Neonatal Immunology

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Learning Objectives

- Review developmental biology of the immune system
- Review specific components and function of the immune system
- Review diagnosis and management of the most common immunodeficiency disorders that may present during the neonatal period

Case

- A 24 year-old mother presented in labor at 24 weeks with premature rupture of membranes and fever to 39C
- Antibiotics and antenatal steroids were given to the mother and the baby delivered vaginally 19 hours later
- The baby required significant ventilatory, oxygen, and inotropic support immediately after birth
- The baby is treated with antibiotics for 10 days for presumed sepsis; BCx/CSF remained negative; CRP peaked at 165 mg/L
- A bilateral grade 3 IVH was diagnosed on DOL3

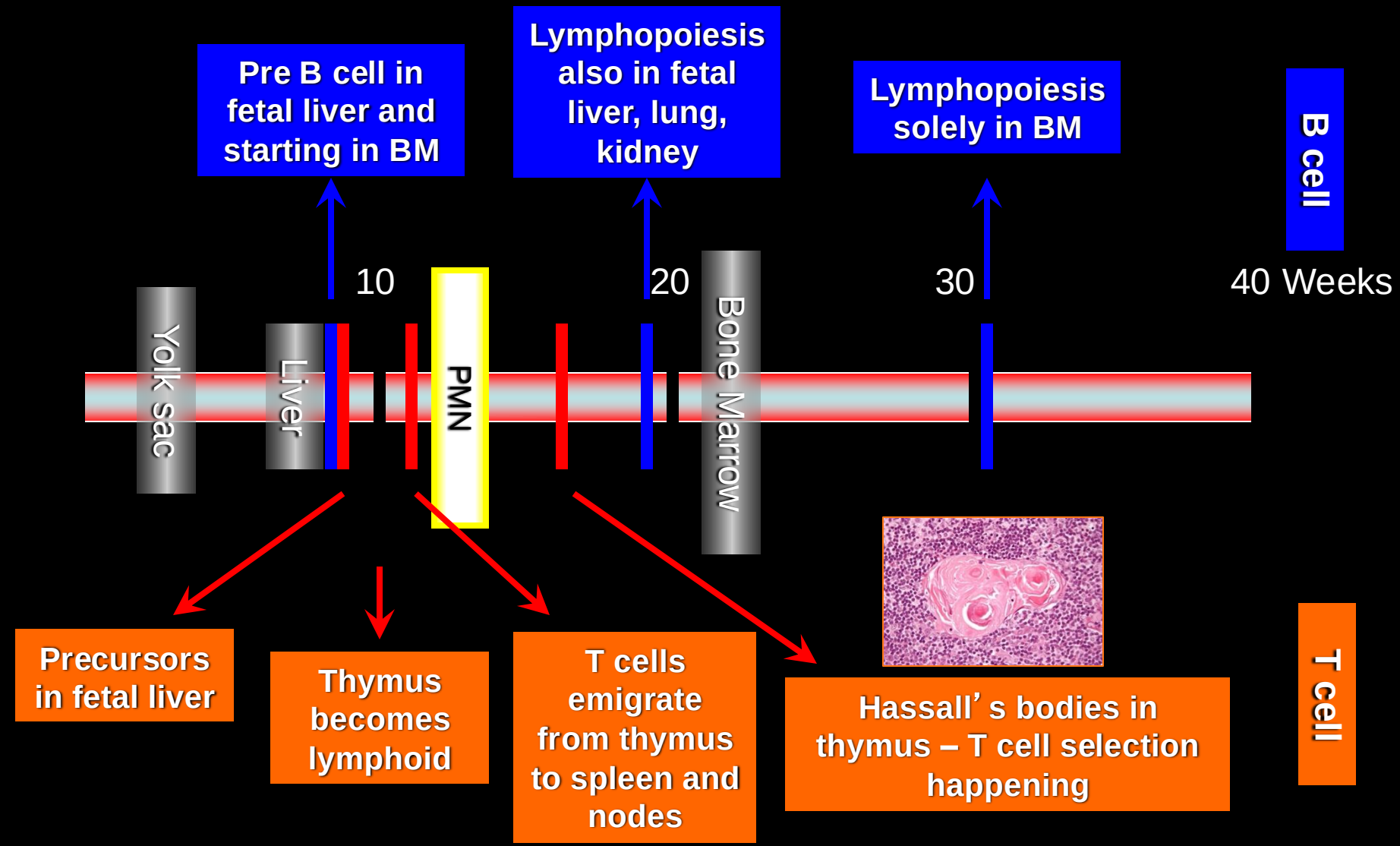
A case-continued

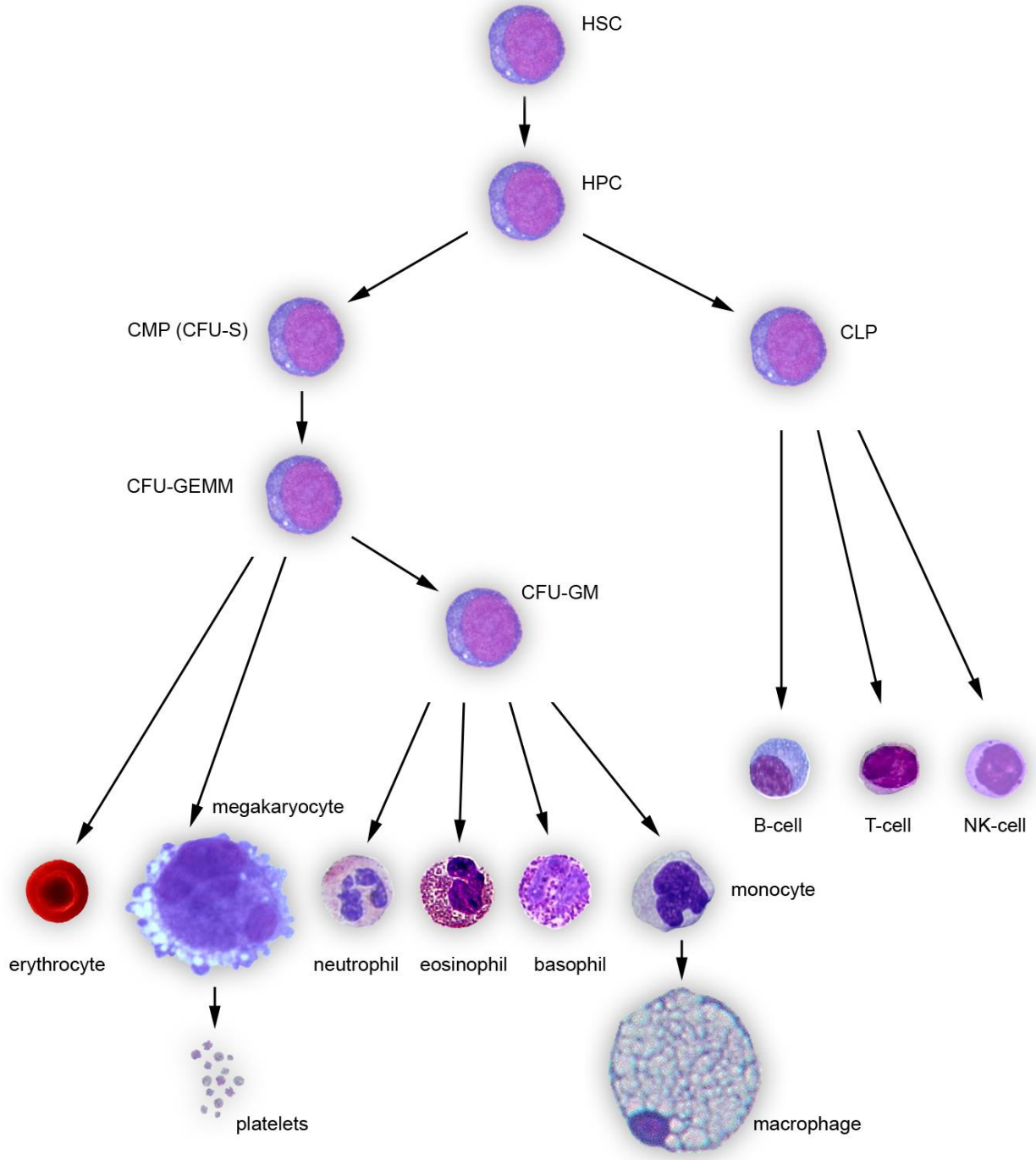
- On DOL 14 the baby experienced a significant clinical decline with worsening respiratory status, increased oxygen requirement and shock
- NEC developed with klebsiella bacteremia, an ex lap was required due to free peritoneal air with necrotic bowel seen, and generalized peritonitis occurred
- The infant survived the episode but the clinical course was tenuous over the next few weeks
- A second surgery was required for GI obstruction due to strictures
- Ultimately the infant developed BPD, ROP, and white matter injury

