WHEN TO CONSIDER AN IMMUNE DEFECT… OR NAME THAT IMMUNODEFICIENCY

Sea Pines Pediatric Infectious Diseases Conference, June 2017

Kathleen A. McGann, MD

Division of Pediatric Infectious Diseases

Department of Pediatrics

Duke University Medical Center

Disclosures:

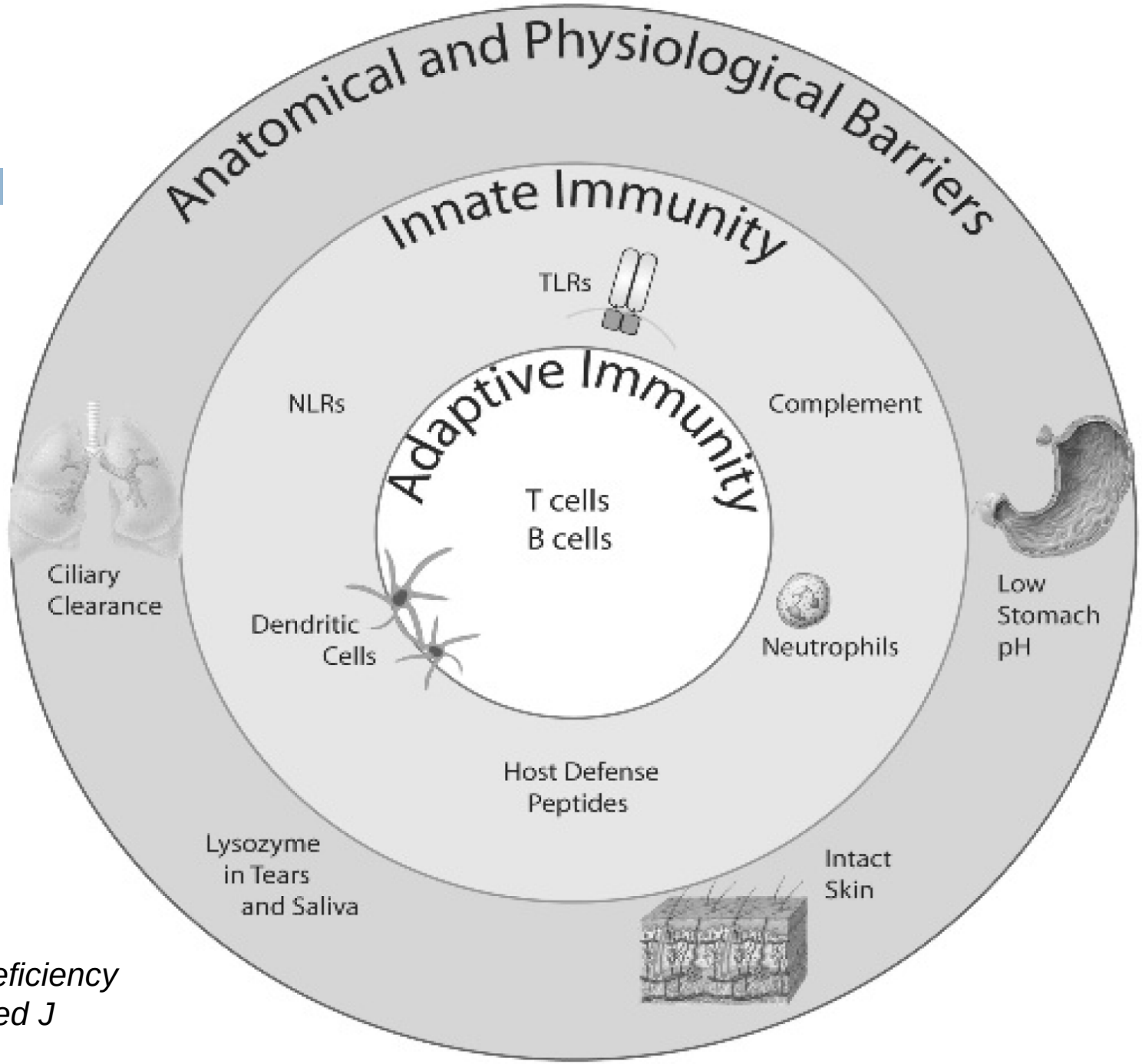
- Shared slides from section of Dr. Rebecca Buckley, MD's (Division of Allergy & Immunology, DUMC) Grand Rounds: *Saving Lives Through Early Diagnosis Newborn Screening for SCID and Other T cell Defects, 3/21/17*

- No other disclosures

Objectives

- List components of the innate and adaptive immune system
- Discuss patient cases with possible immunodeficiencies
  - develop a differential diagnosis
  - describe a diagnostic and management plan
- Discuss a few PIDs in more detail
- Delineate clinical manifestations & laboratory findings that raise suspicion for an immunodeficiency
  - Or when an immunodeficiency should be considered

Three Levels of Human Defense against Infection



Turvey SE, Bonilla FA, Junker AK. Primary immunodeficiency diseases: a practical guide for clinicians. Postgrad Med J ***2009;85:660-666***

Components of the Innate & Adaptive Immune System

Innate immune system

-Surface/Physical barriers (tight junctions, mucus)

-Enzymes in epithelial & phagocytic cells

-Inflammation-related serum proteins (eg, complement, CRP, lectins and ficolins)

-Complement system

-Antimicrobial peptides (AMPs) (defensins, cathelicidins, etc) on cell surfaces & within phagocyte granules

-Cell receptors that sense microorganisms & signal defensive response (eg, toll-like receptors [TLRs])

-Cells that release cytokines & other inflammatory mediators (eg, macrophages, mast cells, NK cells, innate lymphoid cells)

-Phagocytes (neutrophils, monocytes, macrophages)

-The microbiome?

Adaptive immune system

-T Lymphocytes: CD4, CD8, etc

-Gamma delta T cells

-B lymphocytes and antibodies

-Alternative adaptive immune system

-Immunological memory

Passive memory

Active memory and immunization

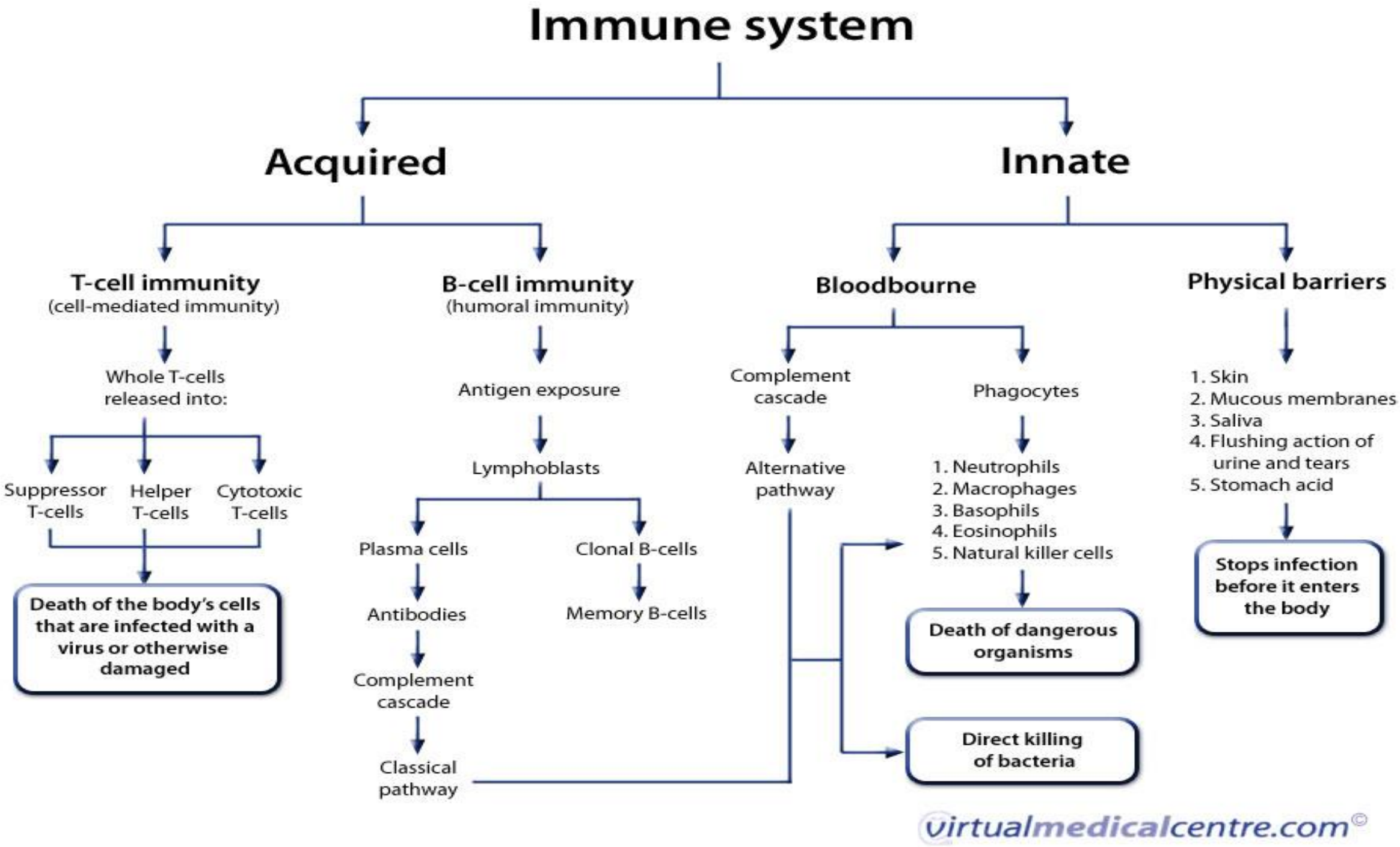
Innate and Adaptive Immunity: Response to Pathogens

Innate System

- Targets pathogens on 1st encounter through recognition of pathogen-specific patterns & tissue damage
- Anatomical barriers (skin, mucosa)
- Sentinel cells (dendritic cells)
- Release cytokines, chemokines
  - Trigger inflammation
  - Attract neutrophils & macrophages
- Complement cascade activation
  - Classical, Alternative or Lectin pathways
- Defects result in rapid progression of infections from Staph or other pathogens

