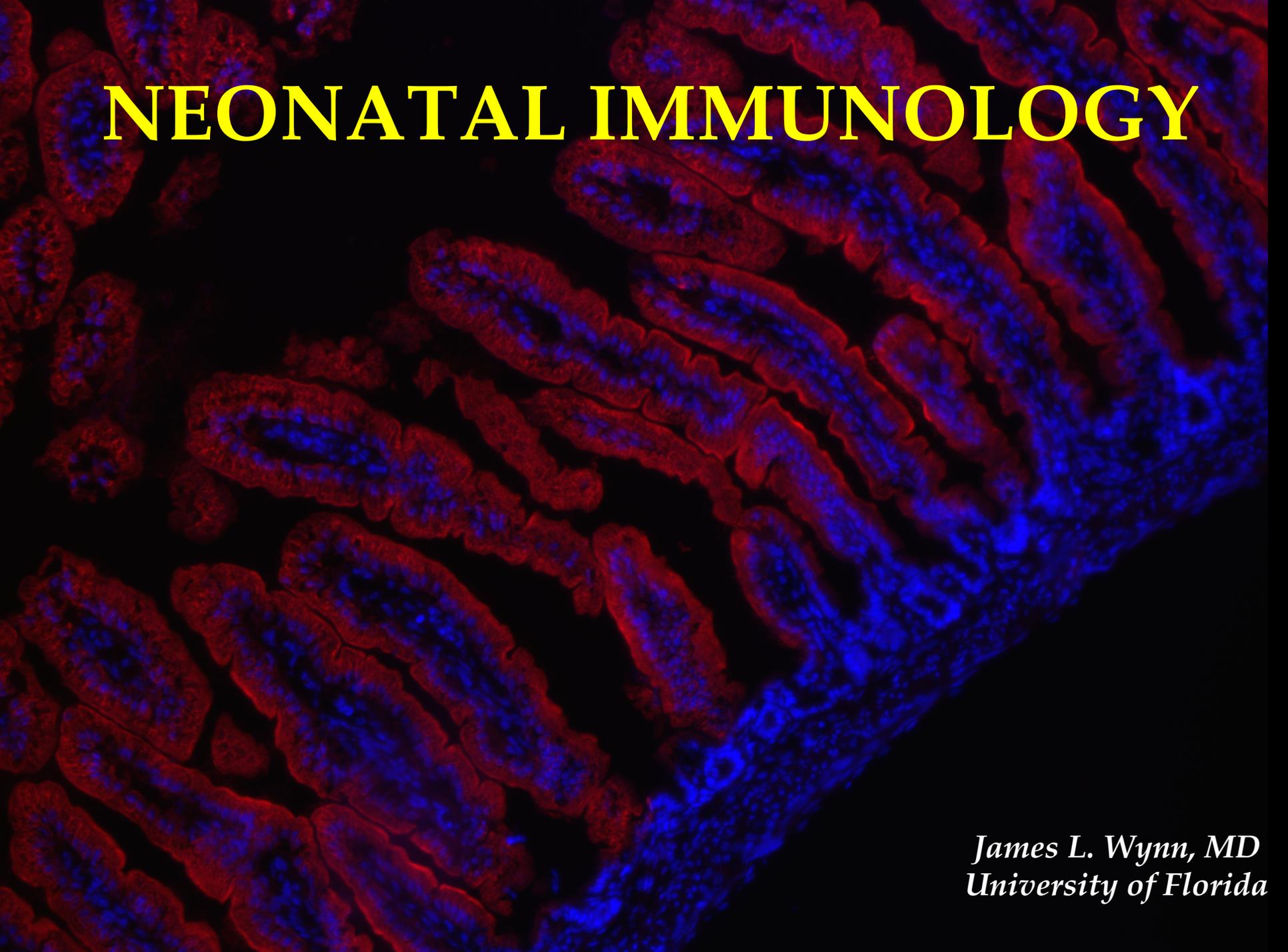
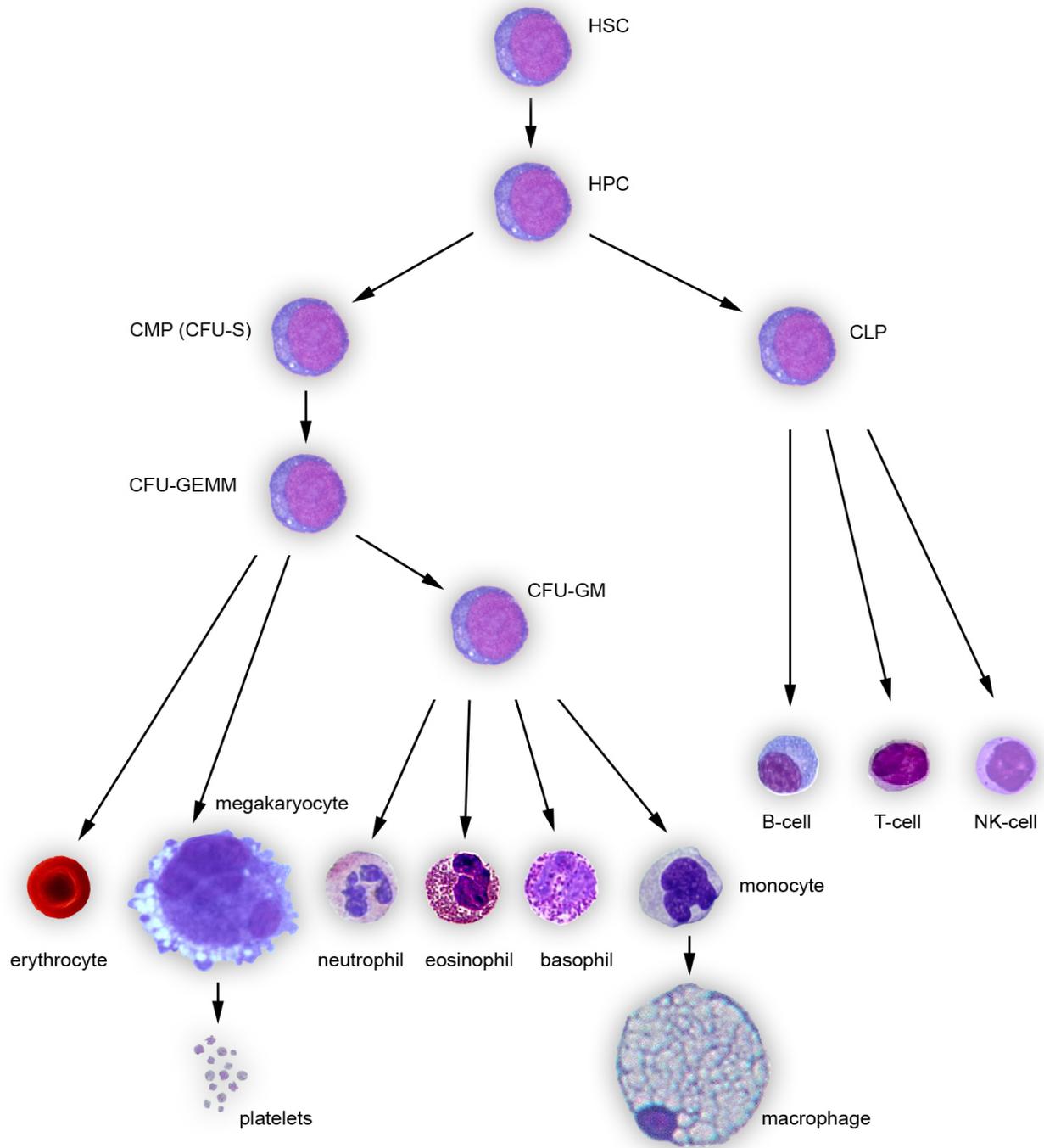


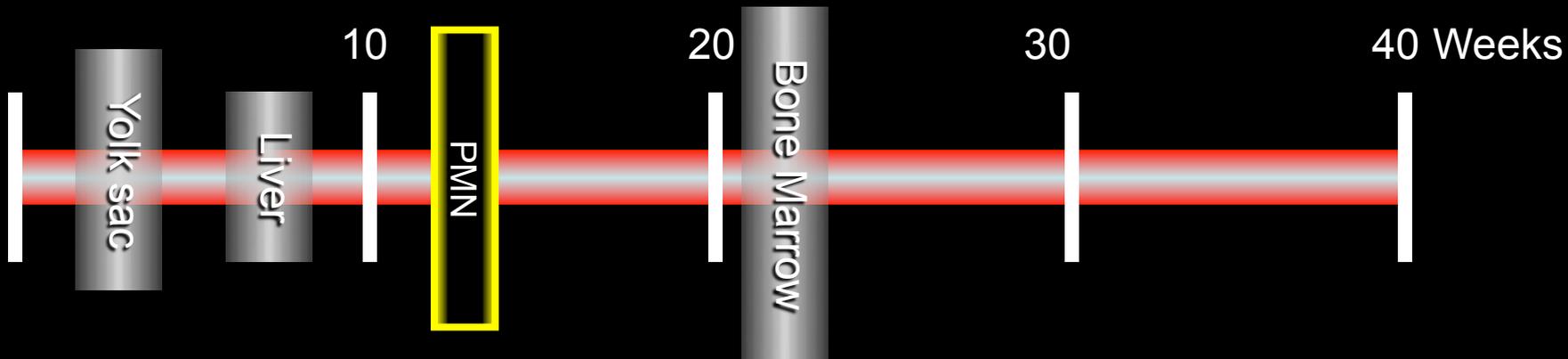
# NEONATAL IMMUNOLOGY

A fluorescence microscopy image of intestinal crypts. The crypts are stained with a red dye, likely DAPI, and a blue dye, likely a fluorescent antibody. The crypts are arranged in a regular, repeating pattern, with the blue staining highlighting the nuclei of the cells within the crypts.

*James L. Wynn, MD  
University of Florida*

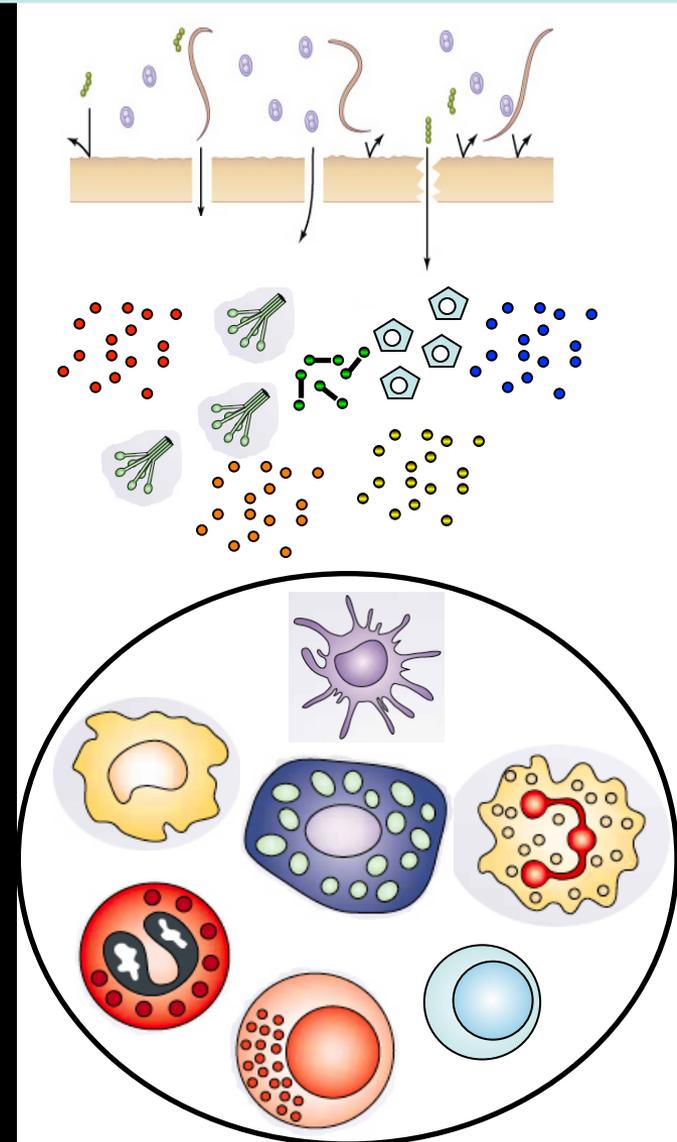


# Development of the Immune System



# Innate Immune System

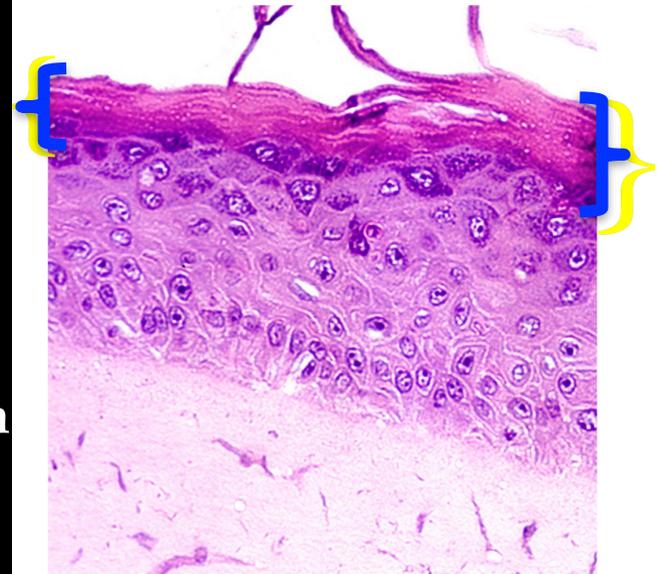
- Preformed non-specific immune response
- Acts within minutes of exposure to pathogens
- Includes multiple elements
  - Barriers
  - Inflammatory response factors
  - Cells
- Interaction with adaptive immune system leads to development of specific immune response
- Critical role in newborn protection



# Barriers

## Skin

- Covered with antimicrobial proteins/peptides present in vernix and amniotic fluid
  - Maintains pH balance
  - Proteins against GBS, E coli, and Candida
- Layers thinner in premature
- Stratum corneum-**{** is primary effector
  - **Disrupted via abrasion and IVs**
- Maturation accelerated in preterm infants
  - Up to 4 weeks to achieve barrier function



