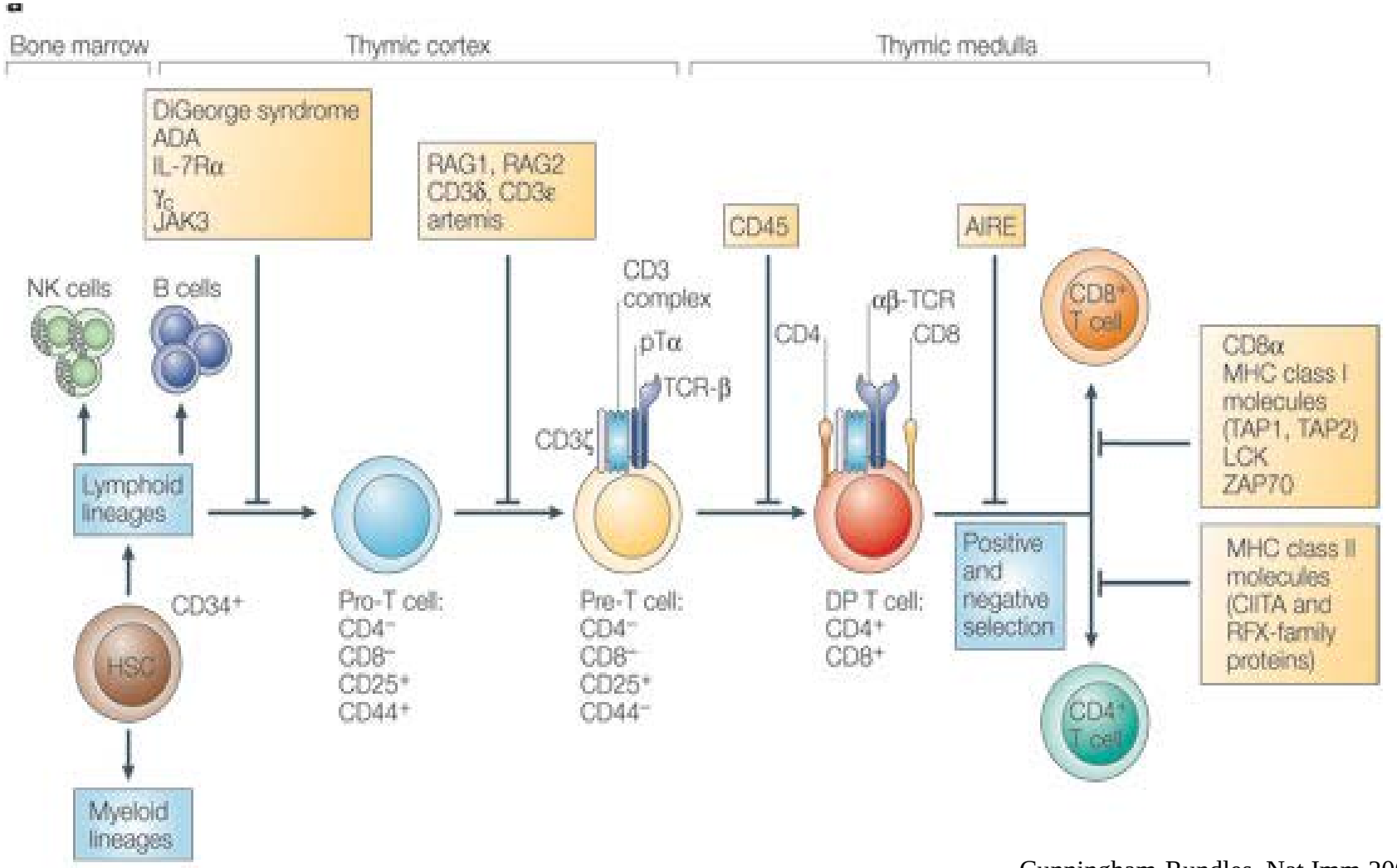
SCID

CID ("leaky" SCID)