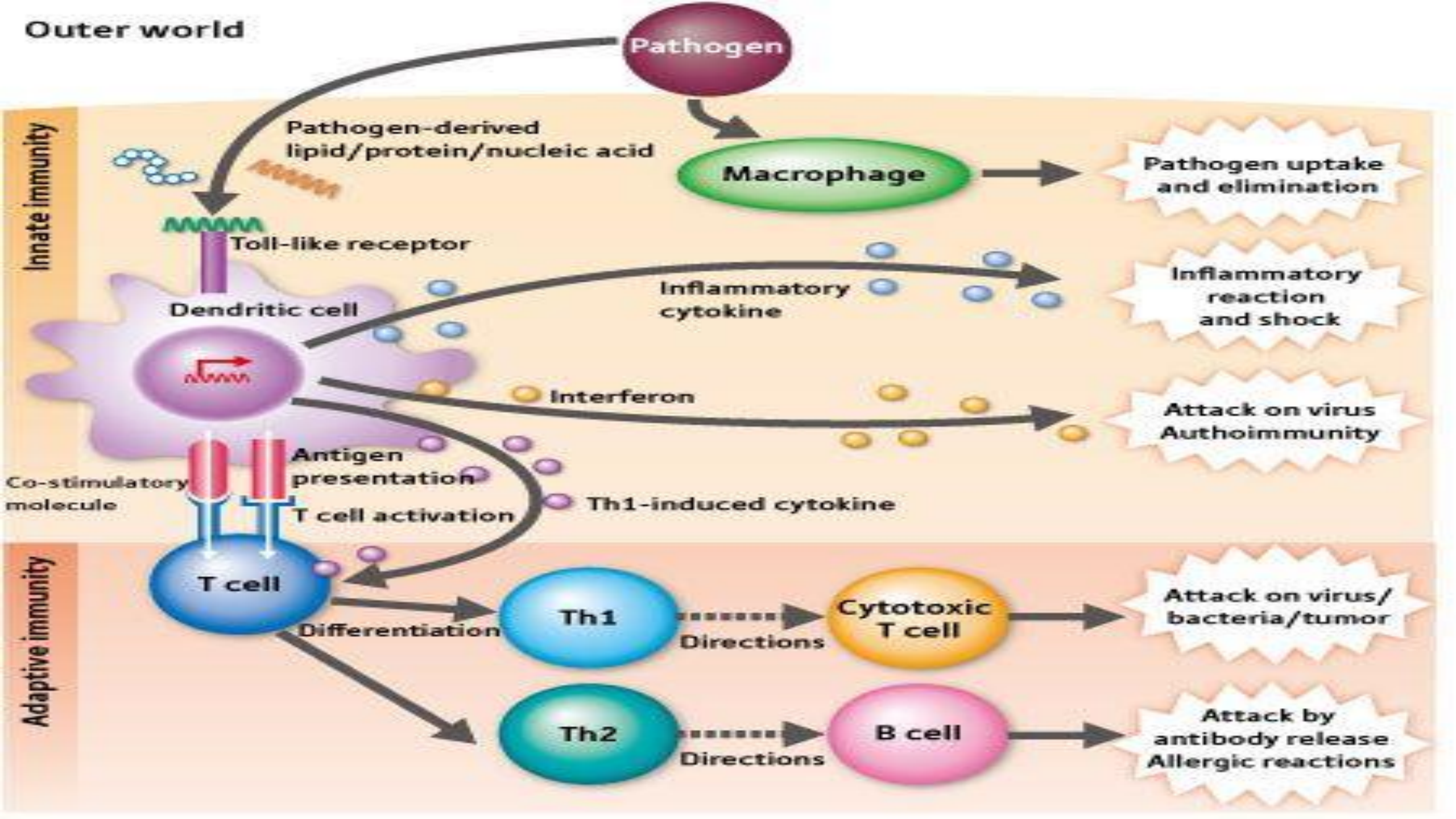
Adaptive Immunity

- Requires days to generate directed response
- Results in immunologic memory
- More rapid response with repeated exposures
- Primary components:
  - B cells
  - T cells


Immune system
Acquired
Innate
T-cell immunity
(cell-mediated immunity)
B-cell immunity
(humoral immunity)
Bloodbourne
Physical barriers
Whole T-cells released into:
Antigen exposure
Complement cascade
Phagocytes
1. Skin
2. Mucous membranes
3. Saliva
4. Flushing action of urine and tears
5. Stomach acid
Suppressor T-cells
Helper T-cells
Cytotoxic T-cells
Lymphoblasts
Alternative pathway
1. Neutrophils
2. Macrophages
3. Basophils
4. Eosinophils
5. Natural killer cells
Death of the body's cells that are infected with a virus or otherwise damaged
Plasma cells
Clonal B-cells
Stops infection before it enters the body
Antibodies
Memory B-cells
Death of dangerous organisms
Complement cascade
Direct killing of bacteria
Classical pathway
virtualmedicalcentre.com©

Defects can be seen in:

- Barriers
- Humoral – B cells or Ab number/function
- Cell-mediated immunity (CMI) – T cell number/function
- White blood cells – number/function
- Non-specific (NK cells, etc)
- Complement


Outer world
Pathogen
Innate immunity
Pathogen-derived
lipid/protein/nucleic acid
Macrophage
Pathogen uptake
and elimination
Toll-like receptor
Dendritic cell
Inflammatory
cytokine
Inflammatory
reaction
and shock
Interferon
Attack on virus
Authoimmunity
Co-stimulatory
molecule
Antigen
presentation
T cell activation
Th1-induced cytokine
Adaptive immunity
T cell
Differentiation
Th1
Directions
Cytotoxic
T cell
Attack on virus/
bacteria/tumor
Th2
Directions
B cell
Attack by
antibody release
Allergic reactions

The relationships among the elements of the nonspecific defenses and the specific defenses (immune response)

Antigens

Trigger

Nonspecific Defenses

Complement system

NK cells
Macrophages

Antigen presentation by APCs

Specific Defenses (Immune Response)

Activation by Class I MHC Proteins

Antigen and Class I MHC Protein

Indicates that the cell is infected or otherwise abnormal

CD8 T cells

Cytotoxic T Cells

Attack and destroy infected and abnormal cells displaying antigen

Memory T_C Cells

Await reappearance of the antigen

Suppressor T Cells

Control of moderate immune response by T cells and B cells

Direct physical and chemical attack

Activation by Class II MHC Proteins

Antigen and Class II MHC Protein

Indicates the presence of pathogens, toxins, or foreign proteins

CD4 T cells

Helper T Cells

Stimulate immune response by T cells and B cells

Memory T_H Cells

Await reappearance of the antigen

Activation of B cells

Production of memory B cells

Production of plasma cells

Secretion of antibodies

Direct physical and chemical attack

Attack by circulating proteins

Destruction of Antigens

Acquired vs Congenital Immunodeficiencies

- Acquired
  - Breakdown of protective barriers – skin integrity, central line, intubation,
  - Secondary to receipt of steroid therapy, chemotherapy, monoclonal Abs
  - Resulting from malnutrition, malignancy (eg, leukemia), etc

- Congenital Immune Defects (Primary Immunodeficiencies=PIDs)

Primary Immunodeficiencies (PID)

- ~130 different heterogeneous disorders resulting from defects affecting immune system development or function
- Originally thought to be rare (incidence 1-2 per 50,000); then 1:2,000 to 1:10,000 live births
- Recent estimated prevalence of 1 per 1200 patients (US survey, 10K households)
  - *How many pts could have a PID in your practice?*

- > 250 individual genetic defects described
- Diagnosed from infancy to adulthood
- Knowledge gaps can delay Dx and treatment, leading to increased morbidity and mortality
- Distinct susceptibility to pathogens dependent on defect(s)

O'Keefe AW, Halbrich M, et al. Primary Immunodeficiencies for the primary care provider. Paediatr Child Health 2016;21:e10
Notarangelo LD. Primary Immunodeficiencies. J Allergy Clin Immunol 2010:125:S182

Immunodeficiencies due to Innate Immune System Defects

Innate Defects

- Phagocyte Defects
  - CGD, Congenital neutropenia
- Complement (rare, < 1% of cases)
  - Severe, recurrent infections with encapsulated organisms
- Pattern recognition receptors
- NK cell defects
- **Presentation** variable but includes:
  - cold abscesses, impaired wound healing
  - Occ severe infections with minimal signs
    - May not mount typical inflammatory responses or have fever

Humoral and Cell-Mediated Immune (CMI) Defects

Humoral / Antibody Defects

- IgA deficiency
- Common Variable Immune Deficiency (CVID)
- X-linked Agammaglobulinemia
- Specific Ab deficiency
- **Pathogens** include:
  - *Strep pneumo, H influenza, others*
- **Management:**
  - *Replacement Ig, timely Rx of infections*
- **Other manifestations:**

  autoimmunity, esp cytopenias, SNHL
- **IgG nadir ~5-6mos, lower in premature infants**

T Cell Impairment

- Combined immunodeficiency syndromes
- SCID
- **Pathogens** include:

  Gram negative, mycobacterial, parasitic, viral or fungal infections
- **Other manifestations:** *autoimmunity*
- **Normal ALC varies with age**

CD4+ Lymphocyte Counts in Children

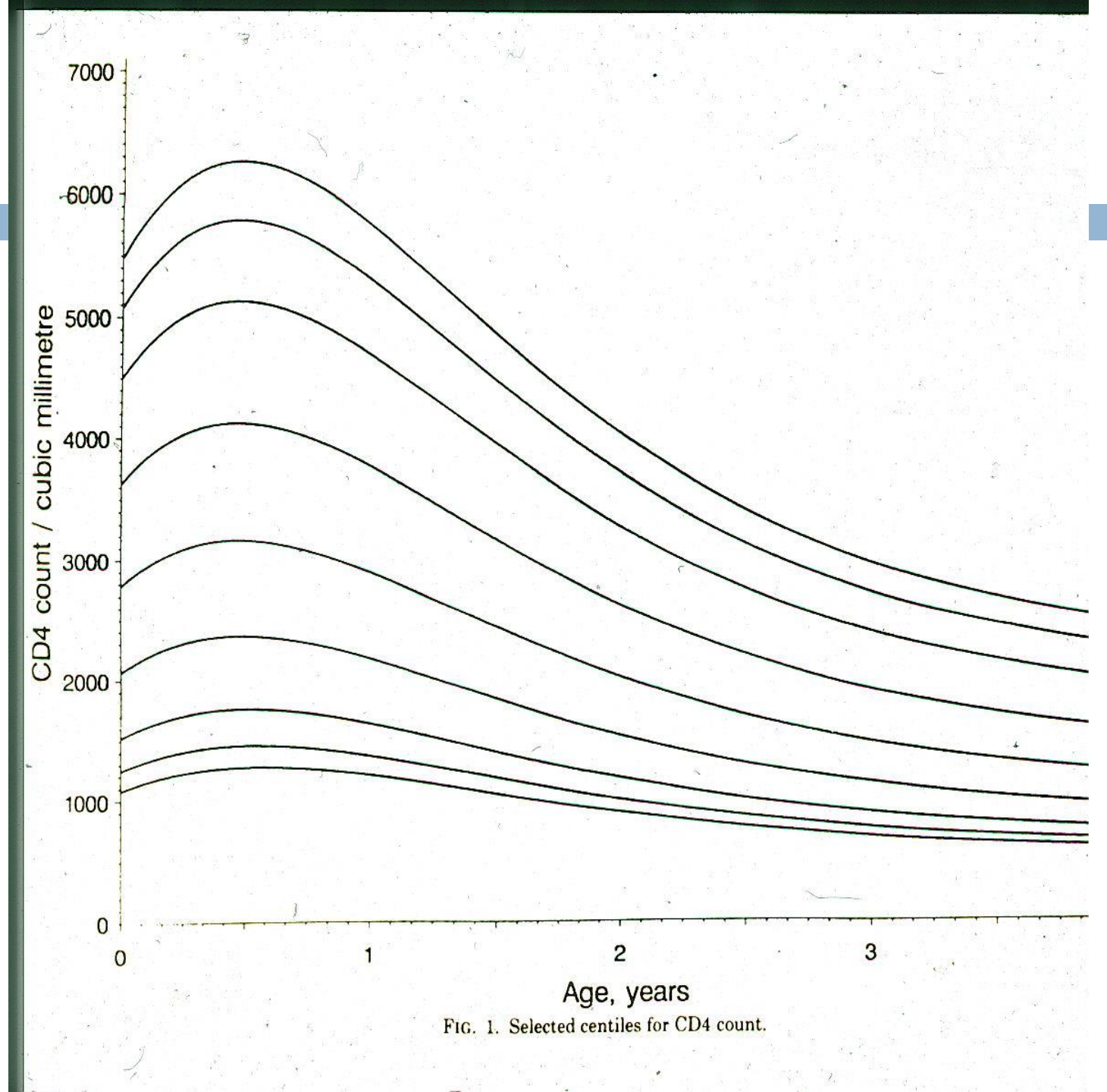


FIG. 1. Selected centiles for CD4 count.