# Barriers

## *Mucosa*

### Gastrointestinal and respiratory tracts

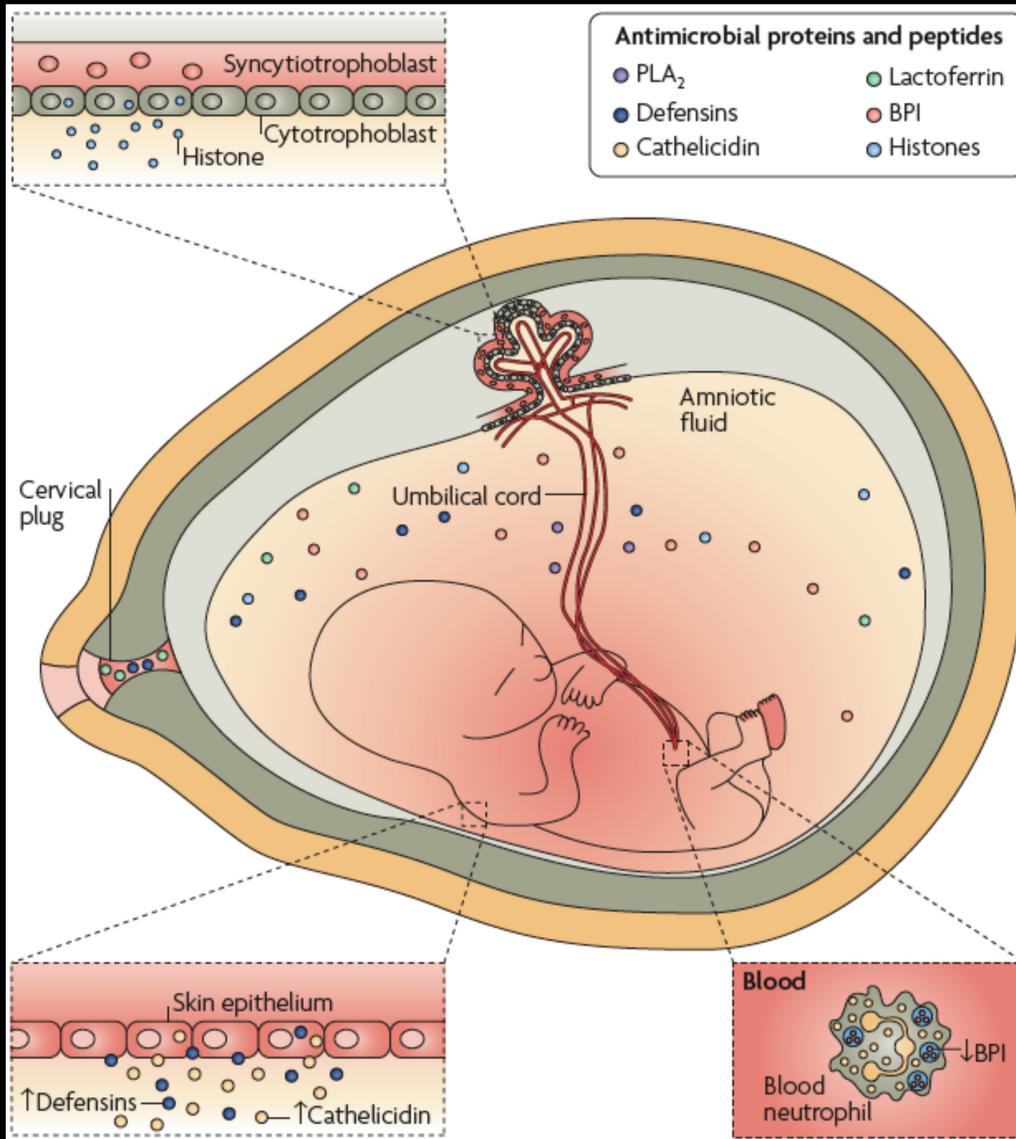
- **Gastrointestinal**

- Colonized with microorganisms
  - Barrier integrity depends on interaction between commensal organisms and host epithelium
- Antimicrobial proteins/peptides, mucus, IgA, cells (epithelial and immune)

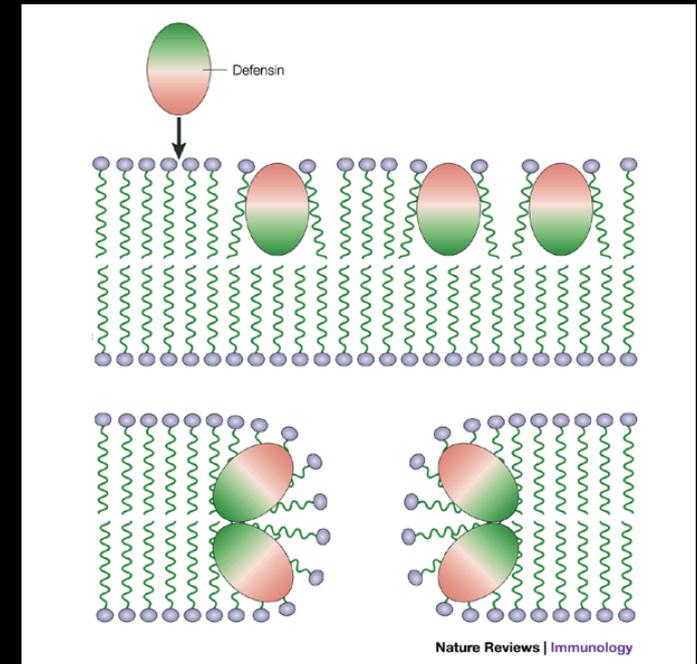
- **Respiratory**

- Colonized with microorganisms
- Antimicrobial proteins/peptides, mucus, cilia, opsonins (surfactant proteins A, D, complement), cells (epithelial and immune)

# Antimicrobial proteins/peptides



- Phylogenetically most ancient
  - “Beginning” of the immune system
  - Bacteria, plants, insects, non-mammalian vertebrates
- Antimicrobial activity against bacteria, viruses, fungi, parasites



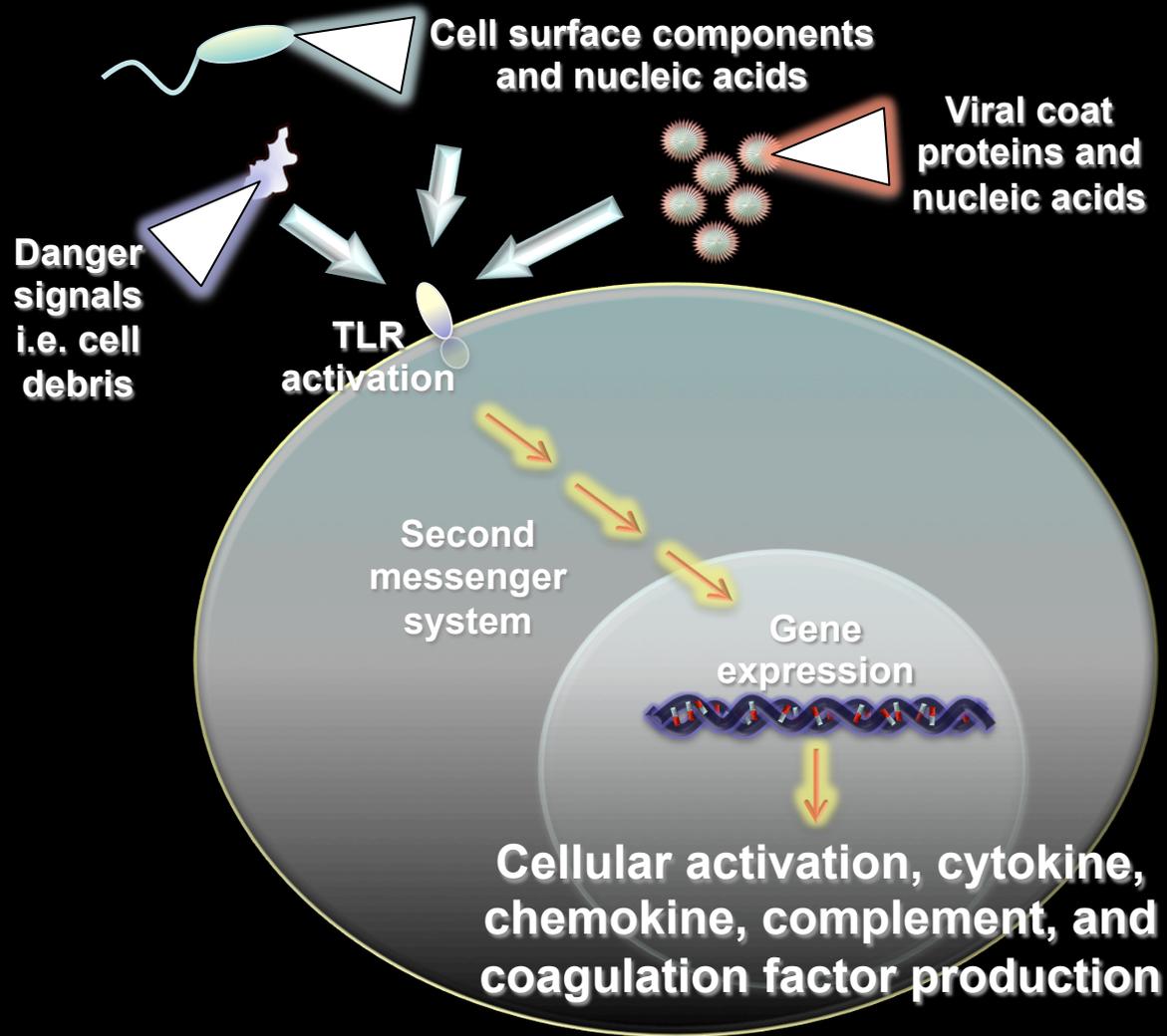
# Pathogen Recognition

Once barrier function compromised, first step towards immune response is pathogen recognition

- Local sentinel cells (tissue-specific macrophages)

Recognition activated by pattern recognition receptors (**PRR**)

- Toll-like receptors (TLRs)



# Cytokines

- Important for cellular signaling
- Mediators of host's response to infection
- Neonates have altered cytokine production compared to adults that persists for months
  - Delayed maturation of cytokine production
  - Decreased IFN- $\gamma$ , TNF- $\alpha$ , IL-12
  - Increased IL-6, IL-10
  - Less receptor responsiveness
  - Impairment of pathway responsible for cytokine activity

# Cytokines and Chemokines

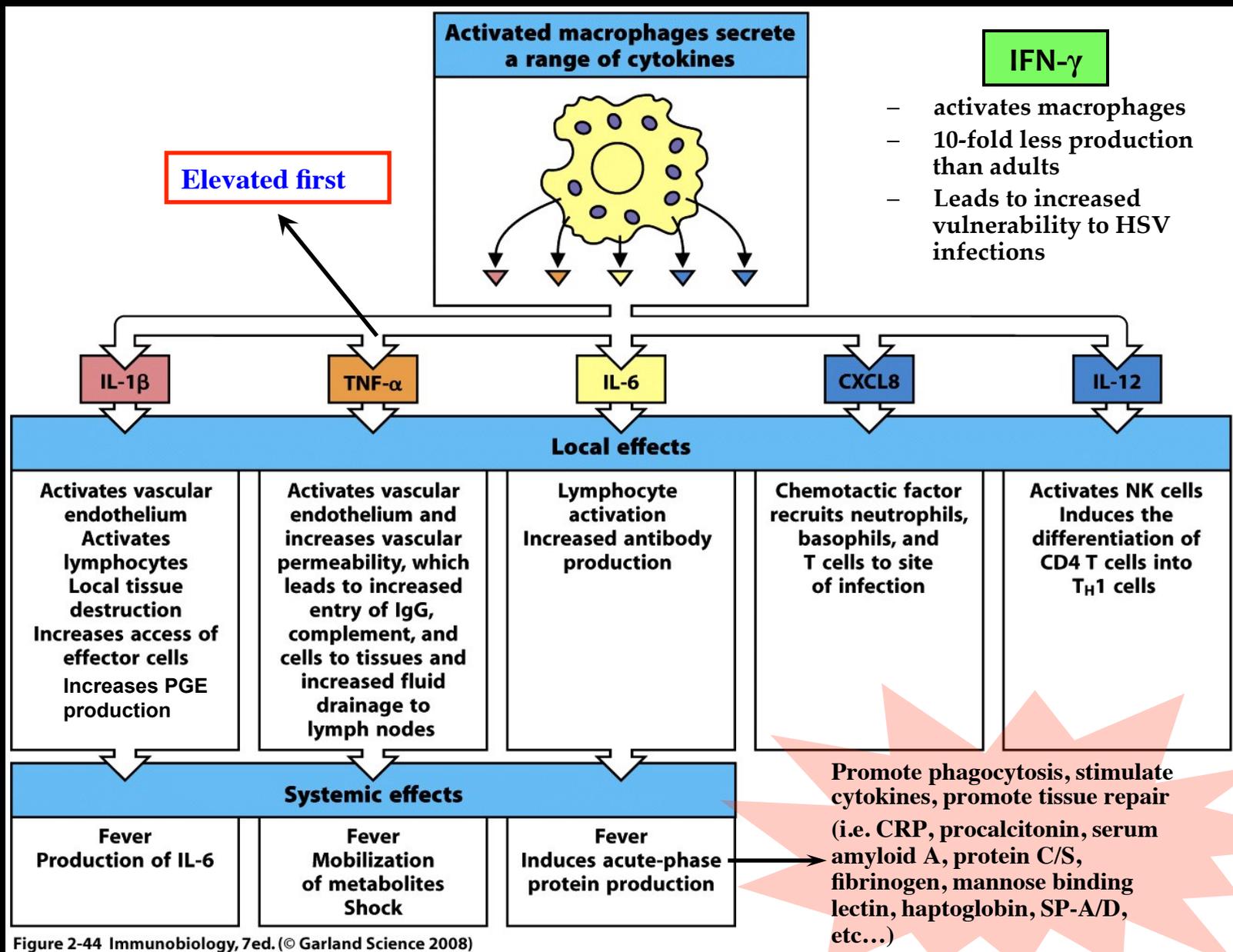


Figure 2-44 Immunobiology, 7ed. (© Garland Science 2008)