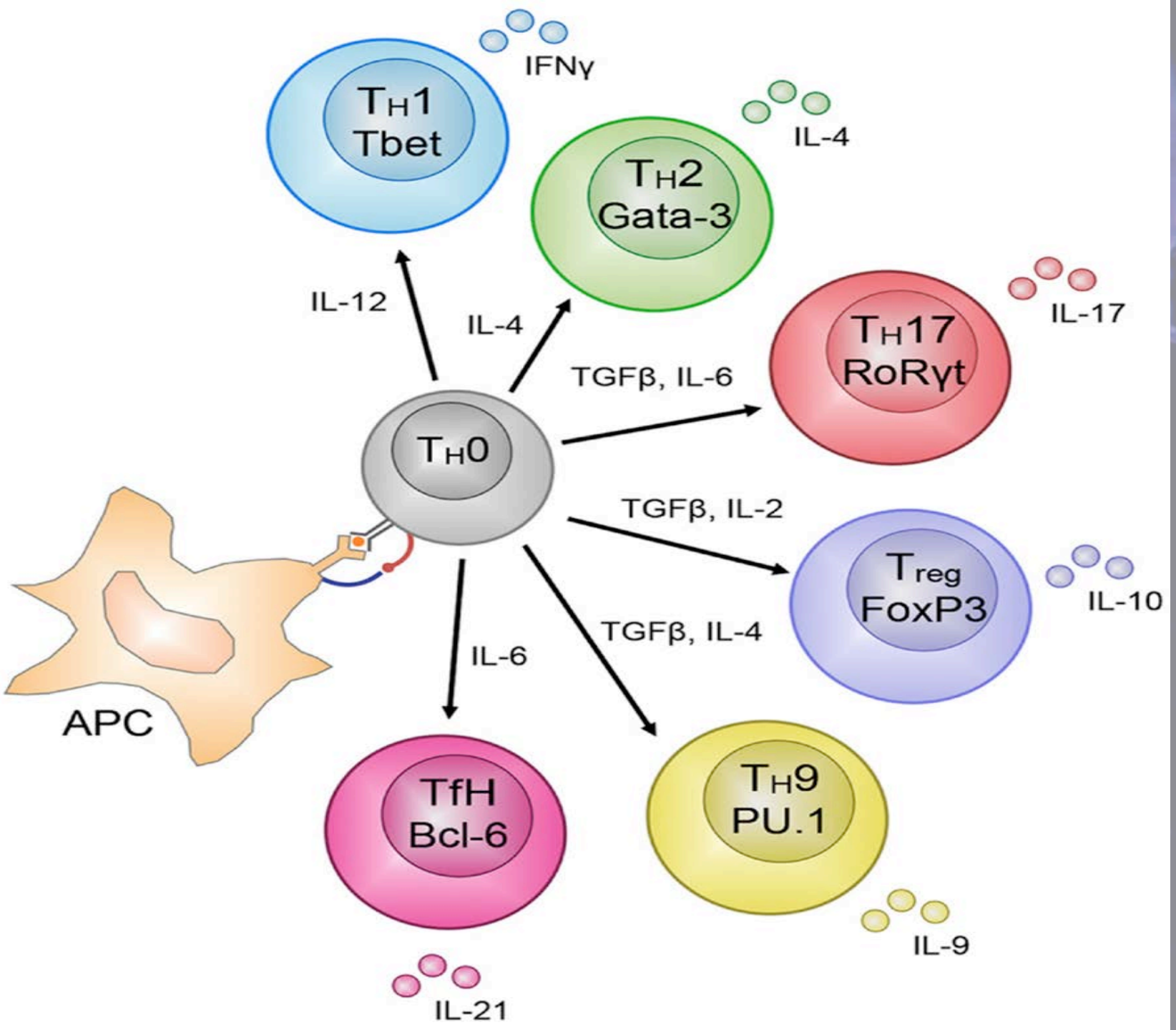
Idiopathic CD4+ lymphopenia

Cunningham-Rundles, Nat Imm 2005

T cell Defects:



Cunningham-Rundles, Nat Imm 2005


IFNγ
TH1
Tbet
IL-4
TH2
Gata-3
IL-12
IL-4
IL-17
TH17
RoRγt
TGFβ, IL-6
TH0
TGFβ, IL-2
Treg
FoxP3
IL-10
APC
IL-6
TGFβ, IL-4
TfH
Bcl-6
TH9
PU.1
IL-9
IL-21