Genetic syndromes associated with immunodeficiency:

- DiGeorge Syndrome
- CHARGE Syndrome
- Ataxia-Telangiectasia

Table 2 Overview of well characterised human primary immunodeficiencies (PIDs)

Representative diseases	Estimated prevalence	Genetic defect(s)
A. Predominantly antibody deficiencies (~65% of all PIDs)		
X-linked agammaglobulinaemia (Bruton's disease)	1:70000 to 1:400000	*BTK*
Autosomal recessive agammaglobulinaemia	Rare	μ heavy chain (*IGHM*), Igα (*CD79A*), Igβ (*CD79B*), λ5 (*IGLL1*), *BLNK*
Common variable immunodeficiency (CVID)	1:25000 to 1:50000	~15% of patients have variants in *ICOS*, *TACI*, *BAFFR* or *Msh5*
Selective IgA deficiency	1:500 (majority are asymptomatic)	Unknown
IgG subclass deficiency	Uncertain as most patients are asymptomatic	Unknown
B. Combined T and B cell (~15% of all PIDs)		
Severe combined immunodeficiency (SCID)	1:65000	*IL2RG, JAK3, IL7RA, ADA, RAG1, RAG2* and several others
Omenn syndrome	Rare	*RAG1, RAG2, Artemis, IL7RA*
C. Phagocytic defects (~10% of all PIDs)		
Chronic granulomatous disease	1:200000	*CYBB, CYBA, NCF1, NCF2*
Severe congenital neutropenia	1:300000	*ELA2, GF11, G-CSF3R, HAX1* (Kostmann syndrome)
Cyclic neutropenia	1:100000 to 1:1000000	*ELA2*
D. Other cellular immunodeficiencies (~5–10% of all PIDs)		
Wiskott–Aldrich syndrome	1:100000 to 1:1000000	*WASP*
DiGeorge syndrome (chromosome 22q11.2 deletion syndrome)	1:4000	Hemizygous deletions of chromosome 22q11.2
Hyper IgE syndrome	1:100000	*STAT3* (in autosomal dominant form)
Ataxia–telangiectasia	1:250000	*ATM*

Turvey SE, et al. Postgrad Med J 2009;85:660

Age at Diagnosis - variable

- SCID - mean age at Dx 97days
  - beyond optimal time for HSCT (<90 dys)
- CGD – age at Dx ranges from infancy to adulthood
- CVID – median delay between Sx onset and Dx 5 yrs
- Combined immunodeficiency syndromes
  - usually present beyond infancy with immune dysregulation
    - infection, autoimmunity or malignancy
  - Often from hypomorphic mutations in SCID-associated genes or partial defects in T cell development

Testing for Immune Deficiencies

Firstline Testing

After Hx, FHx, PE

- CBC with differential
- QUIGs
- Ab response to vaccinations
  - Tet, dipth, pneu
  - Isohemagglutinins (AB)
- UA, Serum Alb and Total Protein
  - For hypogammaglobulinemia

More Specialized Testing

- Flow cytometry-lymphocyte subsets
- T cell receptor diversity
- Lymphocyte proliferation
- Complement levels & function (CH50)
- Neutrophil oxidative burst
- Genetic testing
- Others based on presentation

Table 1 Clinical approach to human primary immunodeficiencies

Clinical presentation	Examples of common causative PIDs	Preliminary investigations	Specialised investigations	Therapeutic options (with evidence base)
Recurrent sinopulmonary infections	Antibody production defects ► common variable immunodeficiency (CVID) ► X-linked agammaglobulinaemia (XLA) ► transient hypogammagobulinaemia of infancy (THI) Complement protein deficiencies	Complete blood count with differential Quantitative serum immunoglobulin (Ig) levels (i.e. IgG, IgA, IgM, IgE) Specific antibody production ► titres against protein (tetanus, diphtheria) and polysaccharide (pneumococcus, blood group isohaemagglutinins) antigens Complement protein function ► CH_{50} and AH_{50}	Enumeration of lymphocyte subsets including T, B and NK cells T and B cell in vitro functional assays Quantification and/or functional assessment of individual complement proteins Genetic analysis	Antimicrobial therapy (III) ► treatment ► prophylaxis Immunoglobulin replacement (IIb) ► intravenous (IVIG) ► subcutaneous (SCIG)
Broad infectious susceptibility and failure to thrive	Severe combined immunodeficiency (SCID) Other combined immunodeficiencies ► Wiskott–Aldrich syndrome ► DiGeorge syndrome ► Ataxia–telangiectasia	Complete blood count with differential Quantitative serum immunoglobulin levels (i.e. IgG, IgA, IgM, IgE) Thorough characterisation of infecting pathogens Urgent consultation with a clinical immunologist	Enumeration of lymphocyte subsets including T, B and NK cells T and B cell in vitro functional assays Biochemical analysis (for adenosine deaminase and purine nucleotide phosphorylase deficiency) Genetic analysis	Antimicrobial therapy (III) ► treatment ► prophylaxis Immunoglobulin replacement (IIb) ► intravenous (IVIG) ► subcutaneous (SCIG) Haematopoietic stem cell transplantation (III) Gene therapy (currently experimental)
Granulomatous infections by catalase producing pathogens	Chronic granulomatous disease (CGD)	Complete blood count with differential	Measurement of phagocyte oxidase activity (preferably using the dihydrorhodamine 123 assay) Genetic analysis	Antimicrobial therapy (Ib) ► treatment ► prophylaxis (typically trimethoprim–sulfamethoxazole and itraconazole) Interferon-γ (Ib) Haematopoietic stem cell transplantation (III)

Turvey SE, Bonilla FA, Junker AK. Primary immunodeficiency diseases: a practical guide for clinicians. Postgrad Med J ***2009;85:660-666***

Potential Patient Diagnoses (Answers to Cases)

- Anatomical defects
- Leukocyte Adhesion Deficiency (LAD)/Variant
- Chronic Granulomatous Disease (CGD)
- Complement Deficiencies/Terminal C Deficiency
- Asplenia
- Cystic Fibrosis

- Severe Combined Immunodeficiency Syndrome (SCID)
- Selective IgA deficiency
- "HyperIgM Syndrome"
- Job's Syndrome (HyperIgE)
- Common Variable Immune Deficiency (CVID) & other Hypogammaglobulinemias
- X-Linked Agammaglobulinemia CVID
- Wiskott-Aldrich Syndrome
- DiGeorge Syndrome
- NEMO
- Human Immunodeficiency Virus (HIV)

Case #1

- 5 mo boy with severe pneumonia, originally treated with IV Ampicillin, then worsened so changed to Vancomycin and Ceftriaxone.
- Still no better, worsening hypoxia (Pulse oximetry 75% - increased to 92% on 50% non-rebreather)
- CXR: mild diffuse bilateral infiltrates
- Respiratory Viral Battery PCR - negative
- Due to lack of improvement, Quantitative Igs sent: IgG = 60
  - DDx??
  - ??CVID or Hypogammaglobulinemia
  - X-linked agammaglobulinemia
  - HIV infection
- However…not responding to V/Ceftx; worsening overall.
- Degree of hypoxia out of proportion to CXR findings

Case #1

- Pulmonary consulted and Bronchoscopy demonstrates…*Guesses?*
  - ***Pneumocystis jirovecii*** (previously *P. carinii*)
- Hmmmm…*what are you thinking?*
- *DDx:*
  - Human Immunodeficiency Virus
  - X-linked agammaglobulinemia
  - CD40 Ligand Deficiency (formerly “HyperIgM syndrome”)

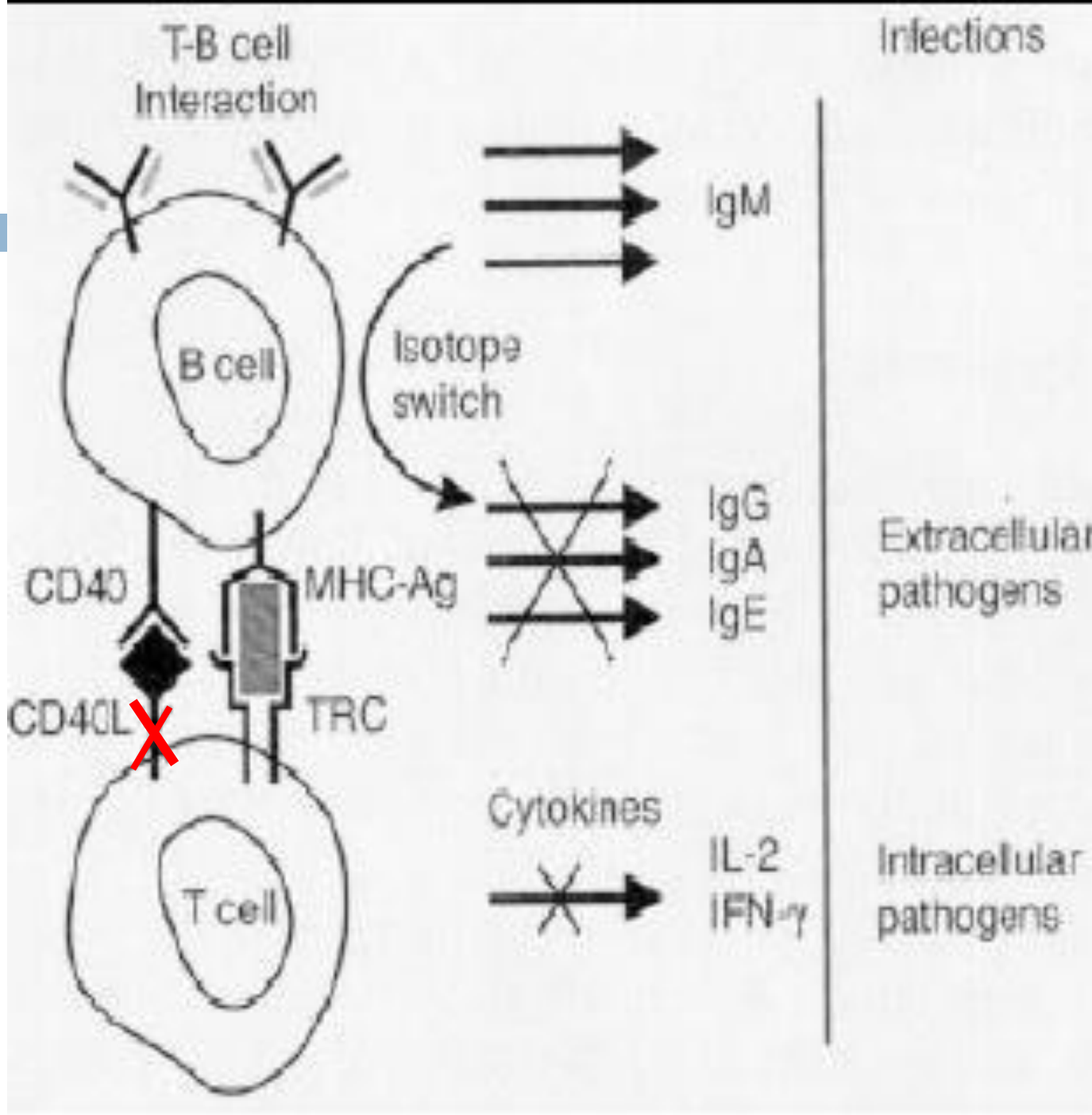
"Hyper IgM syndrome"- Class Switch Recombination (CSR) Defects

- Unable to switch from IgM antibody production to IgG, IgA or IgE antibodies
  - results in decreased IgG and IgA levels with normal or elevated IgM levels
  - lack response to protein antigens

Five Types of "Hyper IgM syndrome":

- Hyper-IgM syndrome type 1 (X-linked) - CD40LG gene mutations
  - **CD40 Ligand Deficiency**
- Hyper-IgM syndrome type 2 (autosomal recessive) - AICDA gene mutations
  - B cells cannot recombine genes to change heavy chain production
- Hyper-IgM syndrome type 3 - CD40 gene mutations
- Hyper-IgM syndrome type 4 -defect in class switch recombination downstream of AICDA gene
- Hyper-IgM syndrome type 5 - UNG gene mutations

"Hyper IgM Syndrome"
CD40 Ligand Defects/
Class Switch Recombination (CSR) Defects



CD40 Ligand Defects: Common Clinical Presentations

- *Pneumocystis jirovecii* pneumonia - common in 1st year of life
  - presentation in many with CD40 Ligand Deficiency (~40% of pts)
- Chronic Enteroviral meningoencephalitis (CD40L)
- Hepatitis (Hepatitis C)
- Chronic diarrhea – failure to gain weight
- Recurrent sinopulmonary infections (eg, pneumonia, sinusitis, OM), primarily due to encapsulated bacteria
- OI's, esp *Cryptosporidium* and *Histoplasma*
- Cryptococcal and Toxoplasma infections, can involve CNS
- Lymphadenopathy and hepatosplenomegaly – often found
- Cholangitis
- Neutropenia
- Arthritis
- Encephalopathy (degenerative)
- Hypothyroidism

"Hyper IgM Syndrome"/CD40 Ligand & CSR Defects: Management

- Immune globulin therapy (sq or IV)
- Antibiotic prophylaxis?
  - Perhaps in the setting of pts with bronchiectasis or recurrent sinusitis
  - TMP/SMZ prophylaxis for all with CD40L or CD40 deficiency
- Good hygiene – for ALL
- Monitor liver function
- HSCT – only definitive cure for CD40L (or CD40) deficiency

Case # 2

- Recurrent episodes of Upper and Lower respiratory infections and of Gastroenteritis

- DDx:
  - HIV
  - Selective IgA deficiency
  - CVID

- Selective IgA deficiency – Most common PID; ~1:700 affected
  - ~2/3rd adults with IgA deficiency asymptomatic
  - Remainder may have recurrent infections, autoimmunity or allergy