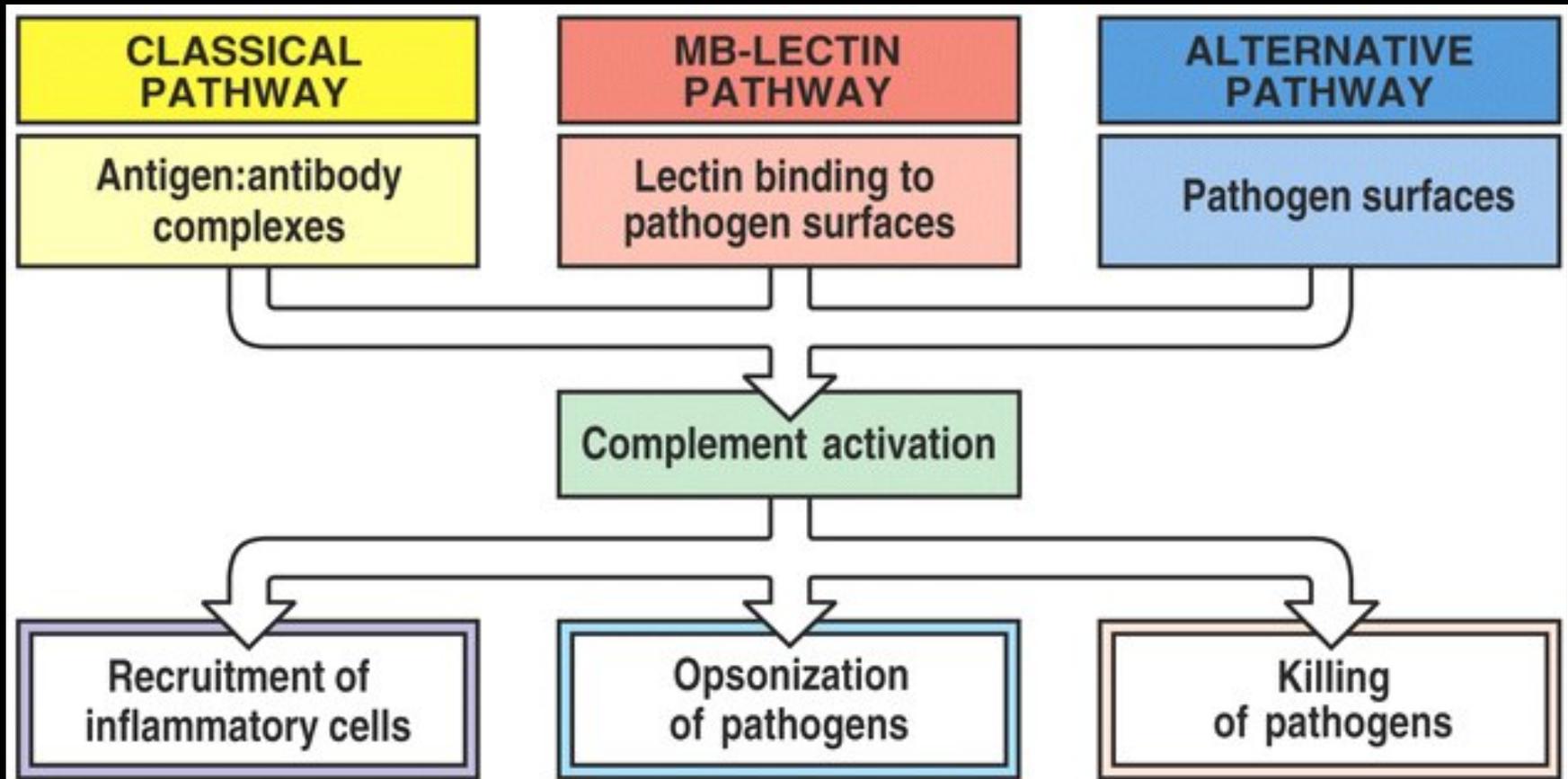
# Chemokines

- **Proteins that facilitate leukocyte migration to sites of inflammation**
  - **Examples: IL-8 (CXCL8) , IP-10 (CXCL10), RANTES (CCL5), MCP, eotaxin, MIP**
- **Diminished chemotactic activity in neonates may lead to slow influx of cells to inflammatory sites**

# Soluble Components & Opsonins

- **C3b**
  - Most critical bacterial opsonin
- **\*\*Fibronectin\*\***
  - Enhance leukocyte adherence and migration
  - Levels are decreased in premature infants
- **C-reactive protein**
  - Produced by the liver following elevated levels of IL-6
  - Bacterial opsonization and classical complement pathway
- **\*\*Lactoferrin\*\***
  - Neutrophil-derived iron-binding and antimicrobial glycoprotein
  - Production is low in neonatal neutrophils
- **Surfactant proteins A & D (Collectins)**
  - Opsonin and inhibit respiratory burst in PMNs
- **Mannose binding lectin**
  - Direct opsonin
  - Activates complement

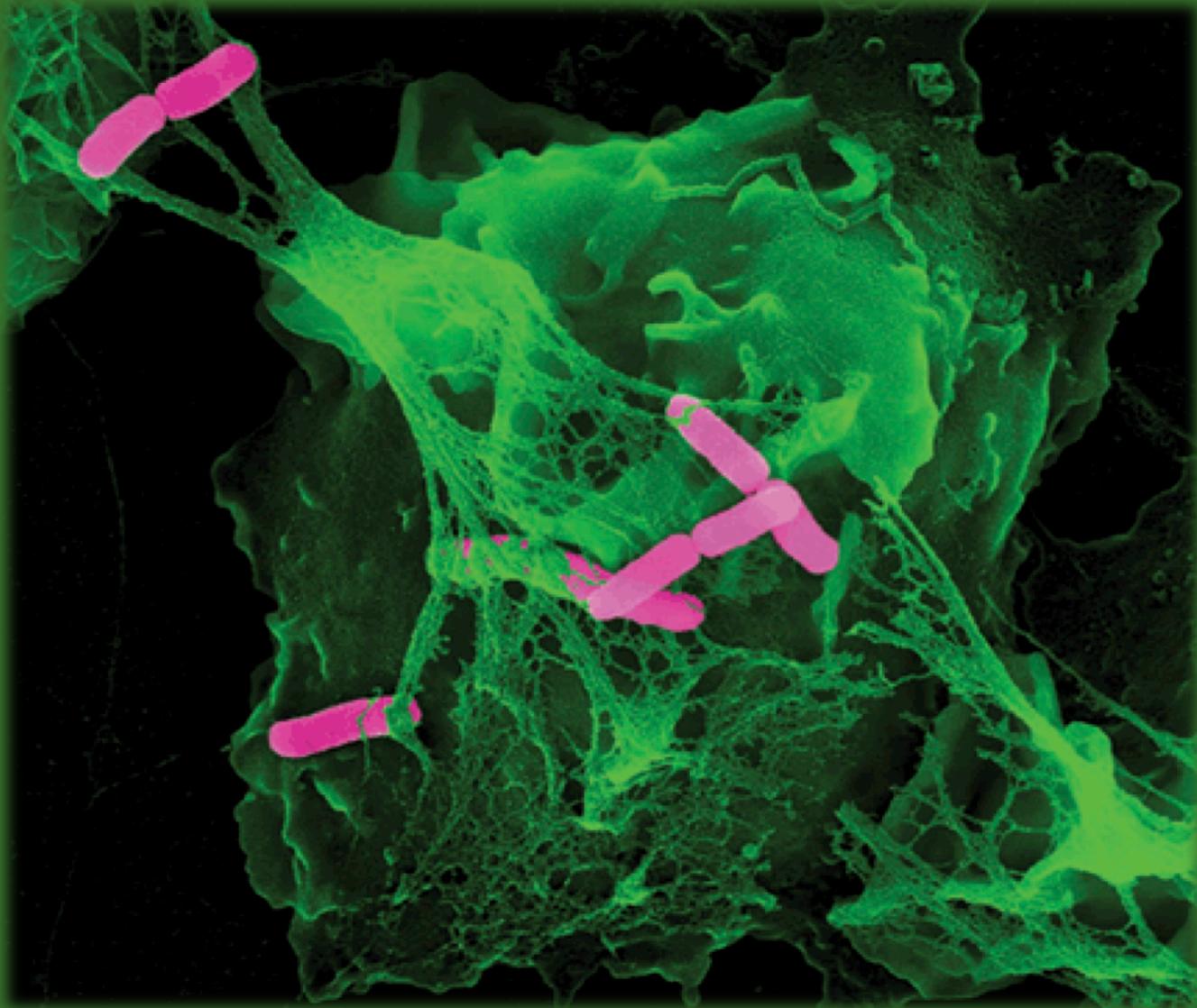
# Complement

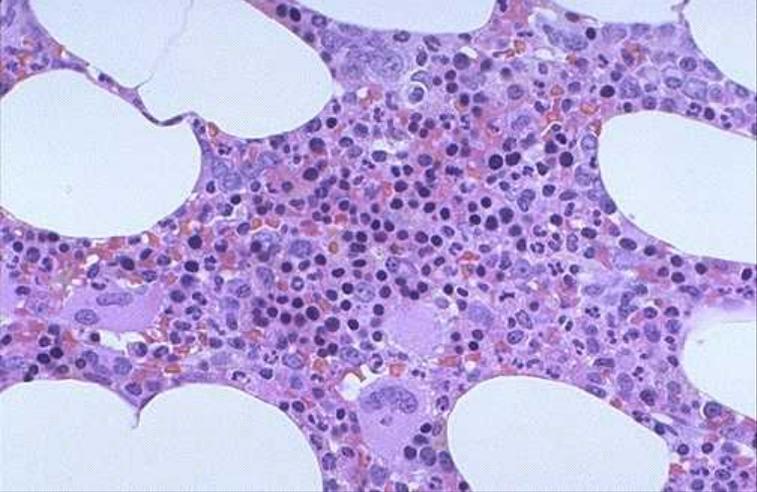


# Defects of Neonatal Complement System

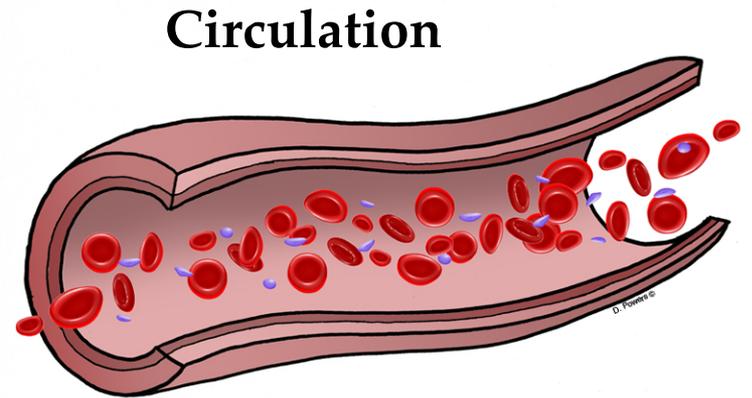
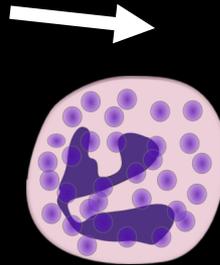
- Reliance on alternate/lectin pathways at birth
  - Limited classical pathway activity due to limited diversity of transplacental antibody
- Complement **does not** cross the placenta
- Adult levels/function ~6-18 months of age
- Complement deficiency disorders do not commonly present in the neonatal period

# Neutrophils & antigen presenting cells





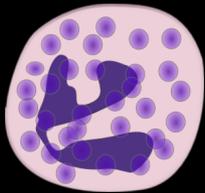
**Bone Marrow**



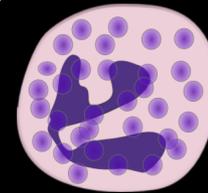
**Circulation**

*1/2 life is 6-7 hours*

**Circulating Pool**



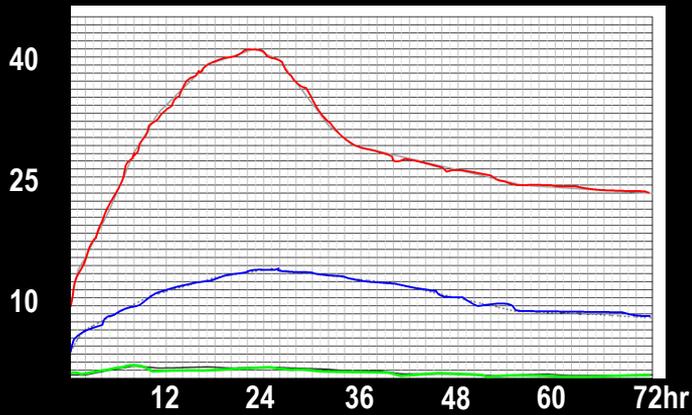
**Marginated Pool**



*Life Cycle of Mature PMN*

# PMN counts

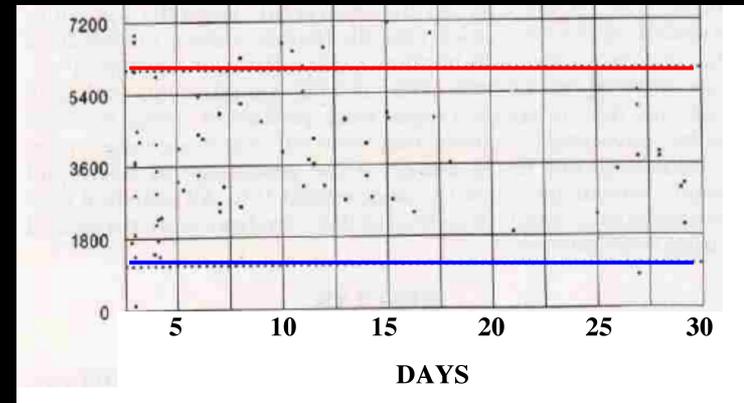
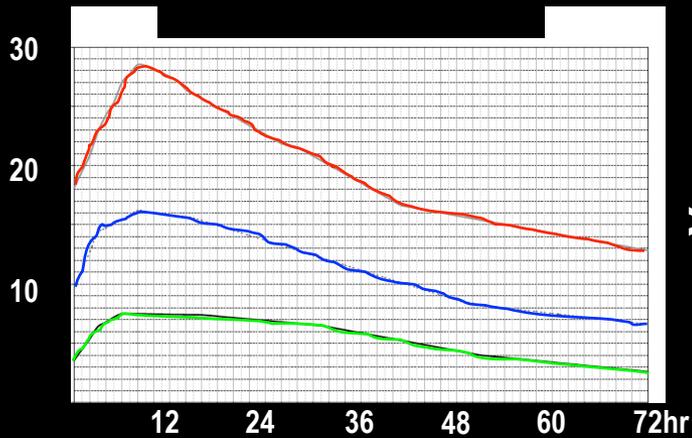
<28wks



28-36wks



>36wks

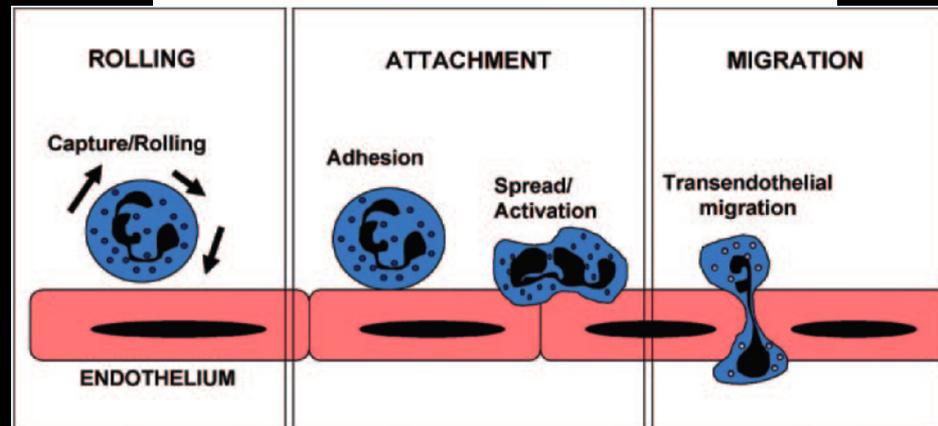
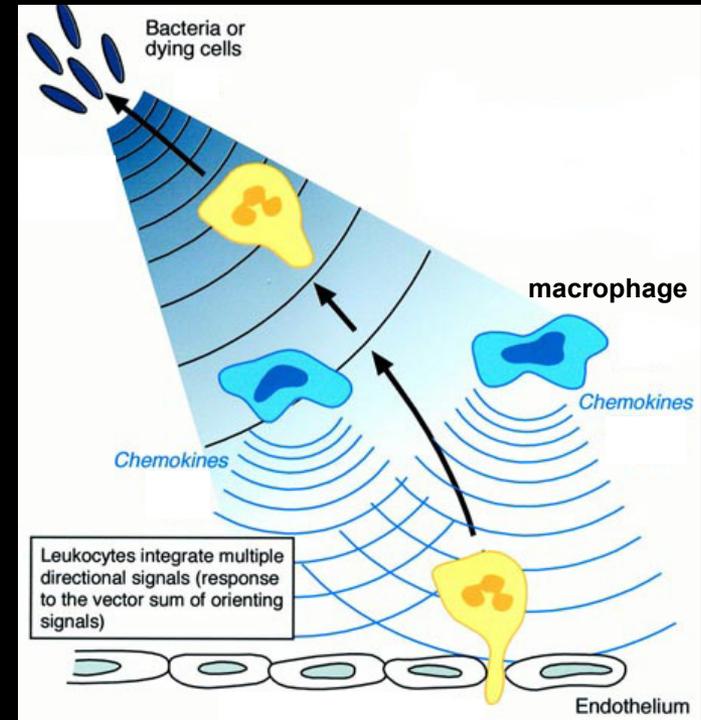