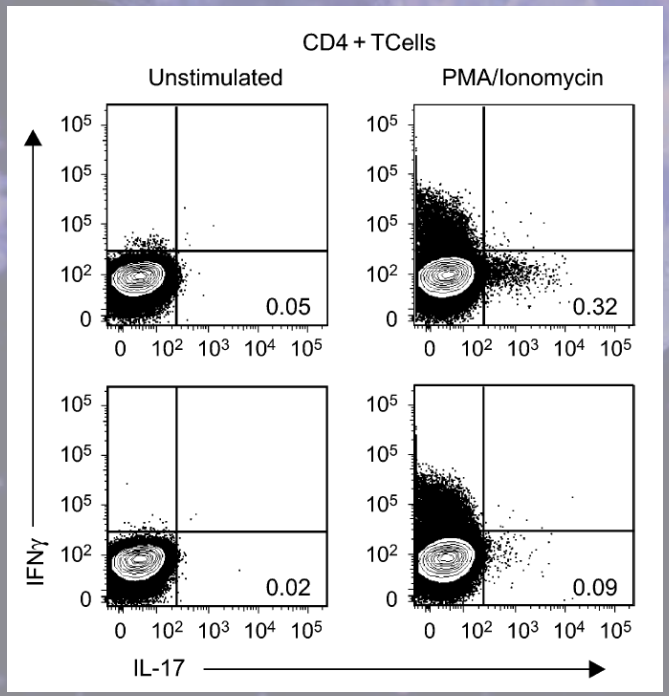
Evaluation of T cells:

- **T / B / NK enumeration:**
- **T**: $CD3^+$ (total)
  $CD4^+$; $CD8^+$
  RA^+ (naive)/RO^+ (memory)
- **B**: CD19 (or CD20)
- **NK**: $CD3^-$,$16/56^+$
- **Monocytes:**
  - Manual > Automated
  - $CD14^+$
- **Ig G / A / M / E**

- **TREC**: T cell maturation through thymus
- **TcR repertoire** (PCR; spectratyping): clonality
- **TB-Quantiferon** (or DTH)

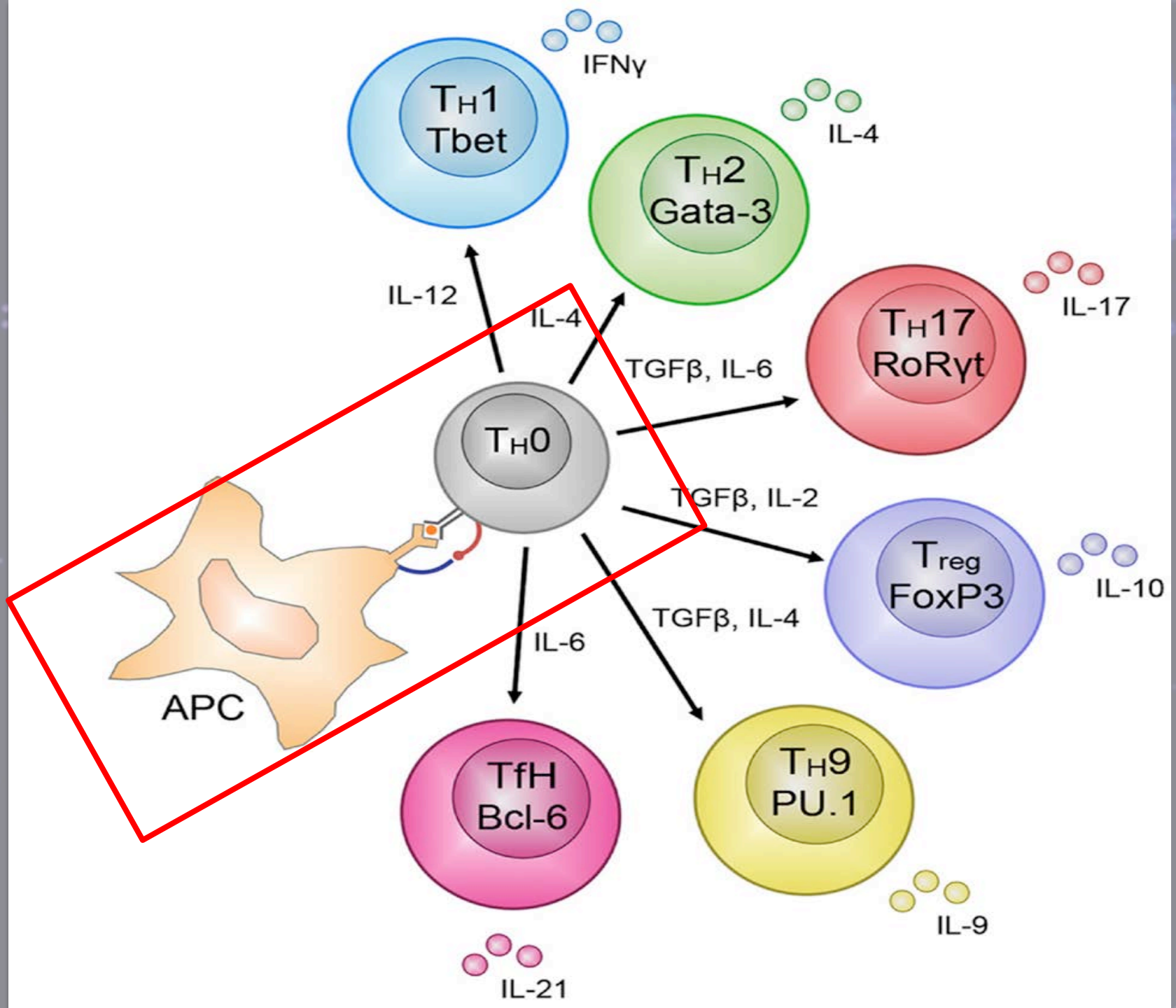
Evaluation of T cells: More Advanced Testing