Notarangelo LD. Primary Immunodeficiencies. J Allergy Clin Immunol 2010:125:S182

Case #3

- 4 month old infant girl presenting with left cervical swelling (~2.5 x 3cm) with warmth and mild overlying erythema. Child cared for in home daycare.
- PMH: Admission at 2 months of age with fever and tachypnea, treated with IV Ceftriaxone until Blood cultures negative. Hospital records obtained, and CXR demonstrated RML & perihilar infiltrates, Hgb 9.4, Plts 250K, WBC 6,000 (65L, 3N, 32M). Thought c/w RSV bronchiolitis; discharged home
- Your thoughts?
  - Recurrent infections due to exposures
  - HIV
  - Hypogamm
  - X-linked agamm – but girl
- ENT performed aspirate of node and growing *Haemophilus aphrophilus*
- *Additional thoughts?*
  - ANC = 180 – DDx neutropenia?
  - BM suppression - viral, medication, etc
  - Transient neutropenia of infancy
  - Congenital neutropenia

Severe Congenital Neutropenia (SCN)

- SCN incidence - est 1 in 200,000
- Due to increased apoptosis of myeloid cells
- Usually present in infancy with very severe neutropenia, ANC <200/µL
- Referred to as "Kostmann Disease" in past

- Genetically heterogeneous group of related disorders
  - at least, 5 different mutations in genes involving neutrophil maturation and function
  - >50% due to ELANE gene mutations
  - ~15% HAX1 gene mutations, encodes mitochondrial protein HCLS1-associated X1 (*HAX1*)
    - Defect in affected Kostmann family descendants

- Inheritance: most sporadic; others AR, AD or X-linked

Clinical manifestations and Treatment

- Oropharyngeal problems, otitis media, respiratory infections, cellulitis, and skin infections, most often due to staphylococci and streptococci
- Oral ulcerations, painful gingivitis
- Diffuse gastrointestinal lesions
- Develop secondary malignancies
- High mortality in year 1 without intervention

- **Treatment**
  - G-CSF therapy –significant reduction in infections and improved QOL
  - Hematopoietic cell transplantation for selected patients
    - high G-CSF requirements or unresponsive to G-CSF

Normal values for white blood count and absolute neutrophil count in neonates and children

Age	WBC (cells/microL)	ANC (cells/microL)	Percent neutrophils (approximate)
Fetus >30 weeks	7710 (range 2720 to 12,700)		23% of nucleated cells including nucleated RBCs
Birth	18,100 (range 9000 to 30,000)	11,000 (range 6000 to 26,000)	61% of WBCs
24 hours	18,900 (range 9000 to 34,000)	11,500 (range 5000 to 21,000)	61% of WBCs
1 week	12,200 (range 5000 to 21,000)	5500 (range 1500 to 10,000)	45% of WBCs
1 month	10,800 (range 5000 to 19,500)	3800 (range 1000 to 9000)	35% of WBCs
1 year	11,400 (range 6000 to 17,500)	3500 (range 1500 to 8500)	31% of WBCs
10 years	8100 (range 4500 to 13,500)	4400 (range 1800 to 8000)	54% of WBCs

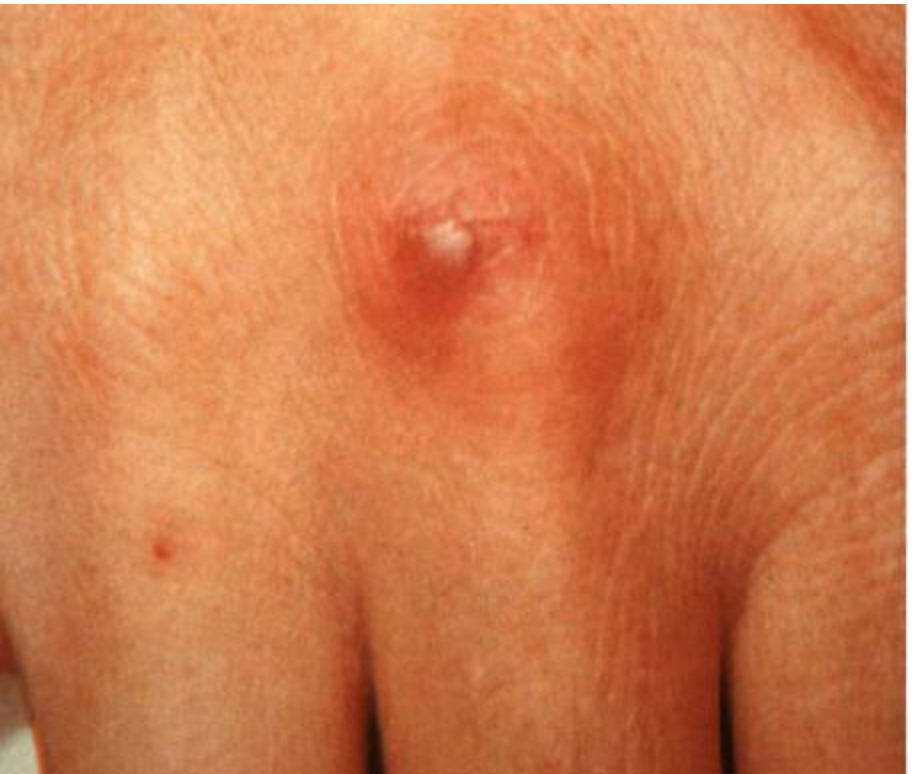
Refer to UpToDate content on neutropenia in children for information about causes of neutropenia and appropriate interventions, and an ANC calculator. Percent neutrophils depends on the percentages of other cells, and ANC should always be used when evaluating neutropenia; this value is presented only as a guide.

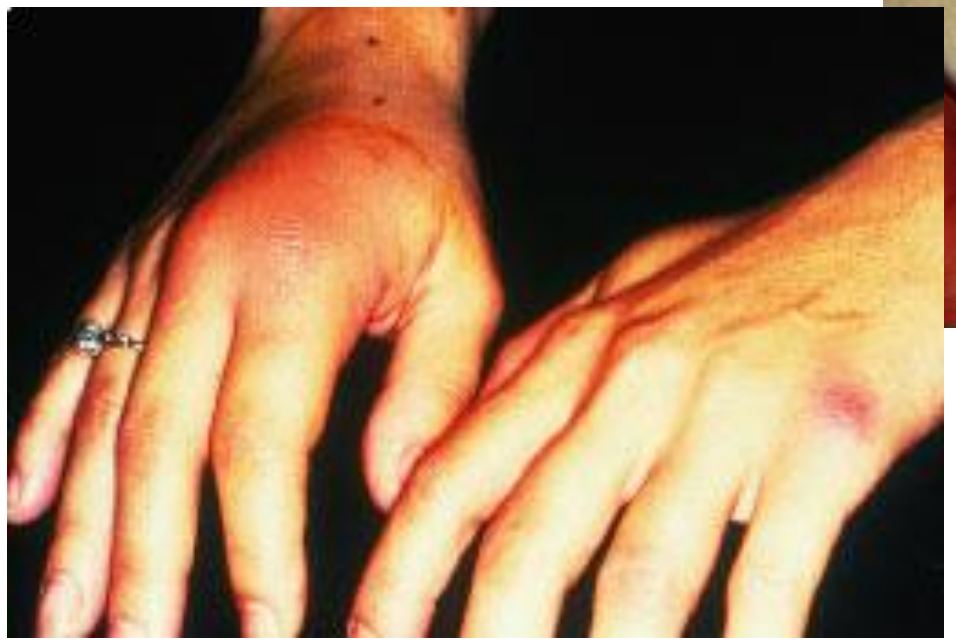
WBC: white blood cell count; ANC: absolute neutrophil count; RBCs: red blood cells.

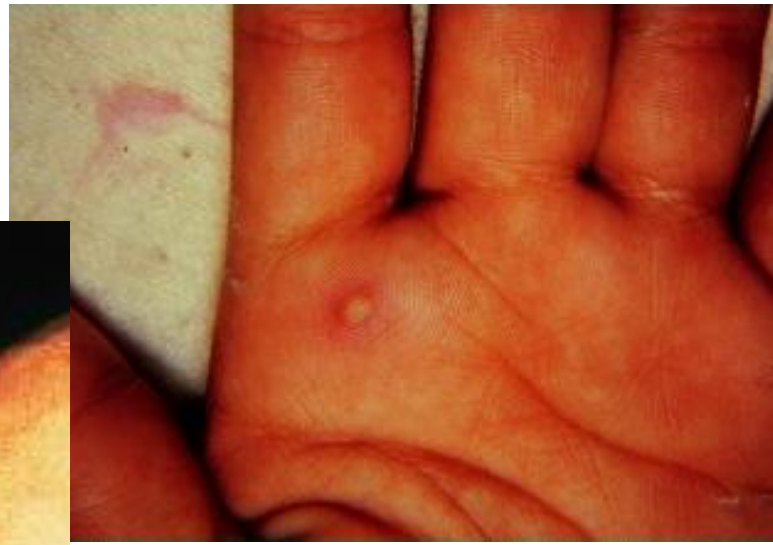
Adapted from Orkin SH, Nathan DG, Ginsburg D, et al. Nathan and Oski's Hematology of Infancy and Childhood, 7th Edition, Saunders, Philadelphia 2009.

Case #4

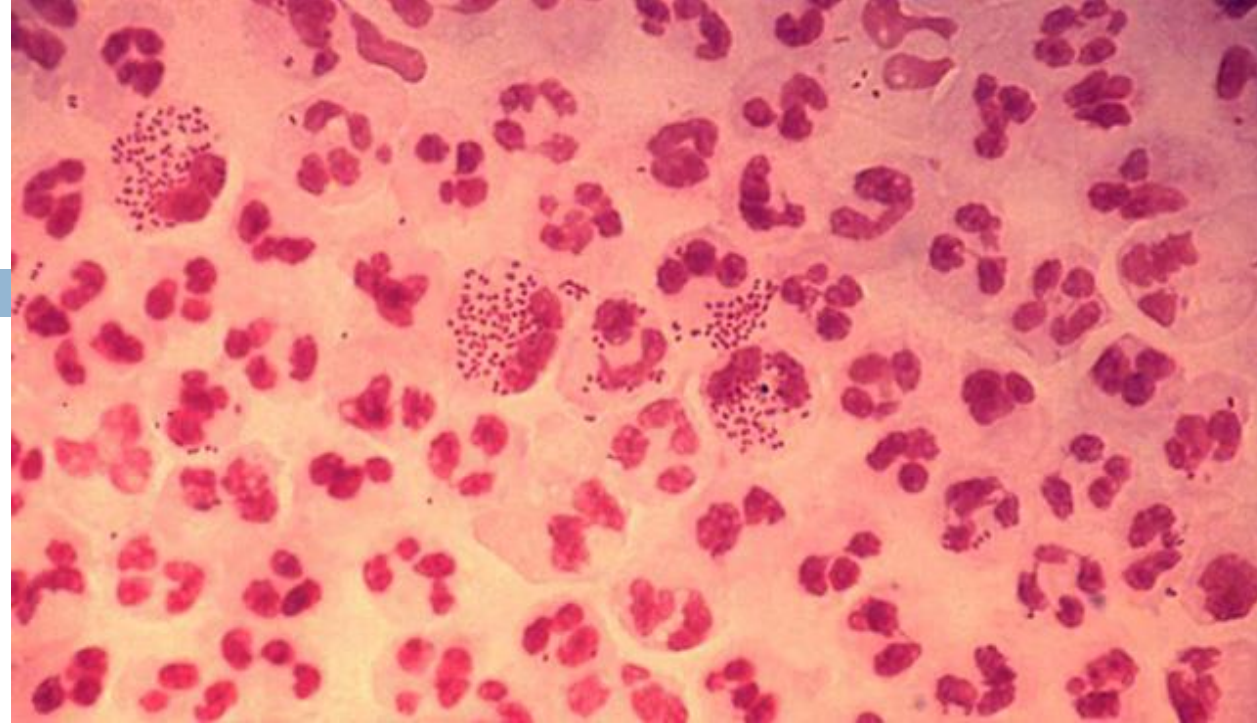
- 16 yo female presents with fever, migratory polyarthralgia (wrists, ankle, hands) and a distal rash (hands and feet), described as pustular rash with erythematous base. Ill appearance







Case 4



- Admitted & now Blood cx + with:

What is the pathogen?