90%

10%

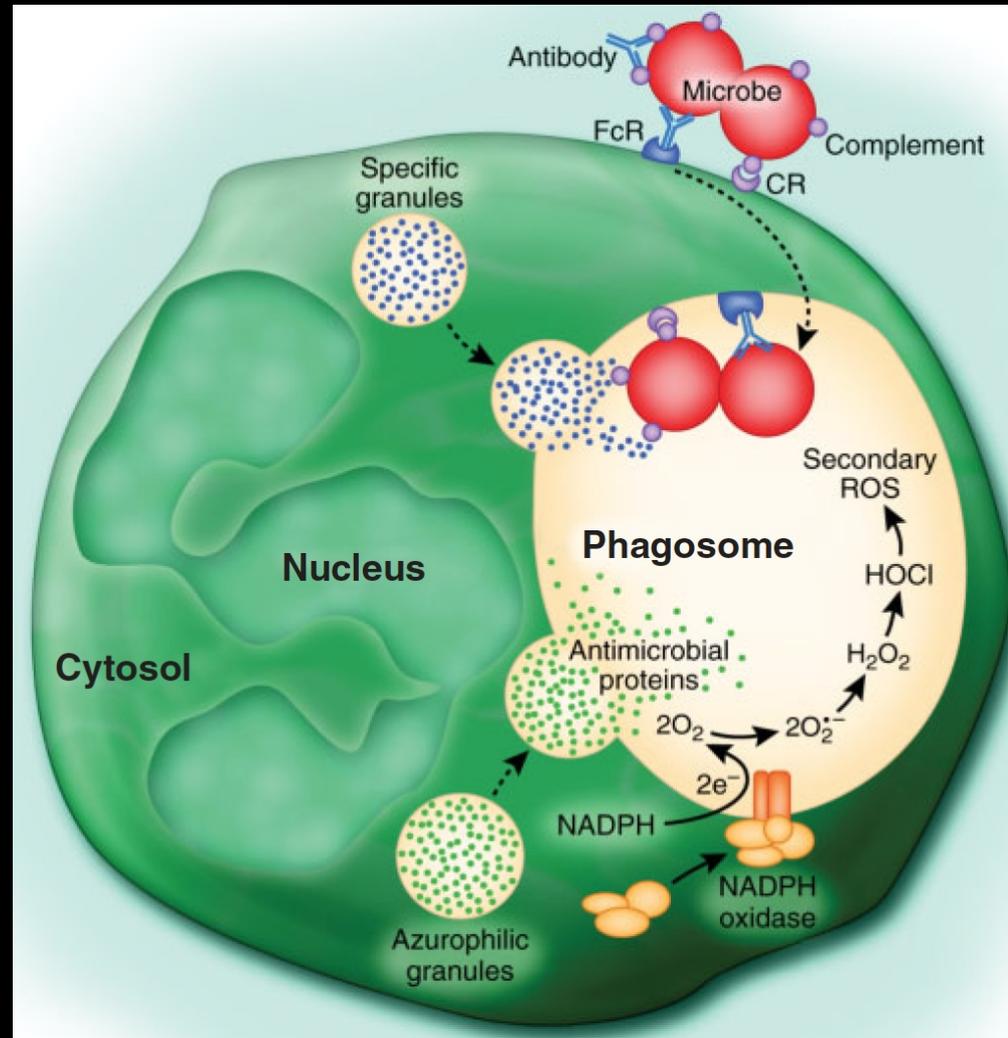
# PMN Chemotaxis and Diapedesis

- **Reduced basal chemotaxis and random migration**
  - ~1/2 speed of adults
  - Worse in post-op and septic neonates
  - Small improvement following labor
  - Impaired signaling down stream of chemokine-receptor binding
  - serum chemokine levels are normal
- **Decreased L-selectin and  $\beta 2$  integrin**
  - Cell adhesion molecules for rolling and diapedesis
- **Deformation reduced**
  - Worse in band forms



# Pathogen killing

- Systems work cooperatively to kill or degrade phagocytosed target
- Oxygen dependent
  - Via reactive oxygen metabolite production by NADPH oxidase)
- Oxygen independent
  - Acid and activities of digestive, enzymatic, or microbicidal components from granules

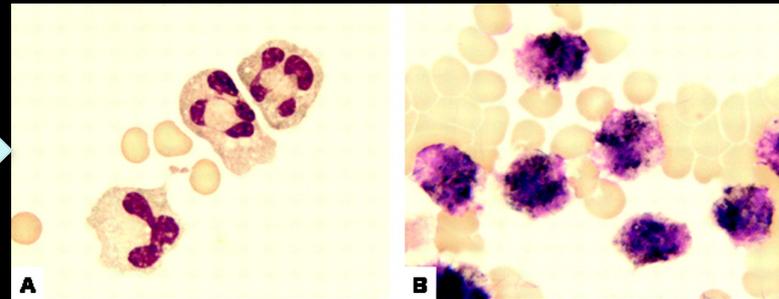


# Neonatal Neutrophils

- Neonatal PMN morphology is *similar* to those from children and adults
- Phagocytic and microbicidal activity of PMNs from healthy neonates comparable to adults
  - Poor phagocytic and killing (respiratory burst) in sick and/or preterm neonates
  - Neutrophil function reduced with prematurity, gram negative sepsis, indomethacin, and intrapartum magnesium
- Numerous *in vitro* functional deficiencies
  - Chemotaxis
  - Adhesion
  - Granule content

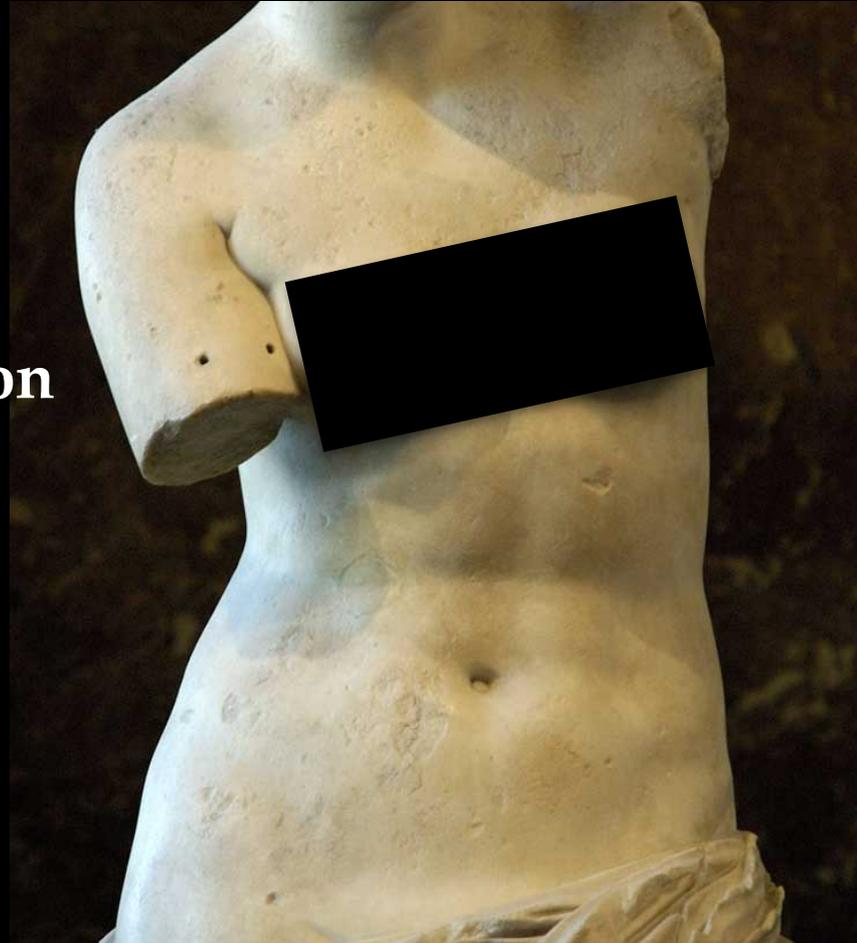
# Chronic Granulomatous Disease

- Deficiency or absent NADPH oxidase function in phagocytic cells
- 80-90% MALE- usually X-linked recessive
- Severe, recurrent infections with catalase-positive bacteria or fungi
- Diagnosis made by NBT → **remains colorless**
  - demonstration of absent or severely deficient respiratory burst activity in phagocytes
- Difficult diagnosis in neonates-reduced respiratory burst at baseline
- Normal NBT test



# Leukocyte Adhesion Deficiency\*

- LAD-1 (predominant type)
  - Deficiency of leukocyte **integrins**
- LAD-2
  - Deficiency of **selectin** function
- Clinical presentation (LAD-1)
  - Recurrent infections
  - Delayed separation of umbilical stump
  - Leukocytosis
  - NO PUS!!



# Other disorders of Neutrophil Dysfunction

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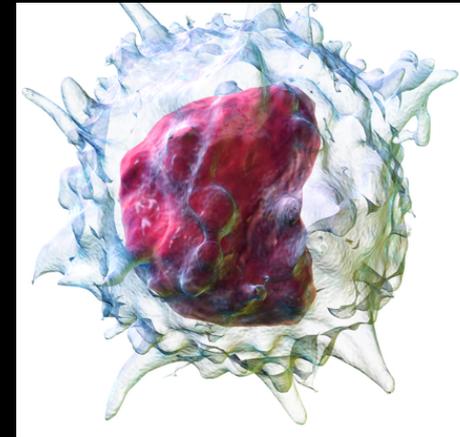
- **Myeloperoxidase deficiency**
  - Most common deficiency (1 in 2-4K people) but can be clinically silent
  - Recurrent candidal infections
- **Chédiak-Higashi syndrome**
  - Abnormal PMN degranulation, recurrent infections, albinism, giant intracellular granules
- **G6PD**
  - Severe form
- **Galactosemia**
  - Inhibitory effects of galactose on PMNs
- **Glycogen Storage Disease 1B**
- **Trisomy 21**
  - Depressed chemotactic activity

# Monocytes & Macrophages

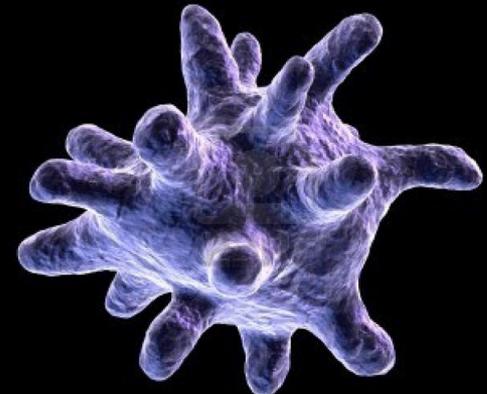
## *(Function)*

- Derived from precursor colony-forming unit shared with PMN
- Macrophages are mature form of monocytes
- Enter circulation as precursor and exit circulation as a long-lived macrophage/histiocyte
- Perform same phagocytic functions as PMNs
- Present antigen to T and B cells