- **Th17 cells**: Candidiasis
- **Tfh cells**: Hypogammaglobulinemia
- **Treg cells**: Autoimmunity



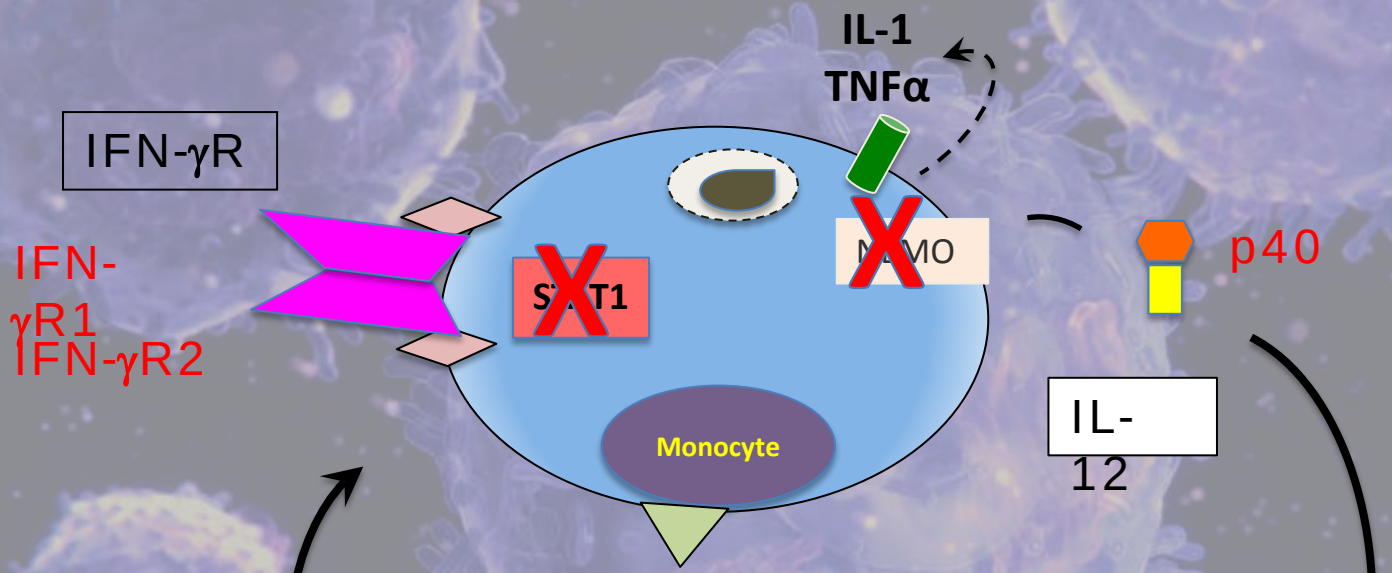
BUT... WHAT IF IT'S ALL "NORMAL" ??