What immunodeficiency might you worry about?

- **PMHx:** mother describes daughter having meningitis during elementary school, and the whole family had to take 4 doses of an Abx over 2 days

- What testing would you send for what immunodeficiency?
- CH50 to evaluate for Terminal Complement (C5b – 9) Deficiency

Case #5

- 10 year old boy presents with focal left lower lobe pneumonia with fever (40C) and hypoxia. Placed on IV Ampicillin without improvement. Changed to Ceftx without improvement, so Vancomycin added & therapeutic levels attained. He continues to worsen and is transferred to the PICU due to worsening respiratory distress and increased hypoxia.
- **PE:** notable for Ill child in respiratory distress on 50% FiO2
  - + rales noted left anterior chest.
  - Extremities with cutaneous scars (mother states from old skin infections)
  - Primary teeth in place even though secondary teeth have erupted
- **CXR**: + dense lobar infiltrate
- On further questioning, she has a **PMH** of suppurative axillary adenitis s/p I&D with growth of *S. aureus*
- **FHx:** Younger brother with Hx of perirectal abscess as infant (UK pathogen) and Staphylococccal hepatic abscess. He also received prolonged therapy for an osteomyelitis.

- *DDx??*
  - Neutropenia / Cyclic neutropenia
  - CVID or other Hypogammaglobulinemia
  - HyperIgE
  - Chronic Granulomatous Disease (CGD)

Chronic Granulomatous Disease (CGD)

- Genetically heterogeneous condition characterized by recurrent, life-threatening bacterial and fungal infections along with granuloma formation
  - Defective neutrophil function
  - X-linked and Autosomal inheritance
- Frequency ~1:200,000 US births
- Present - from infancy to late adulthood; majority diagnosed as children <5yo
- Majority of severe infections in No America due to 5 organisms:
  - *Staphylococcus aureus*
  - *Burkholderia cepacia* complex
  - *Serratia marsescens (infts with bone/joint infections, older with abscesses/sepsis)*
  - *Nocardia species*
  - *Aspergillus spp*
  - Less common pathogens:
    - *Chromobacterium violaceum* (found in brackish water, eg, near Gulf of Mexico), BCGosis, *Mycobacterium tuberculosis*, nontuberculous mycobacteria, Klebsiella, Salmonella
  - Fungal infections - leading causes of mortality

Chronic Granulomatous Disease (CGD)

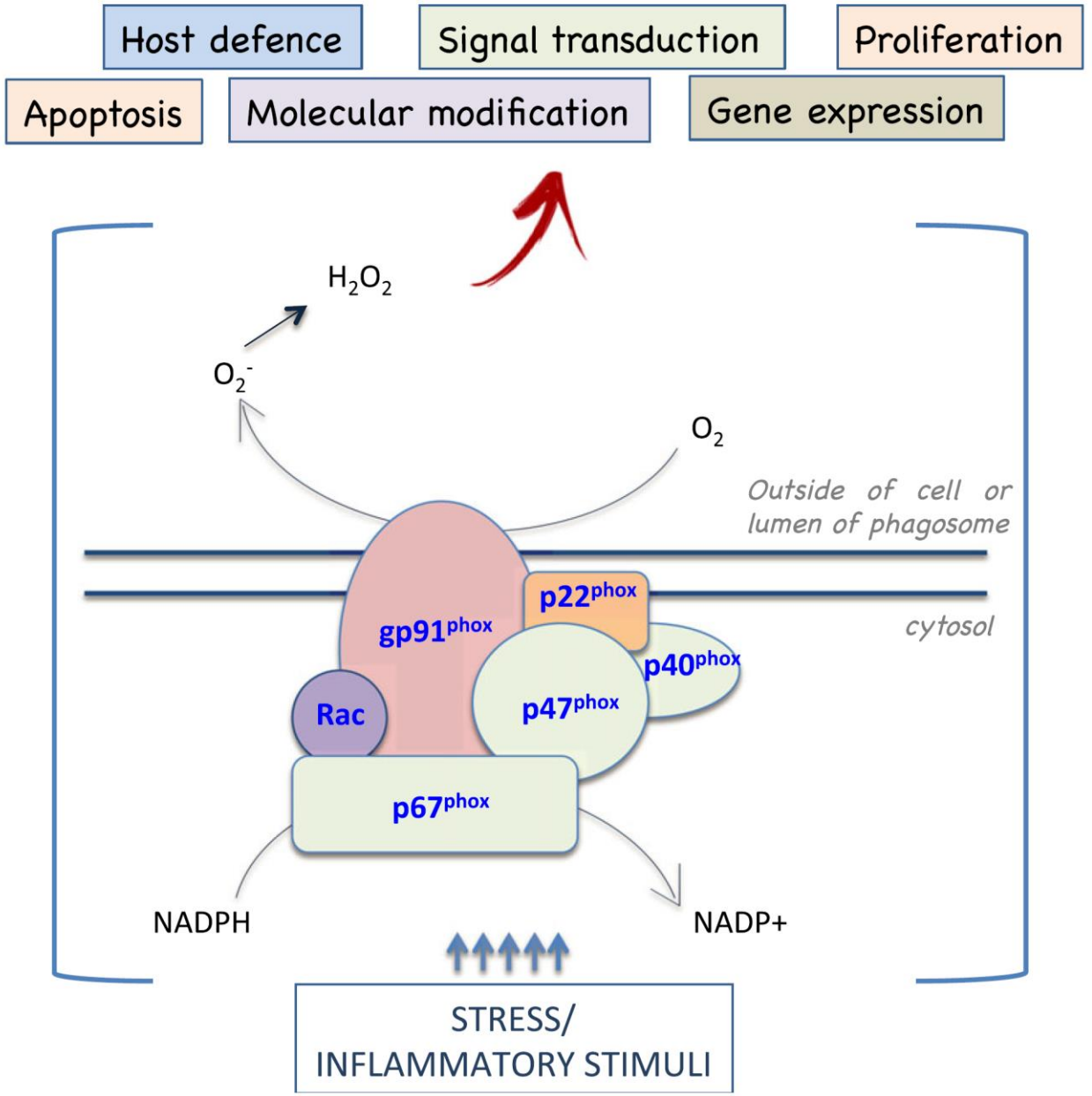
- **Sites / Types of infections** most often seen:
  - Pneumonia
    - Also lung abscesses, empyema, hilar lymphadenopathy
  - Abscesses (skin, tissue, organs)
    - especially perianal/perirectal and liver
  - Suppurative adenitis
  - Osteomyelitis
  - Bacteremia/Fungemia
  - Superficial skin infections (cellulitis/impetigo)
- Gingivitis, stomatitis, gastroenteritis, and otitis also
- Inflammatory granulomas can be seen (eg, gastric outlet obstruction, colitis)

Chronic Granulomatous Disease (CGD)

- Defects in the phagocyte Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the phagocyte oxidase (phox)
  - Enzyme complex responsible for the phagocyte respiratory burst
  - Phagocytes (neutrophils, monocytes, macrophages) unable to destroy certain microbes

- Genetic mutations in the NADPH oxidase complex
  - gp91phox, p47phox, p22phox, p67phox, p40phox
    - gp91phox - X-linked mutation; ~65 to 70% of cases
    - Other 4 - autosomal recessive mutations

CGD



To cite this article: Chiriaco M, Salfa I, Di Matteo G, Rossi P, Finocchi A. Chronic granulomatous disease: Clinical, molecular, and therapeutic aspects. *Pediatr Allergy Immunol* 2016: **27**: 242–253.

CGD: Diagnosis

- Neutrophil function testing - measure superoxide production
  - Dihydrorhodamine oxidation (DHR), Neutrophil oxidative burst, or Nitroblue tetrazolium – initial testing

- Confirmation testing: Genotype

Chronic Granulomatous Disease (CGD): Management

- TMP/SMZ - prophylaxis against *S. aureus, Nocardia*
- Itraconazole prophylaxis
- IFN-gamma
  - some controversy; reduced infections by 70% in large trial [1]

- **AVOID exposure to** mulch/lawn mowing, repotting plants, cleaning cellars, hay rides, smoking marijuana. Avoid swimming in non-chlorinated pools/lakes
  - Why?
  - Risk for life-threatening Aspergillus pneumonia
- BMT / HSCT – comparable survival

1 International CGD Cooperative Study Group. NEJM 1991;324:509

Case # 6

- 5 year old girl with 2 episodes of sinusitis, multiple otitides, pneumonia x 1
- DDx:
  - HIV
  - X-linked Agammaglobulinemia – girl; therefore, unlikely unless highly Lionized
  - Common Variable Immunodeficiency
  - Selective IgA deficiency – most common immunodeficiency

- What if recurrent / chronic diarrhea? Or enteroviral meningoencephalitis?

- DDx?
  - ~~Selective IgA deficiency –~~ not with enteroviral meningoencephalitis
  - HIV
  - X-linked Agammaglobulinemia – girls so unlikely unless highly Lionized
  - Common Variable Immunodeficiency

North Carolina State Laboratory of Public Health

Newborn Screening/Clinical Chemistry Branch

P.O. Box 28047
4312 District Drive
Raleigh, NC 27611-8047
http://slph.ncpublichealth.com
Phone: (919)733-3937
Fax: (919)715-8610

Abnormal

General Information — **SCID PILOT REPORT**

MED. RECORD NO:
BABY'S NAME: [redacted]
DATE OF BIRTH:
RACE: WHITE
MOTHER'S NAME:
ADDRESS:
CITY/STATE: RALEIGH, NC 27604

MULTIPLE BIRTH:
SEX: MALE
WEIGHT:
Feeding Type: Breast
MAIDEN NAME:
MOTHER'S SSN:
PHONE:

Specimen Information

Laboratory Number: 2016000204
RECEIVED DATE: 12/29/2016
DATE OF BIRTH: 02/18/2017
DATE BLOOD COLLECTED:
COLLECTED BY:
FIRST RBC TRANSFUSION:
COMMENTS:

TIME OF BIRTH: 06:00
TIME COLLECTED:
AGE AT COLLECTION:
TIME:

NBS Device Barcode: 79075675
FIRST BLOOD SPOT

SUBMITTER: 111111111
NO SUBMITTER NAME
NO SUBMITTER ADDRESS
UNKNOWN, NC 27610
PHONE: (919)555-1111

Laboratory Results

DISORDER: Severe Combined Immunodeficiency (SCID)
ANALYTE: T-cell Receptor Excision Circles (TREC)

SCID Result: Abnormal

Action Required:
High Risk for SCID. Consultation strongly recommended within 48 hours.

This report is for SCID Pilot only and is separate from the Newborn Screening panel.

* To convert to ng/dl * - Multiply ng/ml by 100

STUDIES SHOULD ALWAYS BE REPEATED WHEN CLINICALLY INDICATED

Report To: Health Care Provider

Report Delivery information:

Abnormal Report

You receive this report and a call about the positive test result in your patient:
TREC Cq is > 35
What do you do?

"High Risk for SCID. Consultation strongly recommended within 48 hrs."