Monocyte



Macrophage



# Monocytes & Macrophages

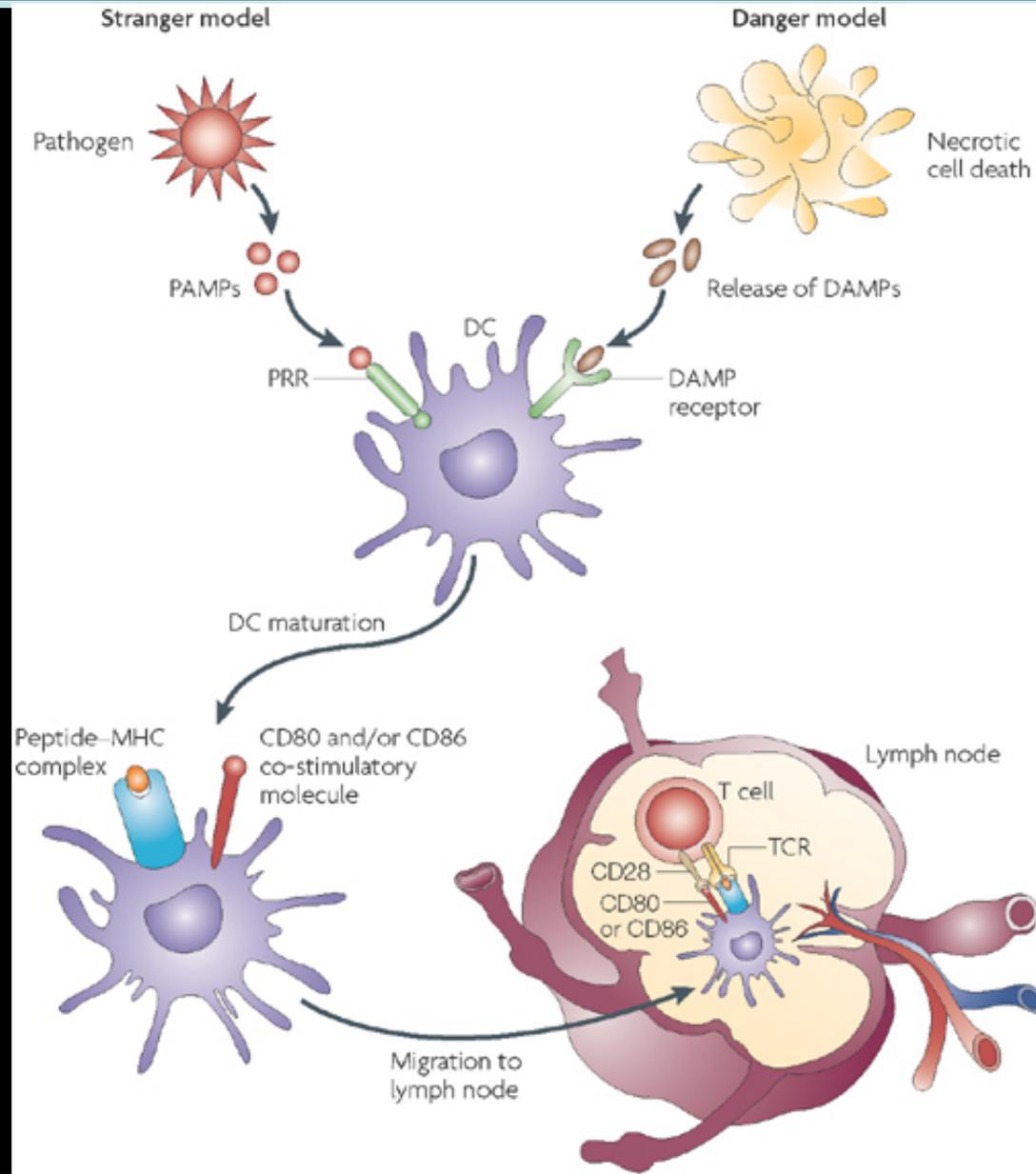
## *(Deficiencies)*

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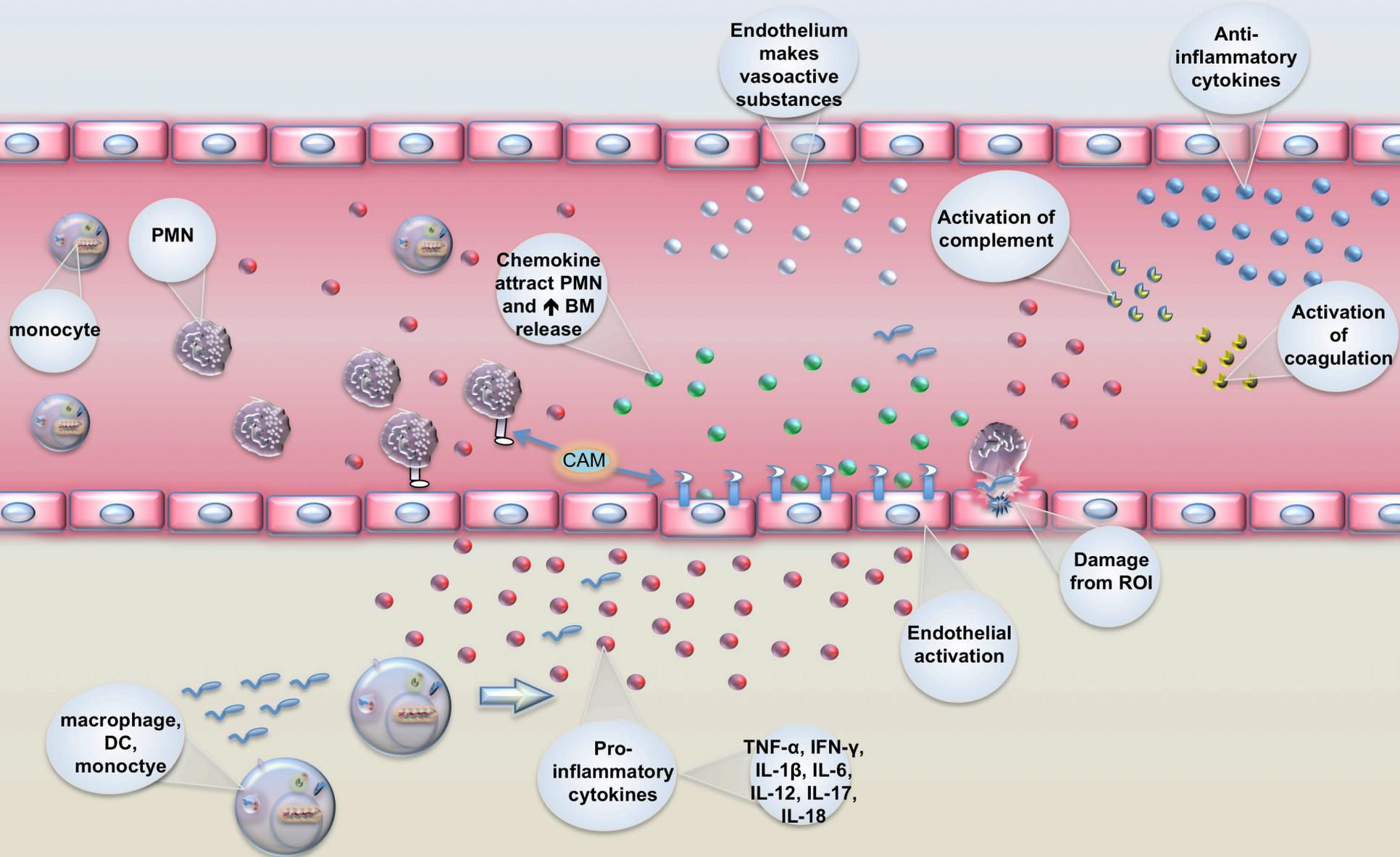
- **Monocytes**
  - Poor cytokine production and antigen presentation well into infancy (~12 months)
  - Low/absent number of monocytes in blood is common for first 5 days
  - Reduced co-stimulatory cell-surface molecules
- **Macrophages**
  - Poorly responsive to IFN- $\gamma$

# Dendritic Cells

- Professional antigen presenting cells that activate T cells
- Neonatal DCs require greater stimulus for activation

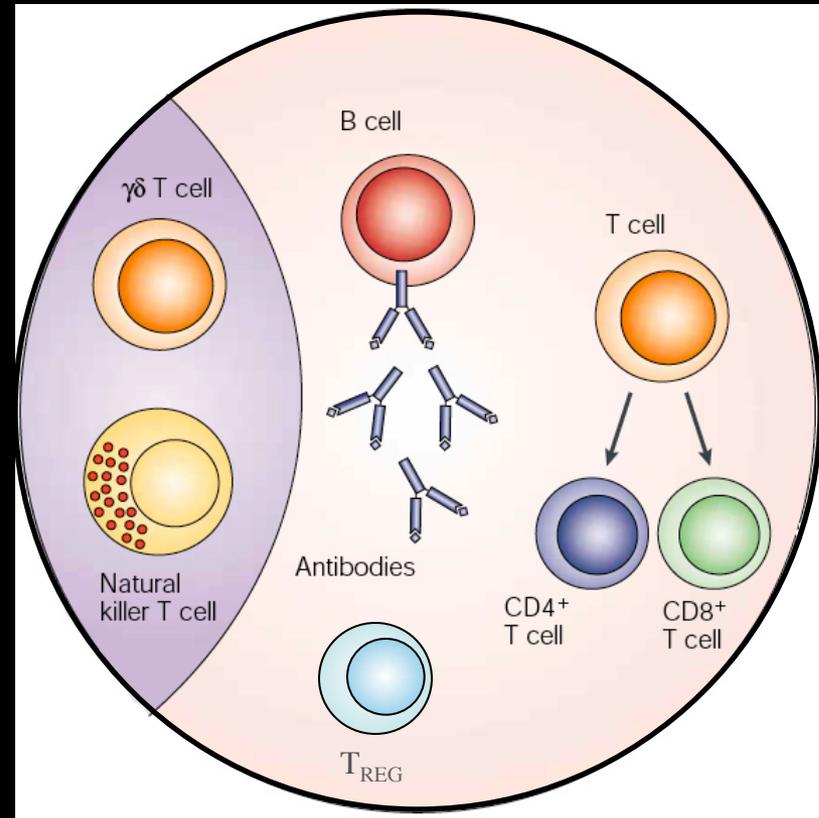


# Inflammation



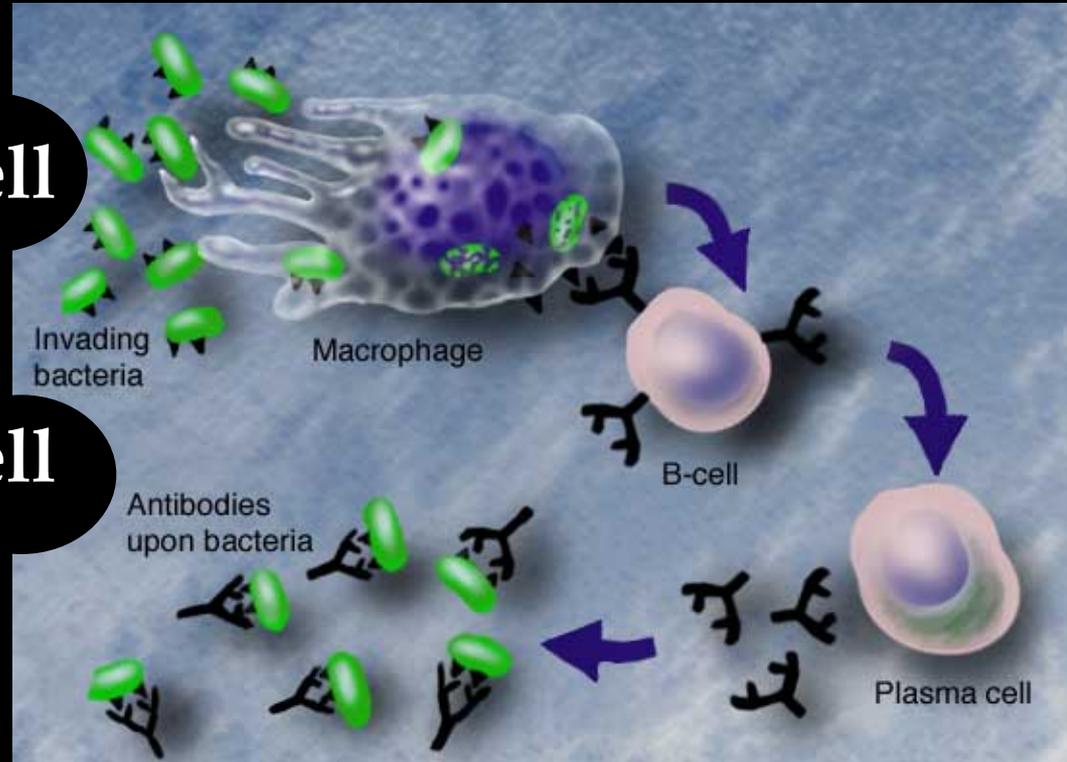
# Adaptive Immune System

- Antigen-specific response
- Takes 4-7 days to develop
- Cell-mediated responses
  - T cells
    - Helper T cells (CD4<sup>+</sup>)
    - Cytotoxic T cells (CD8<sup>+</sup>)
- Humoral responses
  - B cells
    - Plasma cells
      - Secrete immunoglobulin
- Immunoregulatory functions
  - T<sub>REG</sub>



# B cells

- Produce antibodies as mature plasma cells
- Antigen presenting cell function
- Antibody production with and without T cell co-stimulation
- Neonatal B cells differentiate into predominantly IgM-secreting cells



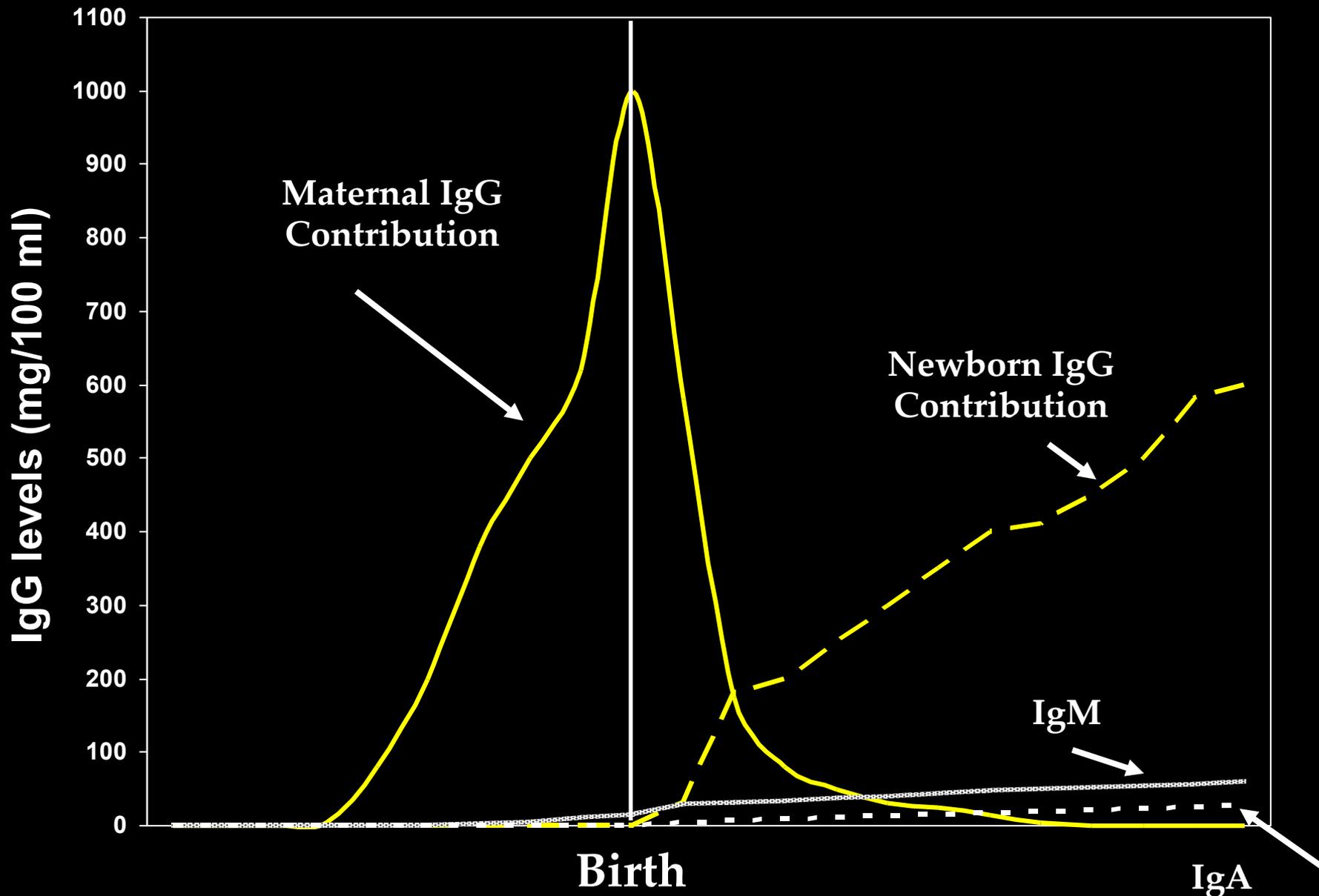
# Immunoglobulin Production

- **In germinal centers: these are absent in the fetus and do not develop until there is antigenic stimulation**
- **Made by B-cells after differentiation to plasma cells**
- **Fully differentiated plasma cells can secrete and produce IgG, IgM, and IgA by 20-weeks gestation**