FUNCTIONAL TESTING


IFNγ
TH1
Tbet
IL-4
TH2
Gata-3
IL-12
IL-4
TGFβ, IL-6
IL-17
TH17
RoRγt
TH0
TGFβ, IL-2
Treg
FoxP3
IL-10
APC
IL-6
TGFβ, IL-4
TfH
Bcl-6
TH9
PU.1
IL-9
IL-21

Functional Testing... an example

IL-12 / IFN-γ axis: Mycobacteria; TDF; *Salmonella*



GENETIC TESTING of the Macrobe (Host)

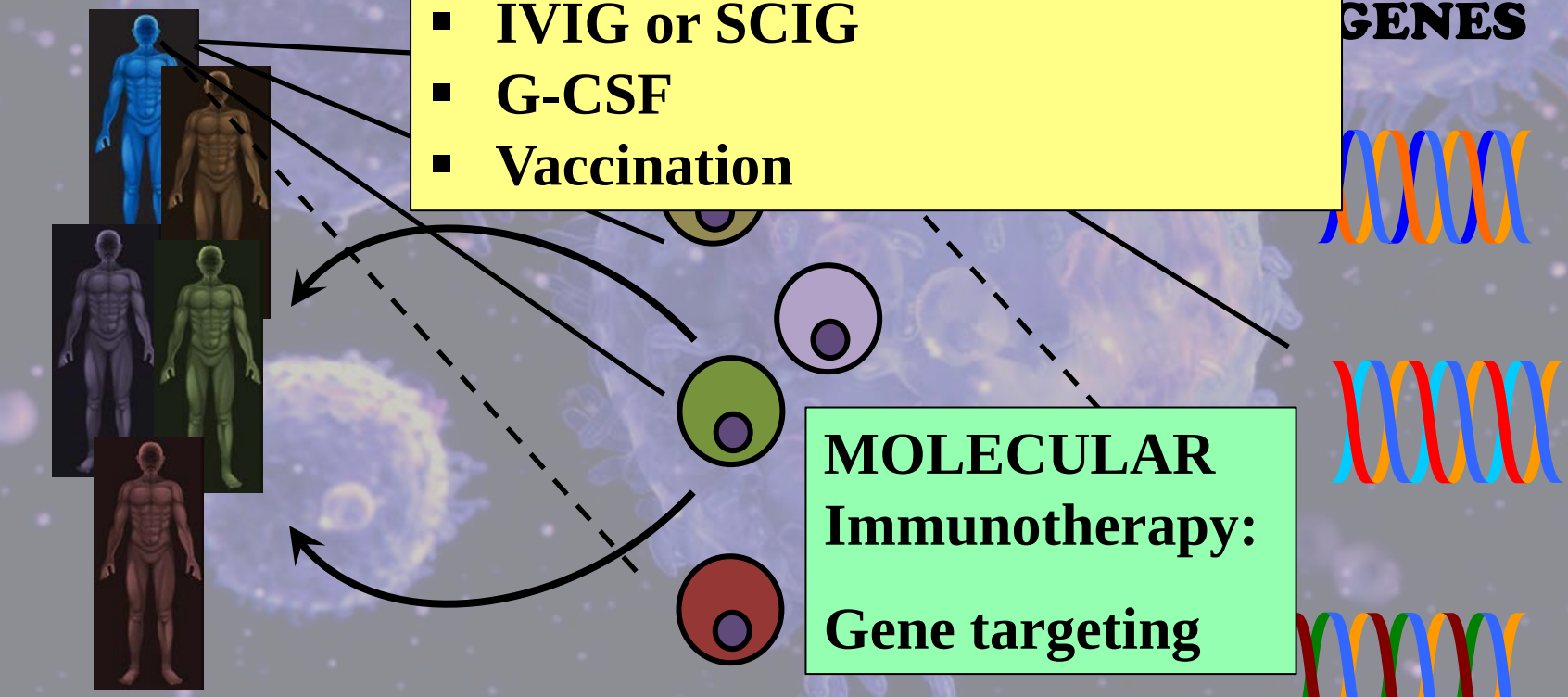
WHO CARES??