T cell receptor excision circle (TREC)

- Overview of TREC screening test — T cell receptor excision circle (TREC) screening identifies infants who have low T cells.
- All typical infants with typical SCID have absent or very low production of T cells from their thymus, affecting both T cell number and diversity.
- Other diseases that have lymphopenia as a feature, such as other genetic syndromes (eg, DiGeorge) or conditions (eg, congenital heart disease), also lead to reduced circulating T cells. Thus, while the primary target of the TREC screening test is to identify infants with SCID, other diseases with TCL are secondary targets of this screening test.
- Formation of TRECs — T cell development occurs in the thymus, where T cell antigen receptor (TCR) gene rearrangements involve cutting and splicing of the DNA encoding the alternate variable, diversity, and joining (VDJ) segments to generate a wide repertoire of unique T cells with diverse specificities.
- Formation of T cell receptor excision circles (TRECs) from excised DNA occurs during programmed gene rearrangements in the thymus. One particular rearrangement, excision of the TCR delta gene locus in precursors of alpha/beta TCR expressing T cells, gives rise to the delta-Rec and psi-Joining segment-alpha TREC. This circular DNA molecule is produced late in maturation and is found in 70 percent of all thymocytes that express alpha/beta TCRs.
- Number of TREC copies per T cell reflects primarily the production of naïve T cells by the thymus, and normal TREC number is a biomarker for adequate autologous T cell production.
- Low or absent TREC numbers indicate either poor T cell production or increased T cell loss, provided adequate DNA

- Higher Cq # means more PCR cycles that did not detect trecs

Abnormal Result from NB Screen

TREC Cq ≥ 35 (Regardless of Birth weight); Cq's = # PCR cycles to detect TRECs

After call from State Lab NBS Follow-up & receipt of FAXed report, **what are your next steps?:**

1. Evaluate by phone immediately; arrange in-person evaluation within 24 - 48 hrs.
2. Determine if baby has Hx cardiac surgery/thymectomy, seizures or low calcium, which would suggest ??
   DiGeorge syndrome
3. Inquire about family history of immune deficiency or early infant death(s)
4. Early in the day, bring baby right back to exam room (don't leave in waiting room).
   PE: abnormal facial features, heart murmur, etc.
5. If baby is sick (unlikely), contact Immunology to decide if admission necessary.
   Do not send to Emergency Dept.
6. Avoid live virus vaccines, such as ??
   Rotavirus vaccines
7. Instruct parents to:
   Boil H2O for formula
   Pump and store breast milk until maternal CMV status known
   Initiate Reverse precautions
   Keep infant at home, Avoid daycare, Sunday school nursery, No sick visitors, etc.
8. Call Immunology (list provided by NBS lab)
9. Immunology to see baby w/in 3 days for evaluation & diagnostic testing
10. Provide educational materials to parents (from NBS Lab or PrimaryImmune.org download)
11. If infant needs a transfusion, what do you order?

Normal Birth Weight Borderline Results (Birth Wt ≥ 2300g)

TREC Cq between 32.5 and 34.9

- NBS Follow-up State Lab will FAX HC Provider **who will**:
  1. Call family to check on infant.
  2. Determine if infant has Hx cardiac surgery/thymectomy, seizures or low calcium to suggest DiGeorge syndrome
  3. Inquire about family history of immune deficiency or early infant death(s)
  4. Instruct parents to boil H20 for formula; pump and store breast milk until maternal CMV status known
  5. Avoid live virus vaccines
  6. Initiate Reverse precautions

- **HCP should repeat NBS within 48 hours**

North Carolina State Laboratory of Public Health

Newborn Screening/Clinical Chemistry Branch

P.O. Box 28047
4312 District Drive
Raleigh, NC 27611-8047
http://slph.ncpublichealth.com
Phone: (919)733-3937
Fax: (919)715-8610

Borderline Full term Preterm

General Information — **SCID PILOT REPORT**

MED. RECORD NO:
BABY'S NAME: [redacted]
DATE OF BIRTH:
RACE:
MOTHER'S NAME:
ADDRESS:
CITY/STATE: RALEIGH, NC 27604

MULTIPLE BIRTH:
SEX: FEMALE
WEIGHT: **2350 grams**
Feeding Type: Breast
MAIDEN NAME:
MOTHER'S SSN:
PHONE:

Specimen Information

Laboratory Number: 2017000016
RECEIVED DATE: 01/04/2017
DATE OF BIRTH: 02/18/2017
DATE BLOOD COLLECTED:
COLLECTED BY:
FIRST RBC TRANSFUSION:
COMMENTS:

TIME OF BIRTH: 04:45
TIME COLLECTED:
AGE AT COLLECTION:
TIME:

NBS Device Barcode: 3674656745
FIRST BLOOD SPOT

SUBMITTER: 111111111
NO SUBMITTER NAME
NO SUBMITTER ADDRESS
UNKNOWN, NC 27610
PHONE: (919)555-1111

Laboratory Results

DISORDER: Severe Combined Immunodeficiency (SCID)
ANALYTE: T-cell Receptor Excision Circles (TREC)

SCID Result: Borderline

Action Required:
Elevated Risk for immunodeficiency screening. Please collect a new filter paper specimen on form DHHS #3105 within **2 Days**. Complete all demographic information and write "SCID pilot" on the top of the form.

This report is for SCID Pilot only and is separate from the Newborn Screening panel.

* To convert to ng/dl * - Multiply ng/ml by 100

*****STUDIES SHOULD ALWAYS BE REPEATED WHEN CLINICALLY INDICATED*****

Report To: Health Care Provider
NO SUBMITTER NAME
ATTN:
NO SUBMITTER ADDRESS
UNKNOWN, NC 27610

Report Delivery information:
EIN: 111111111
Courier:

DATE OF REPORT: 03/07/2017

Normal BW with Borderline Report

*"Elevated Risk for immunodeficiency screening. Please collect a new filter paper specimen on form DHHS #3105 within **2 Days** of receipt of this report. Complete all demographic information and write "SCID pilot" on the top of the form."*

Normal Birth Weight Infant's 2nd specimen: Borderline Results

TREC Cq is between 32.5 to 35

- State Lab NBS Follow-up person calls and FAXes infant's HCP
- HCP to examine baby in person within 24 to 48 hours
  - Early in the day, bring baby right back to exam room (no waiting room exposure)
  - Assess for cardiac surgery/thymectomy, seizures or low calcium, to suggest DiGeorge syndrome
  - Ask about family history for immune deficiency or early infant death
- Instruct parents to boil H20 if formula feeding; if breast feeding, pump and store breast milk until mother's CMV status is known
- Avoid live virus vaccines
- Reverse precautions
  - Keep baby at home, no daycare, Sunday School Nursery, no sick visitors, etc
- Call Immunology (per NBS Lab list)
- Provide educational materials to parents (from NBS Lab or PrimaryImmune.org download)

- Immunology is to see within 7 days for clinical evaluation and testing

North Carolina State Laboratory of Public Health

Newborn Screening/Clinical Chemistry

P.O. Box 28047
4312 District Drive
Raleigh, NC 27611-8047
http://slph.ncpublichealth.com
Phone: (919)733-3937
Fax: (919)715-8610

General Information Borderline Preterm **SCID PILOT REPORT**

MED. RECORD NO:
BABY'S NAME: **E CRADDOCK**
DATE OF BIRTH: **02/18/2017**
RACE: WHITE
MOTHER'S NAME:
ADDRESS:
CITY/STATE: RALEIGH, NC 27604

MULTIPLE BIRTH:
SEX: FEMALE
WEIGHT: **2200 grams**
Feeding Type: Breast
MAIDEN NAME:
MOTHER'S SSN:
PHONE:

Specimen Information

Laboratory Number: 2016000203

NBS Device Barcode: 78786754

FIRST BLOOD SPOT

RECEIVED DATE: 12/29/2016
DATE OF BIRTH: 02/18/2017
DATE BLOOD COLLECTED:
COLLECTED BY:
FIRST RBC TRANSFUSION:

TIME OF BIRTH: 06:45
TIME COLLECTED:
AGE AT COLLECTION:
TIME:

SUBMITTER: 111111111
NO SUBMITTER NAME
NO SUBMITTER ADDRESS
UNKNOWN, NC 27610
PHONE: (919)555-1111

Laboratory Results

DISORDER: Severe Combined Immunodeficiency (SCID)
ANALYTE: T-cell Receptor Excision Circles (TREC)

SCID Result: **Borderline**

Action Required:
Elevated Risk for immunodeficiency screening. Please collect a new filter paper specimen on form DHHS #3105 within **7 Days**. Complete all demographic information and write "SCID pilot" on the top of the form.

This report is for SCID Pilot only and is separate from the Newborn Screening panel.

* To convert to ng/dl * - Multiply ng/ml by 100

*****STUDIES SHOULD ALWAYS BE REPEATED WHEN CLINICALLY INDICATED*****

Report To: Health Care Provider
NO SUBMITTER NAME
ATTN:
NO SUBMITTER ADDRESS
UNKNOWN, NC 27610

Report Delivery information:
EIN: 111111111
Courier:

DATE OF REPORT: 03/07/2017

Borderline Low BW Report

Comment: Elevated Risk for immunodeficiency screening. Please collect a new filter paper specimen on form DHHS #3105 within **7 Days** of receipt of this report. Complete all demographic information and write “SCID pilot” on the top of the form.

Borderline Results Low Birth Weight (BW <2300g)

TREC Cq results are between 32.5 and 34.9

- State Lab Follow-up Person to FAX HCP
- HCP will
  - Check on infant (if discharged), ask about seizures or history of low calcium
  - Determine if infant had cardiac surgery/thymectomy
  - Inquire about family history of immunodeficiency or early infant death
  - Instruct parents to boil H20 for formula or to pump and store breast milk until mother's CMV status known
  - Avoid live virus vaccines
  - Reverse precautions if in hospital
    - If discharged, keep infant at home, no daycare or Sunday school nursery.
    - No sick visitors

  - **Repeat NBS at 2 weeks of age, and every 2 weeks until normal**
  - If not normal by 36th week of gestation, consult Immunology (using list from NBS Lab).
  - If in NICU at non-academic center, neonatologist should contact Immunology
  - Immunologist will see patient within 3 days and order diagnostic tests

Human Severe Combined Immunodeficiency (SCID)

- Fatal syndrome of diverse genetic origin, characterized by absence of T and B cell (and sometimes NK cell) functions

- 13 different genes mutations found to cause syndrome, to date

Thirteen SCID-associated Gene Mutations

- Cytokine Receptor Genes
  - *IL2RG*
  - *JAK3*
  - *IL7Rα*
- Antigen Receptor Genes
  - *RAG1*
  - *RAG2*
  - *Artemis*
  - *Ligase 4*
  - *DNA-PKcs*
  - *CD3δ*
  - *CD3ε*
  - *CD3ζ*
- Other Genes
  - *ADA*
  - *CD45*

SCID Lymphocyte Phenotypes

- T-B+NK-
  - γc-deficient
  - Jak 3-deficient
- T-B+NK+
  - IL-7Rα-deficient
  - CD3δ-deficient
  - CD3ε-deficient
  - CD3ζ-deficient
  - CD45-deficient
- T-B-NK-
  - ADA-deficient
- T-B-NK+
  - RAG1/RAG2-deficient
  - Artemis-deficient
  - Ligase 4-deficient
  - DNA-PKcs

Human Severe Combined Immunodeficiency (SCID)

- AKA the “boy in the bubble” disease
- Usually present before 1st birthday; if unrecognized, often fatal in 1st yr
- Manifestations include:
  - FTT, Chronic diarrhea, respiratory infections, oral/cutaneous Candidiasis
- AVOID giving infants what in your office?
  - Live vaccinations, notably Rotavirus, MMR, varicella
- SCID – medical emergency requiring immediate treatment (Abx, etc), protective isolation, replacement Ig, and immune reconstitution (HSCT or Gene therapy)
  - Early ID and intervention associated with decreased morbidity & mortality
  - HSCT in those Dx with NB screen have survival rates of 90% vs 40% in infants diagnosed later
  - More common than previously thought based on NB screening in CA
    - incidence of SCID & non-SCID immunodeficiencies est at 1 in 66,250 births, higher than thought