# IgG

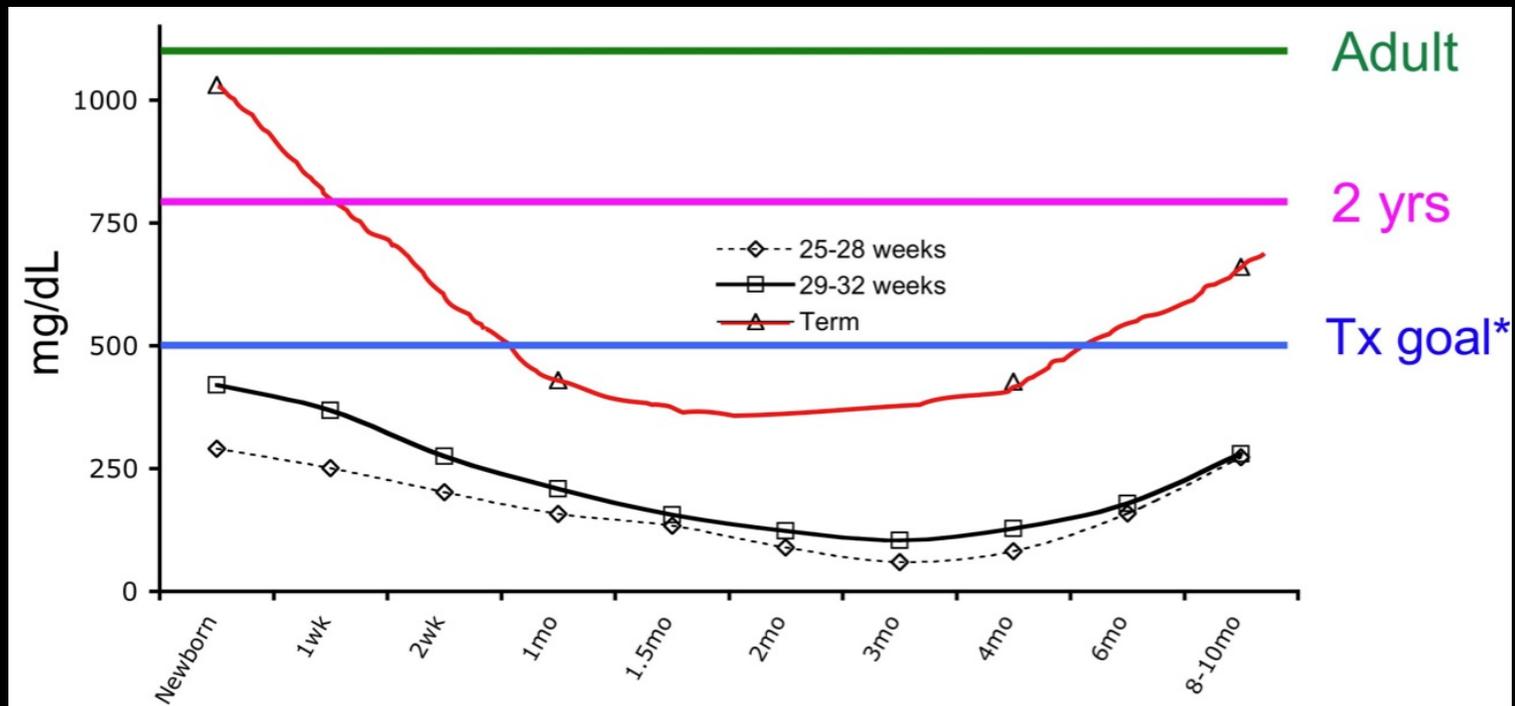
- **The fetus produces very little antibody before birth**
- **Maternally derived IgG is likely important to protect the fetus from infection**
  - **Crosses the placenta by pinocytosis**
  - **Mostly IgG1**
- **Newborns do not have pathogen-specific antibodies unless the mother has been exposed**

# Immunoglobulin Levels With Age

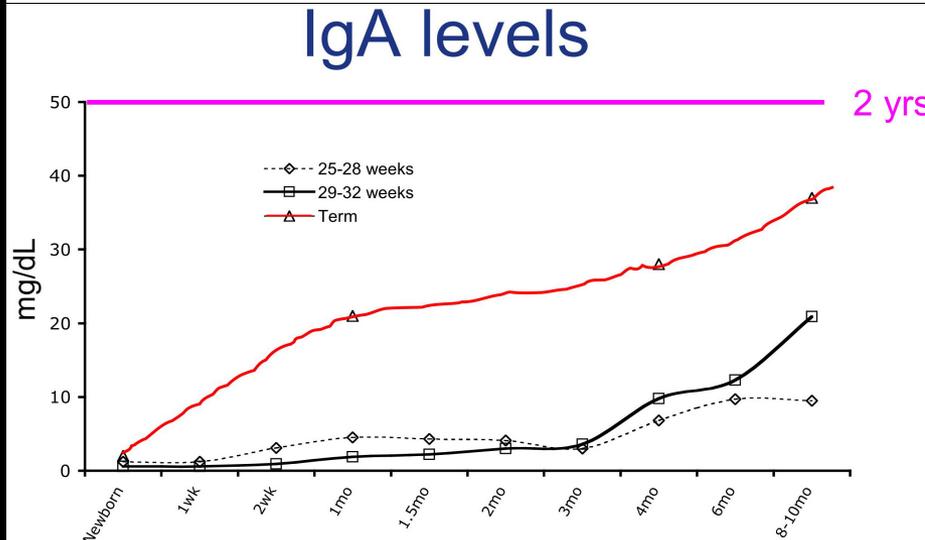
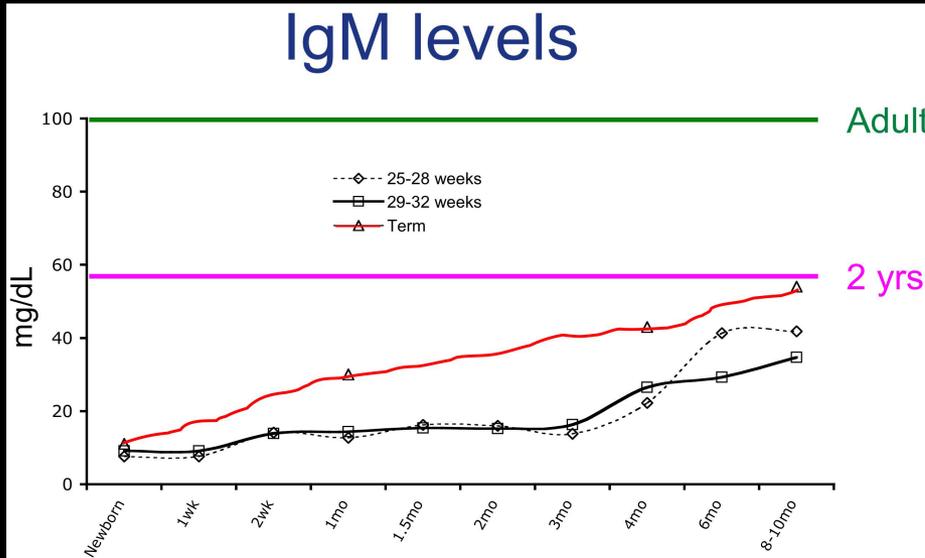


# IgG Levels in Neonates

- Term infants may have IgG levels in excess of maternal IgG levels
- Growth restricted and prematurely born infants have lower levels
- $\frac{1}{2}$  life of maternal antibody is 30 days
  - Maternal IgG still detected until 18 months life
- Nadir at 2-4 months of life



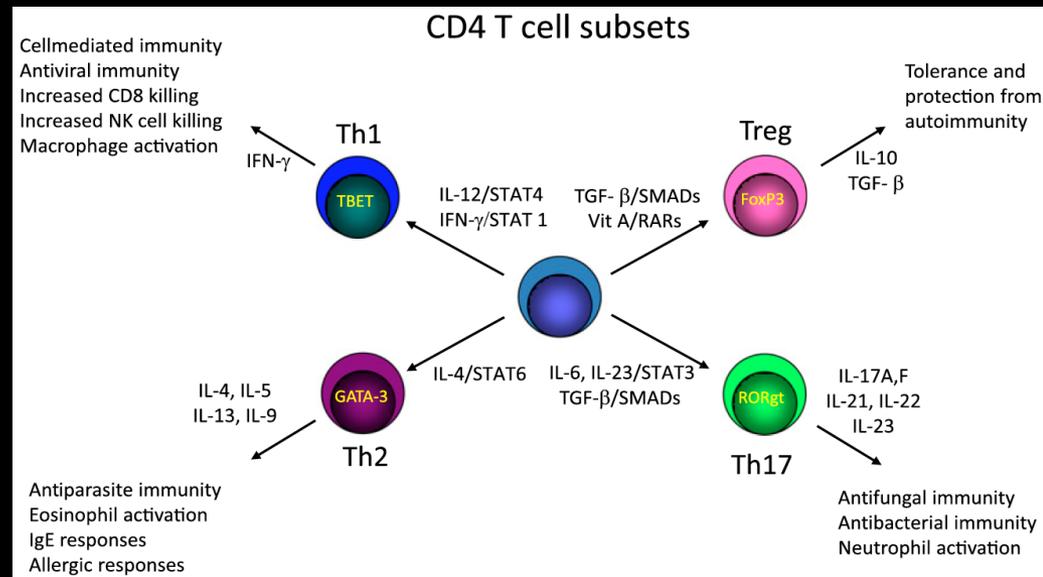
# Other Igs



- IgM and IgA levels are low at birth as they do not cross the placenta
- Infections with CMV, rubella, and Toxo can raise levels
- Only source of IgA is from colostrum

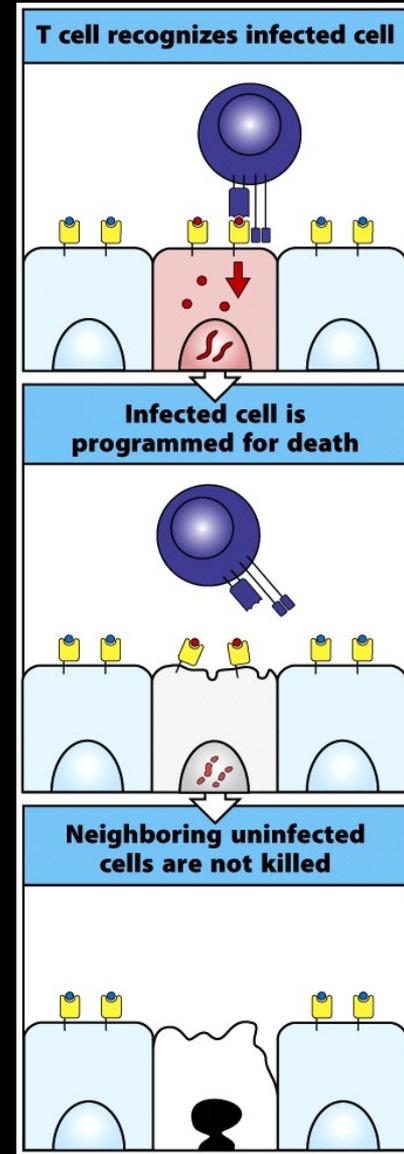
# CD4 T Cells

- “Helper” cell → Coordinate the immune system
- Stimulated by cytokines and antigen presenting cells
- Secrete cytokines
- Stimulate B cells to make immunoglobulin
- Activate macrophages
- Can function similarly to adults if appropriately stimulated

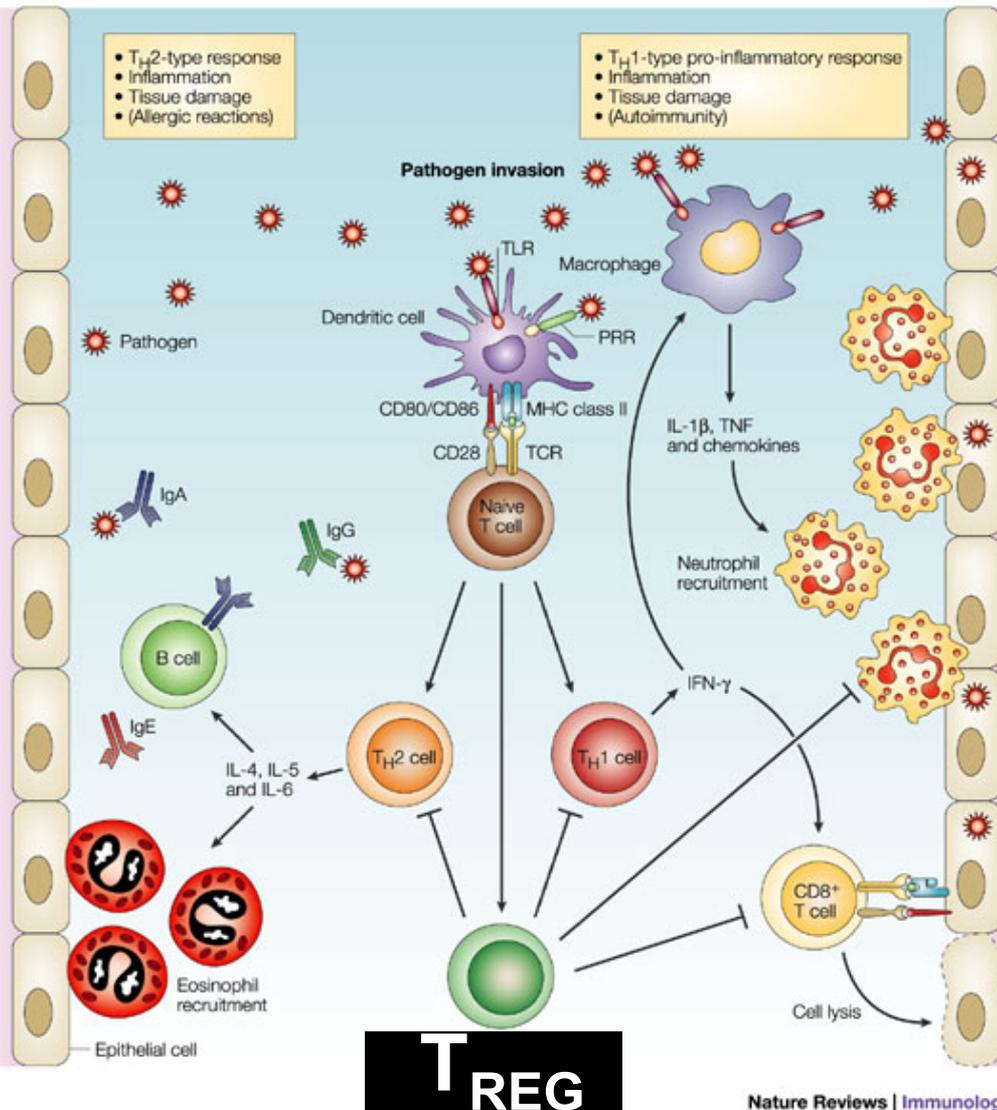


# CD8 T Cells

- CD8
  - Cytotoxic lymphocytes
  - Release perforins, degradative enzymes, and cytokines
  - Cytokine production is lower compared with adults
  - Cytotoxic activity is limited in neonates