YOU DO !!

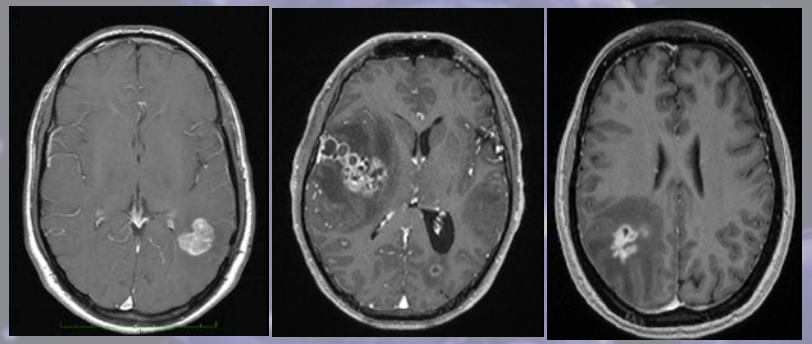
Genetic Testing: WHY you should care

- **CLINICAL:**
  - **MOLECULAR** confirmation of diagnosis
  - Genetic counseling: who else in family is at risk?
  - Prognosis: what complications to monitor
- **DIAGNOSTIC:**
  - Unambiguous
  - "Forme fruste"
    - variable penetrance; variable expressivity
  - Testing
    - Functional = viable cells [fragile]; Genes = stable
- **THERAPEUTIC:**