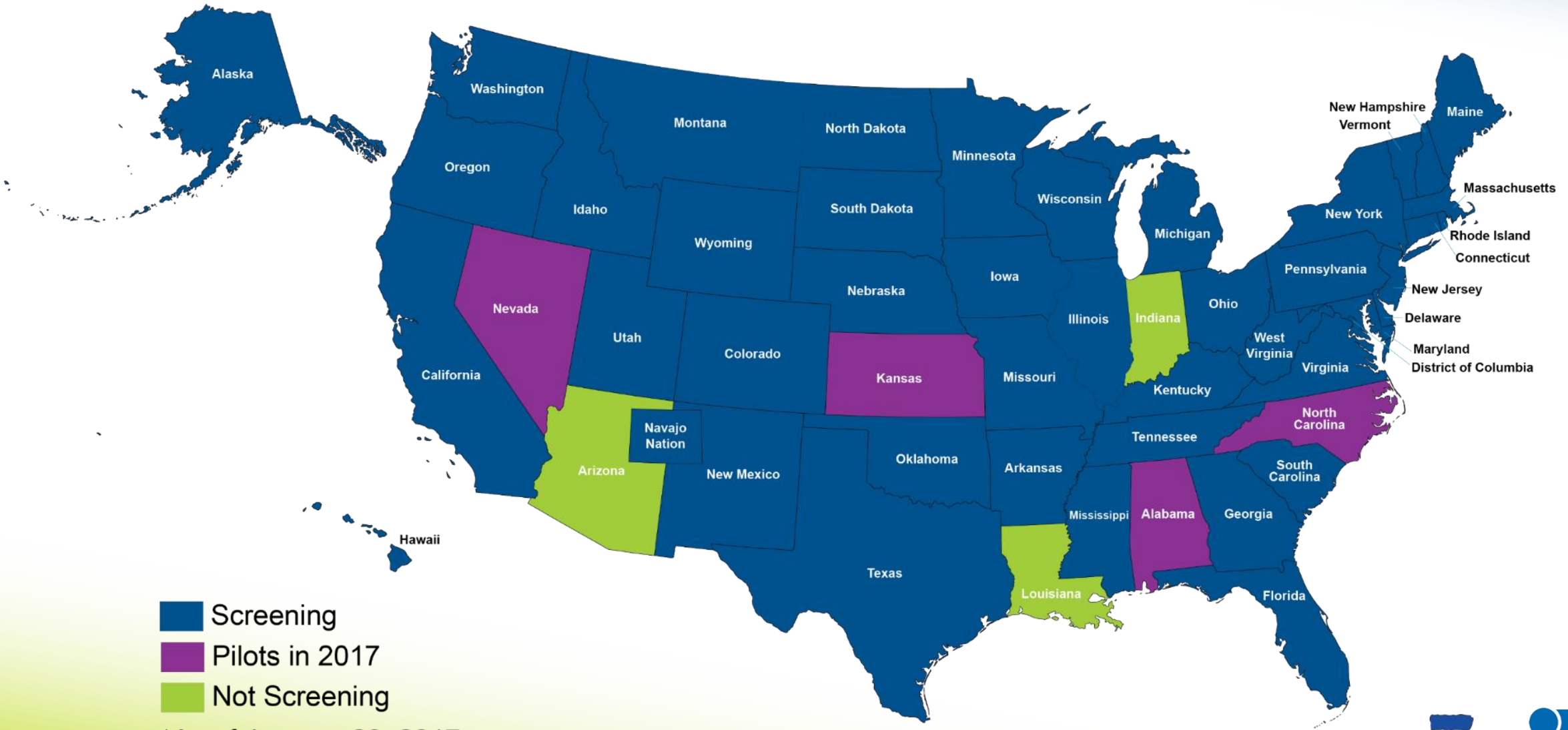
Important Dates

- January 2010 - U.S. Secretary's Advisory Committee for Heritable Disorders of Newborn and Children (SACHDNC) unanimously recommended adding SCID to conditions routinely screened for at birth
-
- May 2010 - HHS Secretary Sebelius adopted Committee's recommendation to add SCID as a core condition, and related T cell lymphopenias as secondary conditions; endorsed as a national standard

- January 2011 - North Carolina Newborn Screening Committee unanimously approved adding SCID to the NC panel. Did not happen for various reasons
  - October 2015 - Governor McCrory signed Baby Carlie Bill to mandate SCID NB screening in NC
  - Pilot began in NC on April 24th, 2017

43 States Currently Screen for SCID

90% of all newborns in the U.S. have undergone SCID screening



Positive Screens: Referral

- Health care provider (HCP) should refer neonate with a positive screen to an immunologist first, not a transplant center
  - TREC testing picks up other T cell lymphopenic conditions besides SCID, many of which do not require transplantation
  - Some infants have received un-necessary or inappropriate transplants

- New NC Committee of ABAI Board Certified Allergists/Immunologists who will perform and evaluate confirmatory testing results of initial positive screens

- No need to hospitalize positively screened newborn but parents should implement reverse precautions at home.

Conditions with Low or Absent T Cells Detected by TREC Screening

Multisystem syndromes with variable T cell deficiency

57% DiGeorge/chromosome 22q11.2 deletion
15% Trisomy 21
3% Ataxia telangiectasia
2% CHARGE syndrome
FOXN1 mutations

Secondary T cell lymphopenia

25% Congenital cardiac anomalies
38% Other congenital anomalies
13% Vascular leakage, third spacing, hydrops
3% Neonatal leukemia

Extreme prematurity alone—T cells normalize over time

“Variant SCID” or Idiopathic T lymphopenia

Low T cells and TRECs, low naïve CD45RA T cells, impaired T cell or antibody responses, no known gene defect

T cell Deficiencies with *Normal* TREC Levels

- Zap70 deficiency
- MHC class II deficiency
- X-linked Hyper IgM (CD40L deficiency)
- Wiskott Aldrich Syndrome
- HIV infection/AIDS

Diagnostic Evaluation of Infants with Low TRECs on NBS

- Diagnostic/confirmatory testing per Immunology:
  - CBC with manual differential
  - Flow cytometry
  - T cell function (mitogen stimulation) testing
  - If all of above are normal, repeat NBS in 1-2 months because some defects, such as ataxia telangiectasia, will still have abnormal TREC testing despite normal results in the above tests

- Immunology will interpret diagnostic results, direct management and determine if transplantation indicated

Screening Immunologic Evaluation for Infants with Low TRECs on NBS (Positive result)

- **Absolute Lymphocyte Count (ALC)** from CBC with manual differential
  - ALC should be >2,500/cmm
- **Flow cytometry:** CD3, CD4, CD8, CD19, CD16/56,
  - Naïve T cells(CD4/CD8/CD45RA)
  - Memory T cells (CD4/CD8 CD45RO)
  - Recent Thymic emigrants: CD4/CD45RA/CD62L
- **T cell function** studies (PHA, Con A & PWM stimulation)

Screening Infants with Low TRECs on NBS (Positive result)

- If ALC not low
  - infant may have **transplacentally transferred maternal T cells, Omenn's syndrome or "leaky" SCID**

- If **no T cells on Flow cytometry**
  - SCID is presumptive diagnosis but could be complete DiGeorge or FOXN1

- Normal infants should have:
  - >90% naïve (CD45RA+) T cells
  - & normal T cell function

Key Flow Cytometry and T Cell Function Study Findings for Screen Positive Infants

- Partial DiGeorge syndrome patients or those with other etiology for T cell lymphopenia may have normal flow cytometry and T cell function

- Classic SCID infants with maternal T cells, “leaky SCID” or Omenn's syndrome would all have a majority of the T cells being **memory (CD45RO+) T cells**

- T cell function studies would be abnormal in classic SCID infants with or without maternal T cells, and in those with “leaky SCID” or Omenn's syndrome.

- ***If flow cytometry and T cell function are both abnormal, parents informed infant will likely need a bone marrow or thymus transplant*.**

Assessment Algorithm for Screen Positive Results

- Screen picks up infants with DiGeorge syndrome, so HCP should inquire about Hx of neonatal hypocalcemia, and note if heart murmur and/or lowset ears or a FISH mouth are present

- Genetic assessments by chromosomal microarray, FISH for 22q deletion (DiGeorge). Molecular testing for FOXN1 mutation.

- Most infants with DiGeorge syndrome will have Partial DiGeorge with only slightly low T cell percentages and have significant or normal T cell function
  - important because partial DiGeorge pts do not require a transplant or specific immune treatment

- Infants with Complete DiGeorge (<1/100 of those with DiGeorge Syndrome) will look like a SCID pt on flow cytometry and T cell function studies
  - will need a thymus transplant, not a stem cell transplant. Same for FOXN1.

Brief Cases: what's your diagnosis?

- Recurrent episodes of pneumonia with residual interstitial changes noted on follow-up CXR
  - Cystic Fibrosis
  - Dysmotile Cilia

- Recurrent pneumonia, always RML or LLL…
  - Anatomical anomaly
  - Foreign body aspiration …..baseball foods

- 3 week old with CHD, fever and seizures, CXR with narrow mediastinum, Hgb 12, WBC 9K (75N, 15B, 10L, 10M)
  - Sepsis, meningitis, endocarditis
  - DiGeorge Syndrome (ALC = 900)
  - DDx Lymphopenia – sepsis, toxin, BM suppression, Ehrlichiosis, steroid therapy, HIV infection, …

- Fully vaccinated 4 ½ yo with a history of Streptococcus pneumonia Bacteremia, presenting now with meningitis due to Haemophilus influenza
  - HIV
  - CVID
  - Asplenic (functional, post-traumatic or congenital asplenia)
  - Complement deficiency - rare

Case:

- 4 week old or 6 week old (check on)
- Parents bring as child's umbilical cord has not fallen off yet. They have been cleaning frequently with alcohol, because grandmother insisted
- child otherwise seems well, without fevers, gaining weight

- What are possibilities?
  - LAD- Leukocyte Adhesion Deficiency, type 1??
  - Vigorous cleaning with alcohol by parents
  - ??neutropenia
  - Other WBC defects…?

LAD- Leukocyte Adhesion Deficiency, type 1??

- Defect in WBCs and ability to migrate – chemotaxis
- Leukocytosis – in all with type ??
- Expand
- Rare

Case:

- 9 yo who presents with a Hx of recurrent boils and abscesses on his skin, ?eczema, also with history of pneumonia
- CXR reveals lobar pneumonia with pneumatocoeles

- Thoughts?
- HyperIgE (AKA "Job's Syndrome")
  - Immune defect
  - Markedly elevated IgE levels (range) – IgE directed against Staphylococcus
  - Rare – incidence
  - Coarse facial features (add photo)

TABLE 1

Key infections and investigations in primary immunodeficiencies

Category	Principle infectious presentations	Key investigations
Humoral (defects primarily in B cells and antibody production)	Encapsulated bacteria, sino-oto-pulmonary infections	IgG, A, M and E levels Antibody response to vaccines
Combined (defects in T and B cells)*	Opportunistic infections with bacteria, viruses and fungi	CBC (lymphopenia) lymphocyte subsets (flow cytometry) lymphocyte stimulation tests T cell receptor diversity Antibody response to vaccines
Innate (including defects in phagocytes, pattern recognition receptors and complement activation)	Pyogenic infections Absence or mild signs of inflammation Neiserria infections	Neutrophil oxidative burst index Assessing classical and alternative complement patheways

**B cells require interaction with T cells to generate a normal response. As such, defects in T cell function also result in humoral/antibody dysfunction. CBC Complete blood count; Ig Immunoglobulin*

Paediatr Child Health Vol 21(2)March 2016
E10-e14, Primary immunodeficiency for the primary care provider. AW O'Keefe, M Halbrich, M Ben-Shoshan, C McCusker

American Academy of Allergy Asthma & Immunology

Table 12. Primary Immune Deficiency

Referral Guideline	Rationale	Evidence Type
Any of the following warning signs: • Eight or more new infections within one year; • Two or more serious sinus infections within one year; • Two or more months on antibiotic with little or no effect; • Two or more pneumonias within 1 year; • Failure of an infant to gain weight or grow normally; • Recurrent deep skin or organ abscesses; • Persistent thrush in mouth or elsewhere on skin after age 1 year; • Need for intravenous antibiotics to clear infections; • Two or more deep seated infections; • A family history of immune deficiency.	Frequent infection, unusual infections or unusual complications of usual infections are the most frequent presentation of immune deficiency[1-7]. Advanced diagnostic strategies are necessary to ensure appropriate diagnosis and treatment.[1,6-9] Allergist/immunologists are trained to diagnose and treat primary immunodeficiency[10]. Immunologic therapy improves immunity[11,12], reduces infections[13-15], improves organ function[16], prevents complications[1], improves quality of life[17], and may be curative[18,19] in patients with primary immune deficiencies.	Diagnostic Indirect outcome (immunologic therapy)