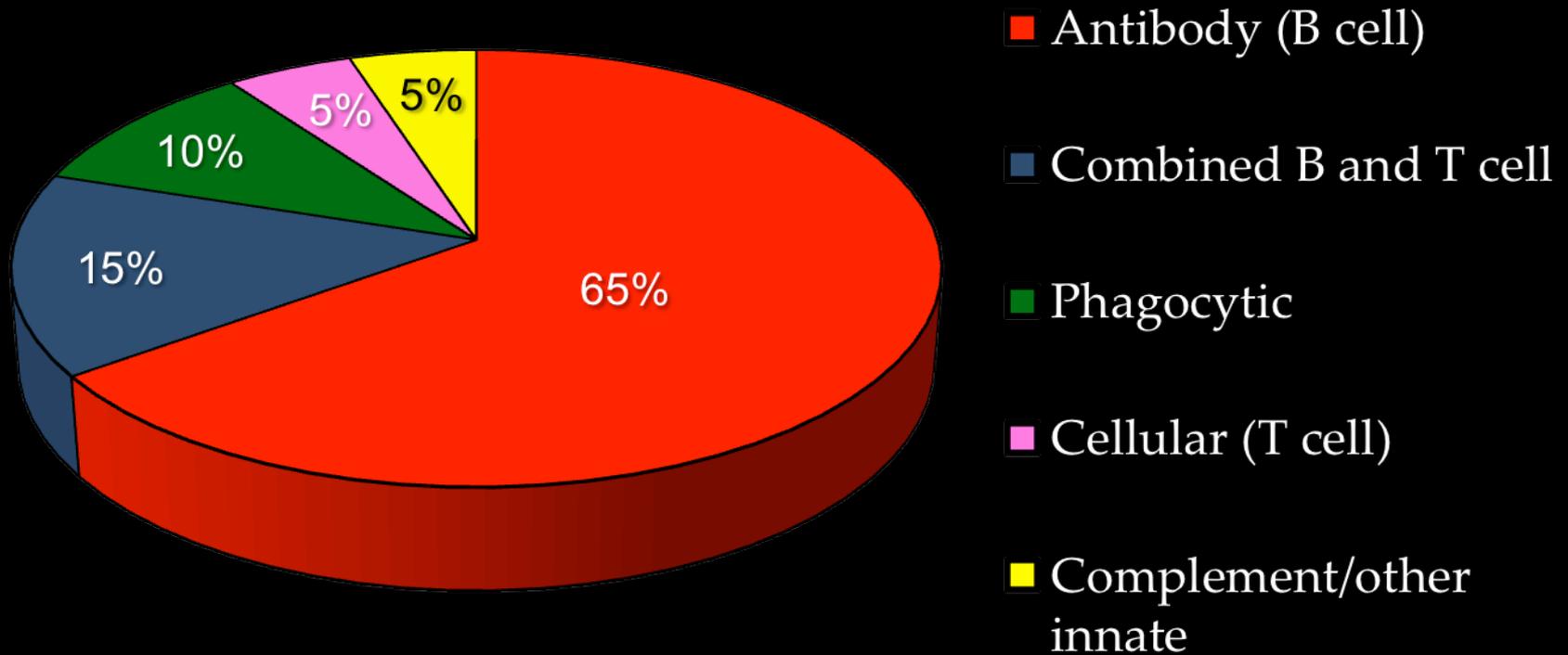


# T Regulatory cells



- High proportion of T<sub>REG</sub> particularly in premature
- Numbers decrease to adult levels by 3-6 years of age
- Necessary for prevention of in utero rejection
- May down modulate APC function

# Primary immunodeficiency



# Predominant Antibody Defects

- **Difficult to diagnose in neonatal period**
  - Ig deficiency secondary to immaturity
- **More apparent >6 months of age**
  - levels of maternal antibody decline
- **Signs of potential disorder of antibody production:**
  - Recurrent sinopulmonary infections
  - Persistent enteroviral infections of the GI tract or CNS
  - Overwhelming bacterial sepsis and/or meningitis

# Primary Antibody Disorders

- X-linked agammaglobulinemia
- Hyper-IgM syndrome
- Transient hypogammaglobulinemia of infancy
- Antibody deficiencies associated with secondary immune disorders

# X-linked Agammaglobulinemia

- **Profoundly diminished Ig** levels of all isotypes
  - IgM levels are undetectable
- B cell-specific src-associated tyrosine kinase mutation → prevents pre-B cells from becoming mature B cells/plasma cells
- **Absence of B cells** in the blood and lymphoid tissue
- **Plasma cells are absent** in GI tract
- **Normal T cell numbers** but dysplastic lymph nodes due to no B cells

# Hyper-IgM Syndrome

- Low/absent levels of IgG/A/E
- *Normal* to elevated levels of IgM
- **Neutropenia** with peri-rectal abscesses and oral ulcers
- Infection with PCP, Cryptosporidium, Salmonella
- Reported in association with congenital Rubella

# Transient Hypogammaglobulinemia of Infancy (THI)

---

- Disorder vs. exaggerated physiologic nadir
- IgG levels are  $> 2$  std dev below normal age-related ranges
- Transient defect in CD4 T helper cell function
- Normal number of B cell numbers
- Can generate normal responses to vaccinations
- **NO TREATMENT NECESSARY**

# Antibody Deficiency Associated with Secondary Disorders

- Turner Syndrome
  - Low IgG and IgM
- Trisomy 21, Monosomy 22, Trisomy 8, Chromosome 18q syndrome
  - Low IgG, IgM, IgA
- Congenital Infections
  - Rubella, CMV, HIV, EBV
    - Impair antibody production and function

# Management of XLA/HIgM

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- **Antibody replacement**
  - q3-4 week IVIg and appropriate antimicrobial treatment
  - Keep IgG levels > 500 mg/dl
  - No evidence supports treatment for THI

# Defects in Cell-Mediated Immunity

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- Chromosome 22 q11.2 deletion syndrome
- Perinatal HIV-1 infection
- SCID
- IPEX syndrome

# Clinical Manifestations of Cell-Mediated Disorders

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- **Opportunistic infections**
  - PCP, Mycobacterium tuberculosis, fungus, disseminated viral infections, GVHD
- **Macular erythematous rash**
- **Hepatitis**
- **Chronic diarrhea**

# Chromosome 22q11.2 Deletion Syndrome\*

- Absent thymic tissue leads to CD3<sup>+</sup> (T cell) lymphopenia
  - Presenting signs usually related to cardiac or calcium issues not infection
  - Viral and fungal infections
  - FTT
  - Chronic diarrhea
  - Blood transfusion associated GVHD\*\*
  - Most patients are only mildly lymphopenic (ALC) but have decreased T cells

# Perinatal HIV-1 Infection\*

- **\*\*Most common global form of combined immune deficiency in neonates\*\***
- Clinical presentation ranges from *asymptomatic* to mucocutaneous candidiasis, splenomegaly, **lymphadenopathy**, lymphopenia, thrombocytopenia
- Elevated IgA and IgE levels
- Decreased CD4 cells, normal number CD8

# Combined (Antibody and Cellular) Immunodeficiency\*

- **Wiskott-Aldrich Syndrome**
  - **Eczema, thrombocytopenia**, and susceptibility to **infection** (decreased lymphocyte count)
  - Platelets are small, defective, and abnormally shaped
  - Lymphocyte numbers are decreased and T cell function is abnormal
  - High IL-4 levels lead to eczema (increases IgE)
  - **Decreased IgM**, Elevated IgA and IgE
  - Normal IgG
  - **X-linked**

# SCID\*

- Mutations in at least ten different genes
- X-linked (IL-2R $\gamma$  chain defect\*) and other forms resulting from defects in cellular signal transduction
- Abnormal purine salvage pathway (ADA)
- Omenn syndrome (RAG1/2)

Ten abnormal genes in human SCID

### Cytokine-receptor genes

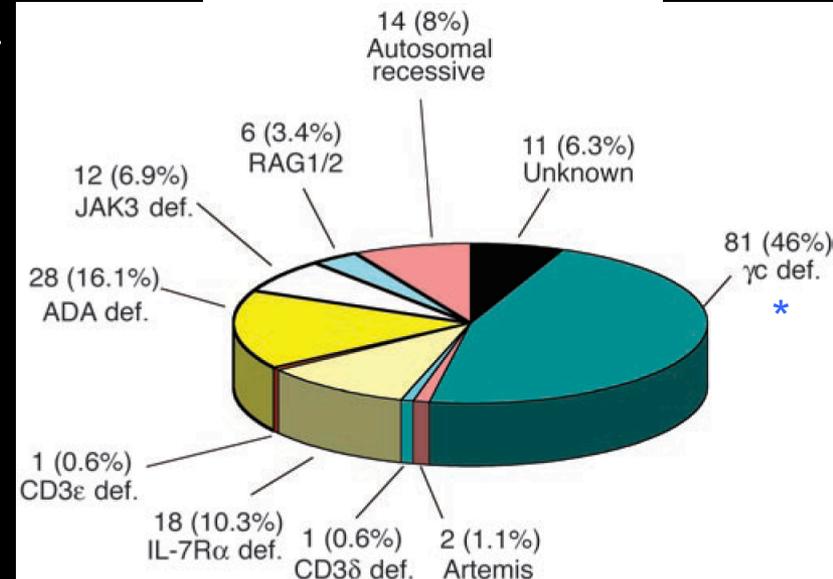
*IL-2RG* \*  
*JAK3*  
*IL-7R $\alpha$*

### Antigen-receptor genes

*RAG1*  
*RAG2*  
*Artemis*  
*CD3 $\delta$*   
*CD3 $\epsilon$*

### Other genes

*ADA*  
*CD45*

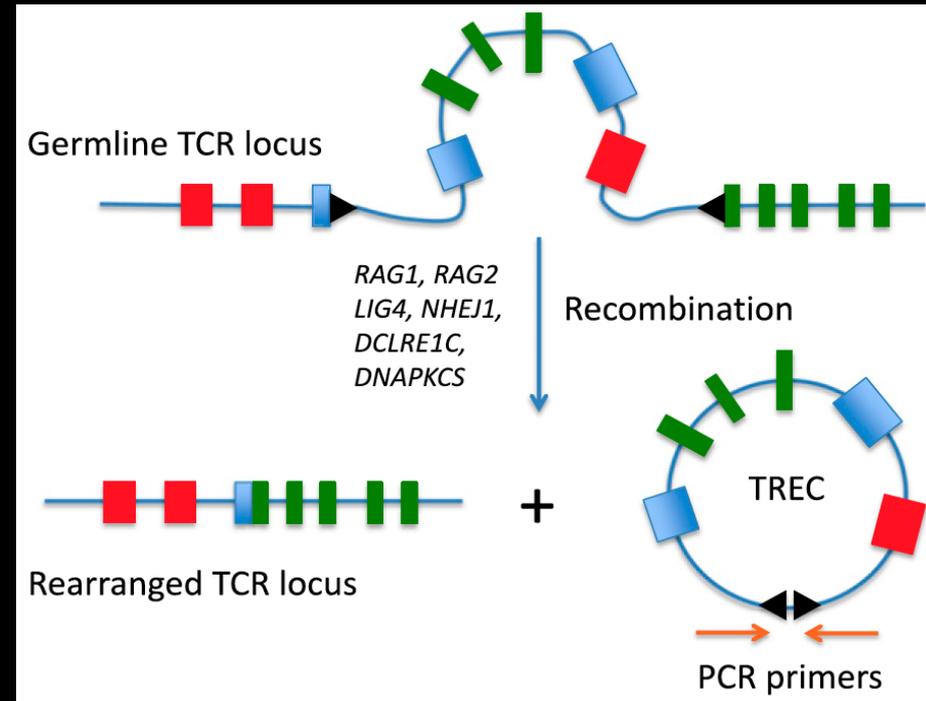


# SCID

- Deficiency of both antibody and cell-mediated immunity (low/normal # B cells, decreased T cells/NK cells)
- Diarrhea, pneumonia, otitis, sepsis, cutaneous infections, eosinophilia
- CMV, PCP, Gram negative sepsis, mucocutaneous candidiasis
- GVHD
  - following blood transfusion
  - 20-30% risk of maternal cell-mediated GVHD (placental transfer of maternal T cells)

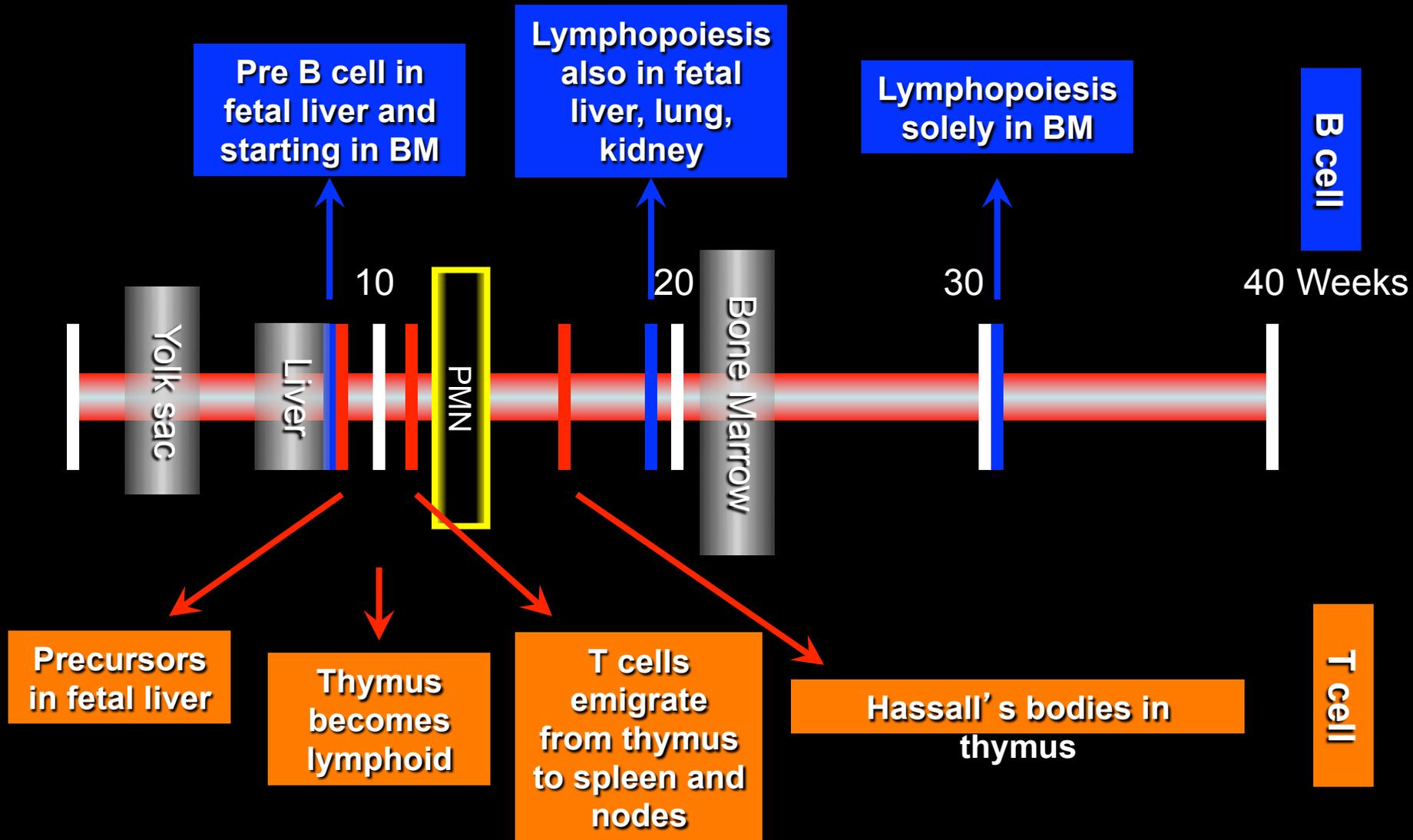
# SCID Management

- **EARLY DIAGNOSIS CRITICAL**
  - newborn screening
  - TRECs
- Immunology consult
- Bone marrow transplant
- Appropriate enzyme replacement therapy
- Irradiated blood products only
- IVIg if combined defect
- PCP prophylaxis
- No live vaccines