Genetic Testing: WHY you should care

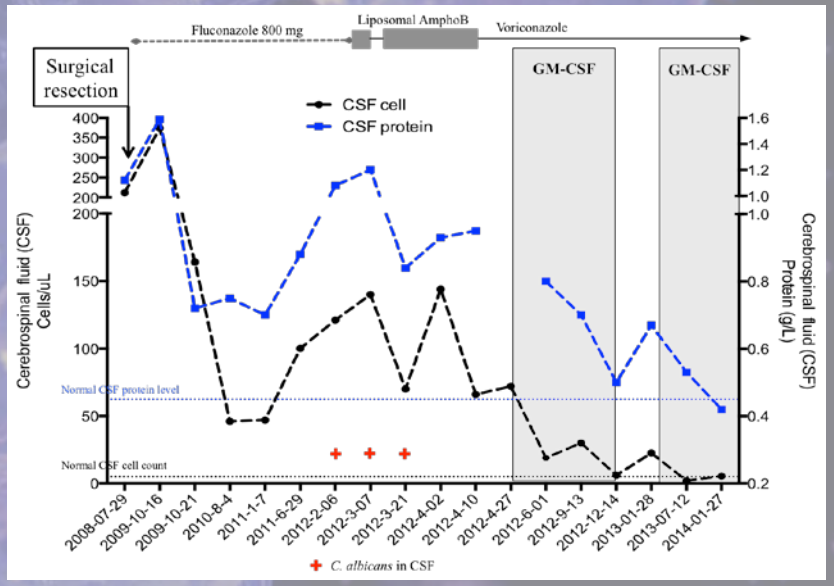


Genetic Testing: the CARD9 example

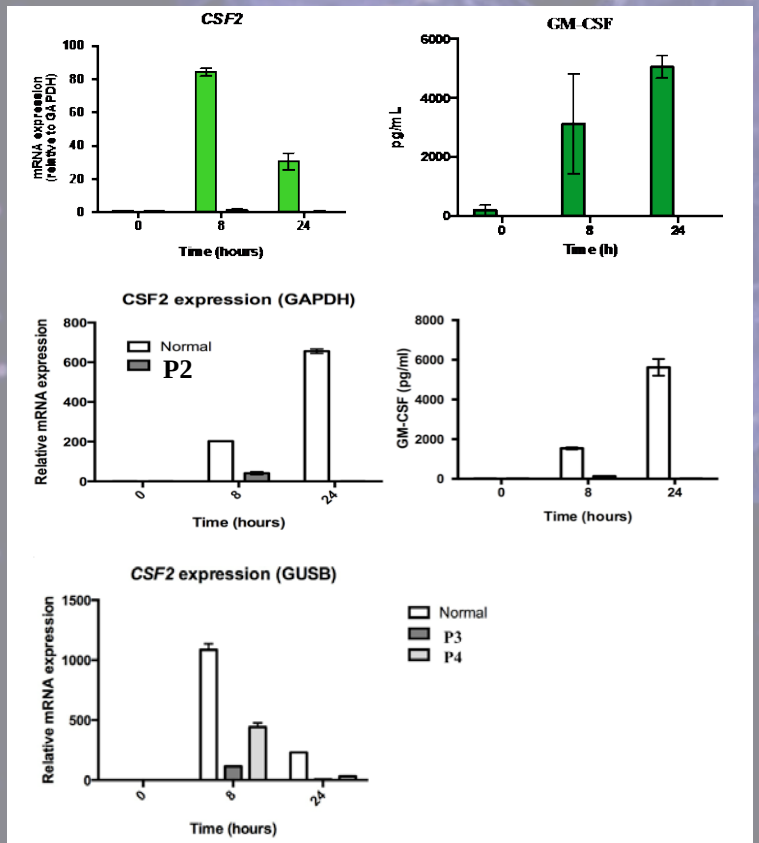


Spontaneous CNS candidiasis (*C. albicans*)

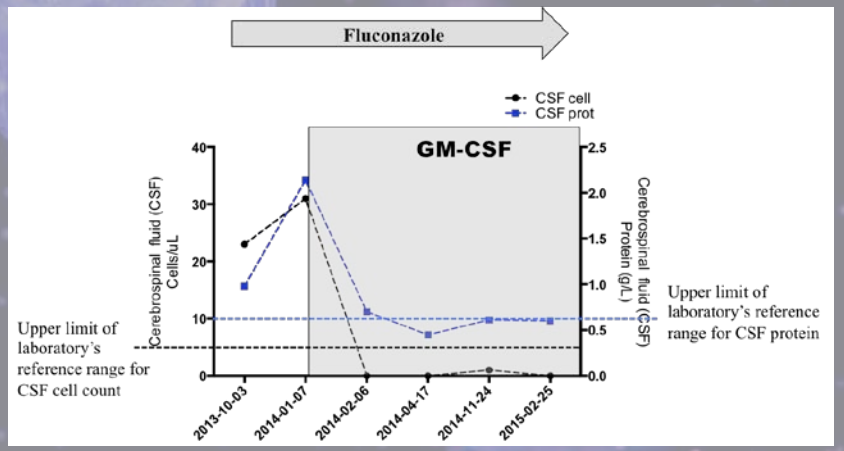
Recessive mutations in *CARD9*



qPCR



Cytokine



Genetic Testing: How?

- Commercial Labs
  - Provincial regulations
- Research Labs

- Targeted: 1 gene
- Panel: Multiple genes
- Whole gene vs. gene regions

- Agnostic
  - "Don't know what I'm looking for"
  - **<u>Discovery of new genes</u>**
- Next-generation sequencing
  - Whole-exome
  - Whole-genome
  - Transcriptome (RNA-seq)

COSTS = Pre-Analytical
+ Analytical
+ Post-Analytical

Key Points:

- Infections (Recurrent / Severe / Unusual)
  → THINK OF IMMUNODEFICIENCY
  (NOT Abx deficiency)
- Immunodeficiency: Primary (Genetic) or Secondary
- Testing:
  - Clinical + Microbiological
  - Immunological: **Quantitative** vs. **Qualitative** (Functional)
    - Myeloid: Neutrophils ; Monocytes/Macrophages
    - Lymphoid: T / B / NK
    - Humoral: Complement; Immunoglobulins
  - Genetic
- The type of immunodeficiency → Management
- And….

KEEP AN OPEN MIND

IF YOU FIND YOURSELF IN AN AWKWARD PREDICAMENT...

WE ARE JUST A PHONE CALL / EMAIL AWAY & WOULD LOVE TO COLLABORATE WITH YOU

THANK YOU!