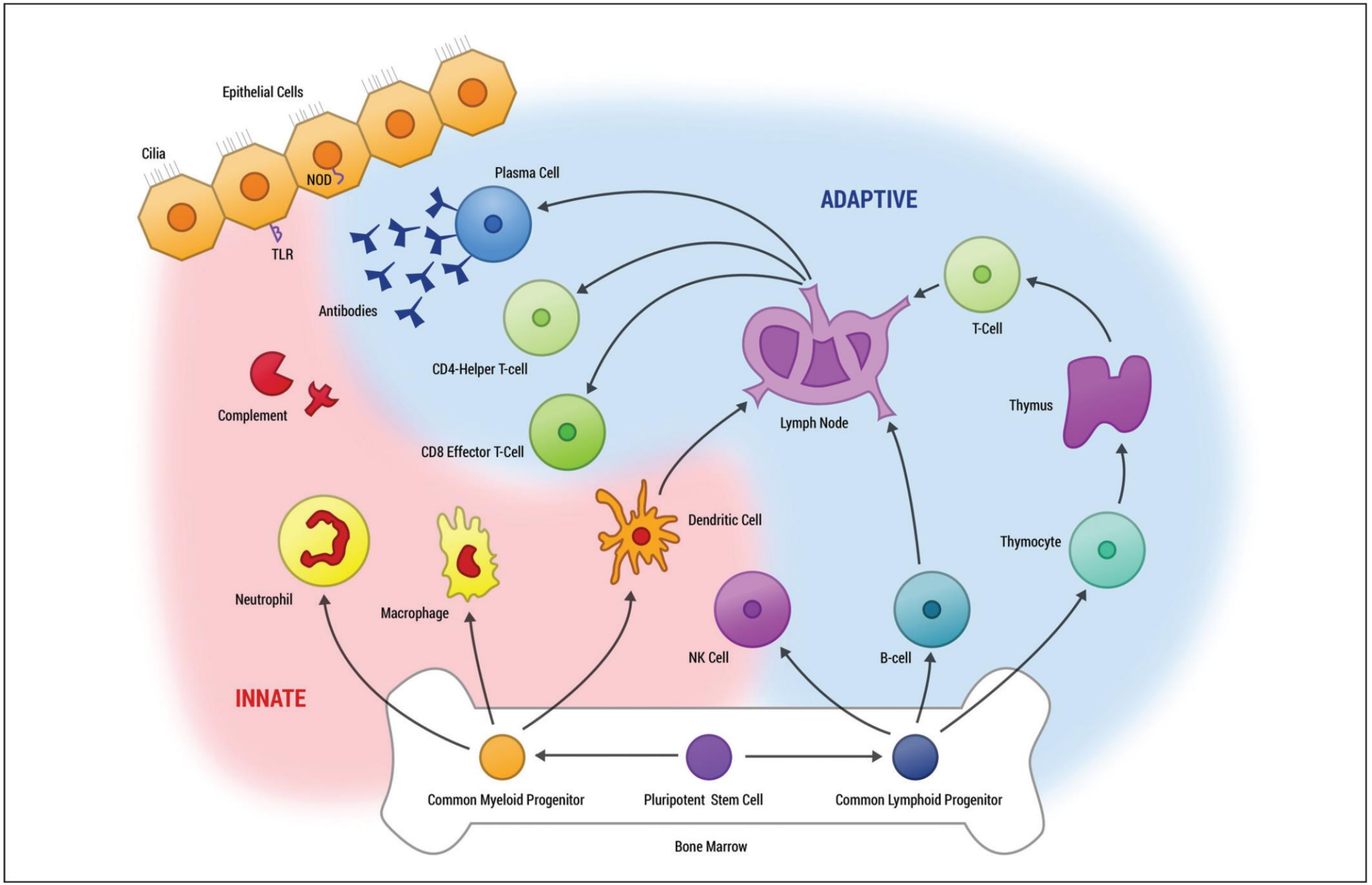


Figure 1) *The immune system can be broadly divided into two arms: innate and adaptive. Innate components include barrier defences (such as epithelial cells), complement, neutrophils and macrophages. Adaptive immunity is composed of B cells, the antibodies they produce, as well as helper and cytotoxic T cells. Immunodeficiency impacts one or more of these components. NK Natural killer; TLR Toll-like receptor*

Table 1
Basic laboratory testing of the immune system.

Type of immunity	Name of test	Description of test	Disease with abnormal results
Phagocytes	Absolute neutrophil count	Evaluation of neutrophil number in the peripheral blood	SCN (low neutrophils), LAD-1 (high neutrophils)
	Bone marrow biopsy	Aspiration and biopsy of marrow space	SCN
	Dihydrorhodamine (DHR) assay	Oxidation of DHR by superoxide to assess the respiratory burst	CGD
	CD18 expression	Flow cytometry to detect CD18	LAD-1
Complement	CH50	Functional assay of the classic complement pathway	Disorders of individual complement proteins
	AH50	Functional assay of the alternative complement pathway	Disorders of individual complement proteins
B-cell	Immunoglobulin levels	Measurement of serum levels of IgA, IgM, and IgG; IgG will reflect maternal transfer	XLA, CD40L, CID/SCID, 22Q11.2DS, WAS, X-ED-ID, IPEX
	B-cell maturation panel	Flow cytometry to detect different subsets of maturing B-cells	XLA, CD40L, CID/SCID, 22Q11.2DS, WAS, X-ED-ID
	Antibody titers	Antibody response to protein and polysaccharide vaccines	XLA, CD40L, CID/SCID, 22Q11.2DS, WAS, X-ED-ID, IPEX
T-cell	Absolute lymphocyte count	Evaluation of lymphocyte number in the peripheral blood	CID/SCID, XLA, 22Q11.2DS, WAS
	Lymphocyte immunophenotype	Flow cytometry to enumerate number of T-, B-, and NK-cells; additional markers including RA/RO can be used to further classify maturity of T-cells	XLA, CID/SCID, 22Q11.2DS, WAS, IPEX
	T-cell receptor excision circle (TREC) assay	TRECs are produced during thymic maturation of T-cells; used in newborn screening of T-cell deficiencies	CID/SCID, 22Q11.2DS, WAS
	Mitogen stimulation	Functional assay measuring proliferation of T-cells to mitosis-inducing agents	CID/SCID, 22Q11.2DS, WAS, X-ED-ID
	Antigen stimulation	Functional assay measuring proliferation of memory T-cells to specific antigens; abnormal in infants until at least six months secondary to lack of exposure	CID/SCID, 22Q11.2DS, WAS, X-ED-ID
	HIV	Nucleic acid PCR of HIV	Perinatal HIV
Autoimmune and autoinflammatory	Eosinophil count and immunoglobulin E	Evaluation for increased levels of eosinophils and IgE	WAS, Omenn syndrome, IPEX, NOMID/CINCA

Take Home Points

- Maintain strong index of suspicion for PIDs in patients with recurrent, difficult to treat, or unusual infections, along with autoimmunity and malignancy.
  - Consider an immunodeficiency if recurrent or unusually severe presentation due to a typical pathogen **OR** infection due to an unusual pathogen
  - Other flags: poor growth, multiple po/IV antibiotic courses, recurrent abscesses, FHx of PID, adenopathy, splenomegaly, autoimmunity
- Order CBC with differential; calculate the ANC and ALC
- Additional testing based on presentation
- If in doubt, no live vaccinations and only irradiated, filtered blood products

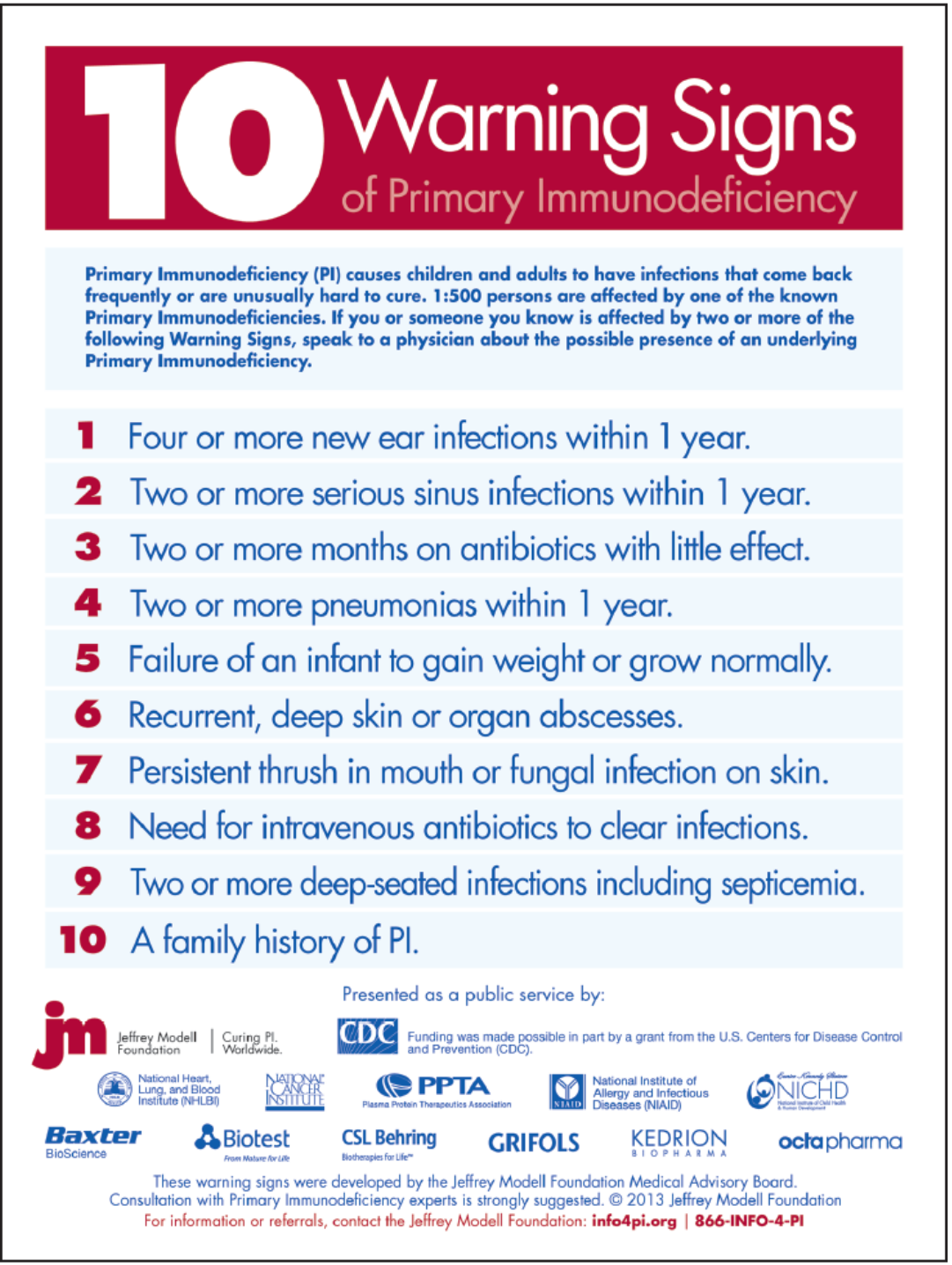
Characteristic features of genetically determined hyperimmunoglobulin M syndrome*

Disease	Gene defect	Inheritance	Type of infections	Autoimmunity	Lymphoid hyperplasia	Defect of CSR	Defect of SHM	Cellular defect
CD40L deficiency (HIGM1)	*CD40LG*	XL	Bacterial, opportunistic	Rare	No	Yes	Yes	T cells
CD40 deficiency (HIGM3)	*CD40*	AR	Bacterial, opportunistic	No	No	Yes	Yes	B cells, DC, monocytes
AID deficiency (HIGM2)	*AICDA*	AR	Bacterial	Yes	Yes	Yes	Yes	B cells
AID deficiency, C-terminus variant	*AICDA*	AD	Bacterial	Yes	Yes	Yes	No	B cells
HIGM4	Unknown	AR	Bacterial	Yes	Yes	Yes	No	B cells
UNG deficiency (HIGM5)	*UNG*	AR	Bacterial	No	Yes	Yes	Yes¶	B cells

CSR: class-switch recombination; SHM: somatic hypermutation; CD40L: CD40 ligand; HIGM: hyperimmunoglobulin M syndrome (hyper-IgM); XL: X-linked; AR: autosomal recessive; DC: dendritic cells; AID: activation-induced cytidine deaminase; AD: autosomal dominant; UNG: uracil N-glycosylase.

* Hyper-IgM syndrome may also occur in some patients with post-meiotic segregation increased 2 protein (PMS2) deficiency, NF-kappa-B essential modifier (NEMO) deficiency, ataxia-telangiectasia, or Nijmegen breakage syndrome.

¶ Biased pattern of somatic hypermutation, in which mutations at dC/dG pairs are almost all transitions (G>A and C>T).



Evaluate child if ≥ 2 warning signs – strongest predictors being +FHx, IV Abx for sepsis, and FTT

But doesn't identify all….