# Reference slides

# Development of the Immune System



# Neutropenia

- Immune
  - Auto/allo-immune
- Maternal
  - Preeclampsia
  - IUGR
- Infection
  - Bacterial
  - Viral
- Perinatal factors
  - Asphyxia
  - IVH
- Ineffective granulopoiesis
  - Nutritional
  - TPN-associated
- Metabolic disorders
  - Isovaleric acidemia
  - Propionic acidemia
  - Methylmalonic acidemia
  - Glycogen storage disease 1B
  - Orotic aciduria
  - Hyperglycinemia
- Congenital
  - Kostmann's disease
  - Pure white-cell aplasia
  - Reticular dysgenesis
  - Cartilage-hair hypoplasia
  - Hyper-IgM syndrome
  - Schwachman-Diamond
  - Dyskeratosis congenita

# Neutrophilia

- Term: > 7000 cells/ $\mu$ l
- Premature: > 13000 cells/  $\mu$ l
- Nonspecific response to stress
- Causes (predominantly demargination)
  - Infection
  - Hemorrhage
  - Asphyxia
  - Seizures
  - Pneumothorax
  - MAS
  - Hemolytic disease
  - Hypoglycemia
  - Leukomoid reaction
  - Leukocyte adhesion deficiency
  - Steroids
  - Leukemia

# Key Screening Immune Function Assays

## Innate

### **CBC with manual differential**

→ Absolute Neutrophil Count (ANC)

### **Nitro blue tetrazolium (NBT)**

→ normal result: colorless → blue

### **Flow cytometry**

→ cell surface antigen measurement

→ CD18 (Leukocyte adhesion deficiency)

### **CH50**

→ C3, C4

## Adaptive

### **CBC with manual differential**

→ Absolute Lymphocyte Count (ALC)

### **Chest radiograph**

→ evaluate for presence of thymus

### **Flow cytometry**

→ T cell subsets (CD3, CD4, CD8)

→ B cell count (CD19, CD20)

### **Quantitative Immunoglobulins**

(IgG, IgA, IgM)

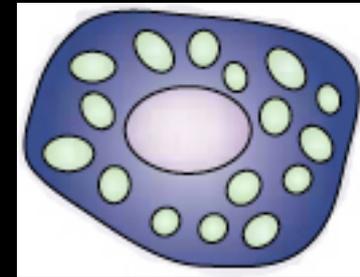
### **Antibody response to T-cell dependent antigens**

→ Tetanus toxoid, anti-HepBsAg, conjugated HIB

# Eosinophils and Mast cells



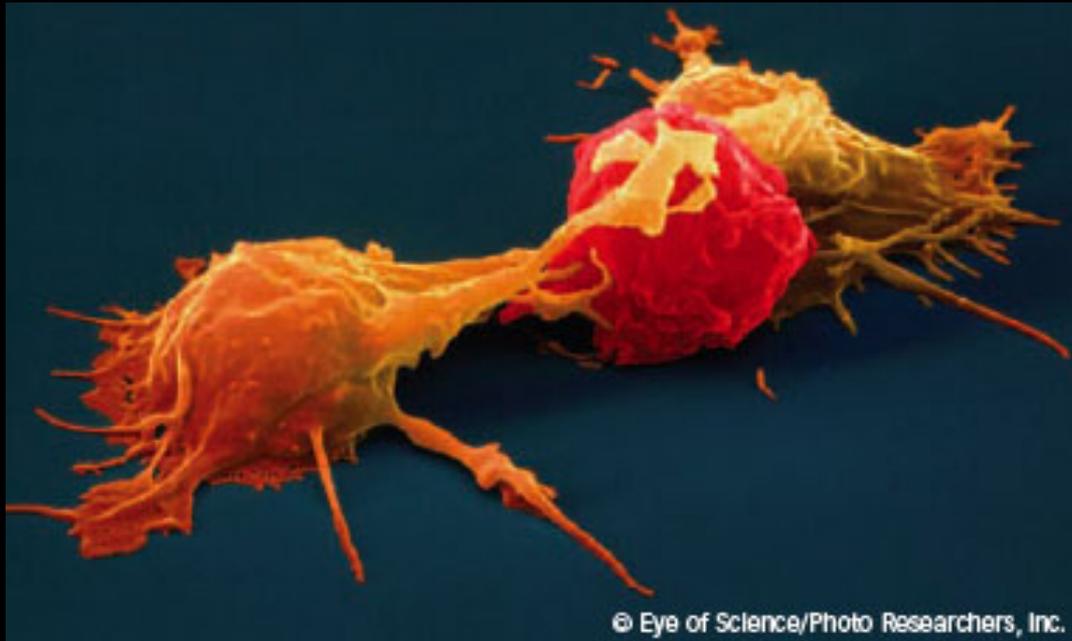
- Produce cytokines, phagocytose Ag-Ab complexes, produce HDP/ cytotoxic molecules, vasodilatory substances
- Expansion associated with sepsis in the very premature
- Associated with erythema toxicum
- Extracellular trap production?
- Role in immune response is incompletely defined



- Produce histamines, cytokines, phagocytose pathogens, and participate in Ag presentation
- Associated with erythema toxicum
- Extracellular trap production?
- Role in immune response is incompletely defined

# Natural Killer Cells

- Role in viral infections
- Direct cellular killing and cytokine production
- Increased numbers at birth
- Decreased cytotoxic function
- Decreased numbers with SCID



# Key Screening Immune Function Assays

## Innate

### **CBC with manual differential**

→ Absolute Neutrophil Count (ANC)

### **Nitro blue tetrazolium (NBT)**

→ normal result: colorless → blue

### **Flow cytometry**

→ cell surface antigen measurement

→ CD18 (Leukocyte adhesion deficiency)

### **CH50**

→ C3, C4

## Adaptive

### **CBC with manual differential**

→ Absolute Lymphocyte Count (ALC)

### **Chest radiograph**

→ evaluate for presence of thymus

### **Flow cytometry**

→ T cell subsets (CD3, CD4, CD8)

→ B cell count (CD19, CD20)

### **Quantitative Immunoglobulins**

(IgG, IgA, IgM)

### **Antibody response to T-cell dependent antigens**

→ Tetanus toxoid, anti-HepBsAg, conjugated HIB

# Classification of Immune Disorders that Manifest in Neonates

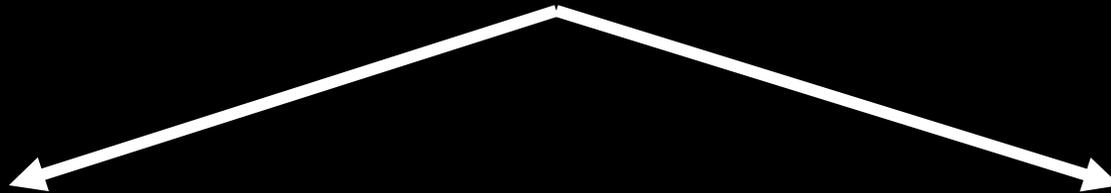
<b>Immune System Component</b>	<b>Immune Disorder (s)</b>
<b>Predominant Antibody Defects</b>	<b>X-linked agammaglobulinemia Hyper IgM Syndrome Transient hypogammaglobulinemia of infancy</b>
<b>Predominant Cell Mediated Defect</b>	<b>Chromosome 22q11.2 deletion syndrome Neonatal HIV infection IPEX syndrome</b>
<b>Combined Immunodeficiency</b>	<b>Wiscott-Aldrich Syndrome</b>
<b>Severe Combined Immunodeficiency</b>	<b>X-linked SCID Zap-70 deficiency Adenosine Deaminase Deficiency Omenn Syndrome</b>

## Clinical Findings



### Screening Evaluations:

- 1) Absolute lymphocyte count
- 2) T cell subsets (CD3, CD4, CD8, CD19/CD20)
- 3) Immunoglobulin Profile (IgG, IgA, IgM, IgE)



### Normal:

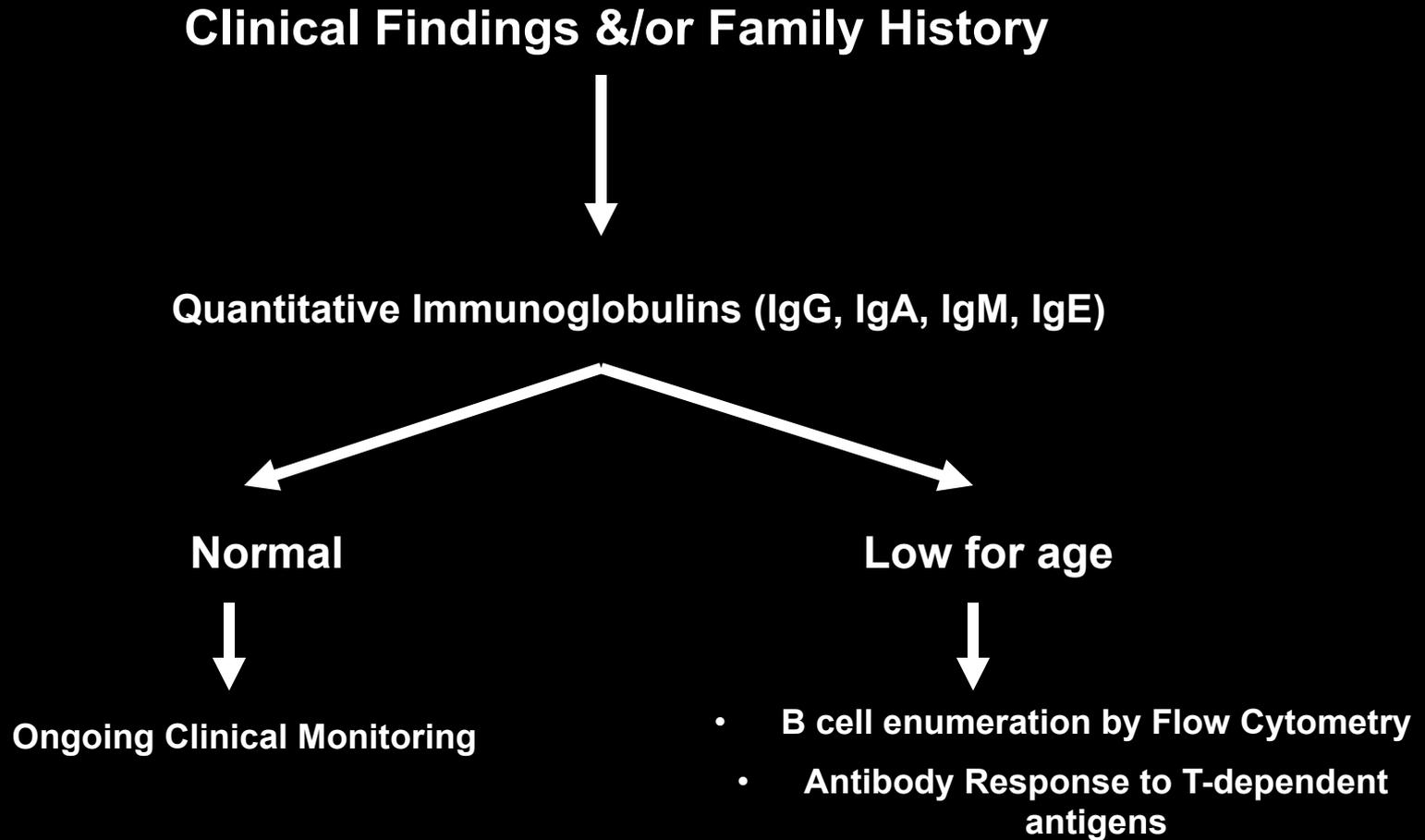
- Continue Clinical monitoring
- Evaluate for Secondary Immunodeficiency

### Abnormal:

- Assay of T cell function (Mitogen proliferation)
- Extended Flow Cytometry
- Evaluate for specific disorder
- Chromosome analysis

Unhelpful Tests: DHST

# Approach to the Neonate with Suspected B cell Deficiency



**Unhelpful Lab Assays during Neonatal Period:**

**Pneumococcal titers**

**Serum Protein Electrophoresis**

**IgG subclass determination**

# Ig Values for Term Infants

Age	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
Newborn	598-1672	0-5	5-15
1-3 months	218-610	20-53	11-51
4-6 months	228-636	27-72	25-60
7-9 months	292-816	27-73	12-124
10-18 months	383-1070	27-169	28-113

# Immunoglobulin and Lymphocyte Profiles in Immunodeficiency Disorders

	IgG	IgA	IgM	IgE	CD3	CD4	CD8	CD19
XLA	Low/nl	Absent	Absent	Absent	Normal	Normal	Normal	Absent
HIGM	Low/nl	Low	Elevated	Low	Normal	Normal	Normal	Normal
THI	Low	Low	Normal	Normal	Normal	Normal	Normal	Normal
22q11.2	Low/nl	Low/nl	Normal	Normal	Low	Elevated	Low	Normal
HIV	Normal	Elevated	Normal	Elevated	Low	Low	Normal	Normal
WAS	Normal	Elevated	Normal	Elevated	Low	Low	Normal	Normal
XSCID	Normal	Low	Low	Normal	Absent	Absent	Absent	Normal
Zap70	Low/nl	Low	Low	Low	Low	Normal	Absent	Normal
ADA	Low/nl	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Omenn	Low/nl	Absent	Low	Elevated	Low	Low	Low	Low

# IPEX Syndrome

- Immunodysregulation, Polyendocrinopathy, Enteropathy
  - **VERY rare** → **But presents in neonatal period**
- Mutation in forkhead box protein 3 located on the **X** chromosome
- Impaired T<sub>REG</sub> suppressor function
- Decreased IL-2 and IFN- $\gamma$  production
- Severe watery diarrhea, FTT, dermatitis, type I DM
- Increased IgE levels and eosinophilia