A case-continued

- All conditions this infant experienced are directly impacted by the immune response that occurs with them
  - Chorioamnionitis
  - Sepsis
  - Intraventricular hemorrhage
  - Acute lung and gut injury
  - Necrotizing enterocolitis
  - Retinopathy of prematurity
  - Bronchopulmonary dysplasia
  - White matter injury

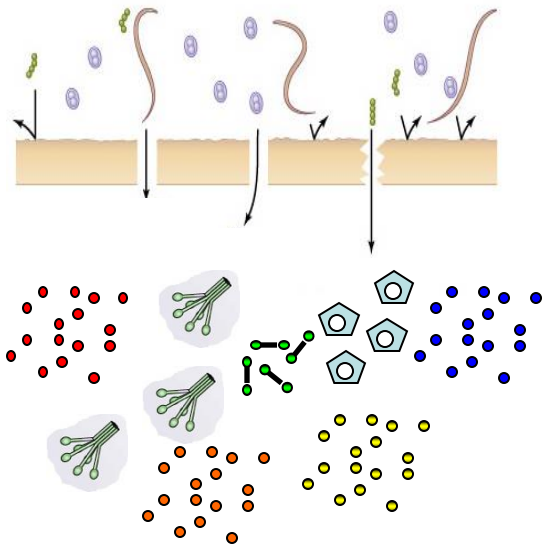
Development of the Immune System

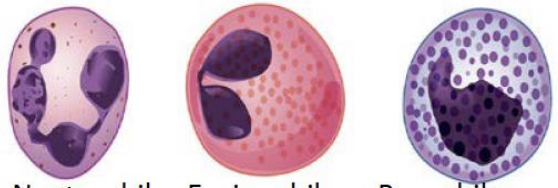


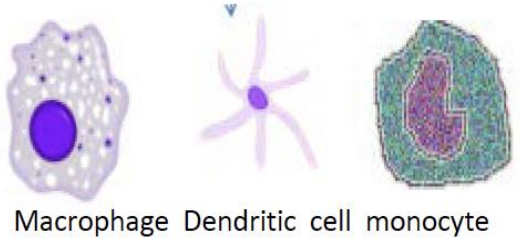

HSC
HPC
CMP (CFU-S)
CLP
CFU-GEMM
CFU-GM
B-cell
T-cell
NK-cell
megakaryocyte
monocyte
erythrocyte
neutrophil
eosinophil
basophil
platelets
macrophage

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

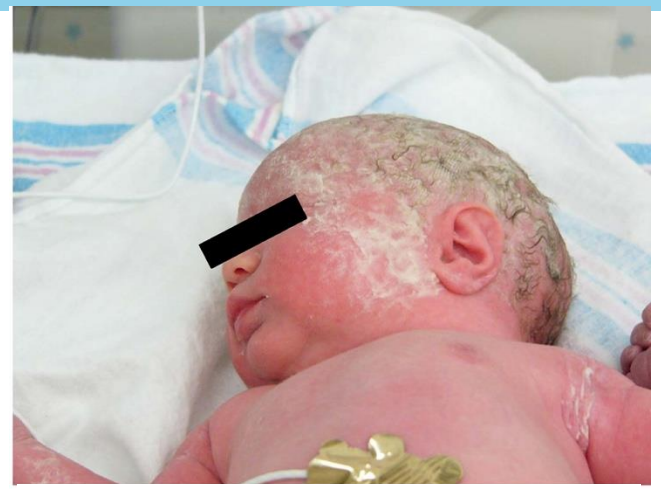




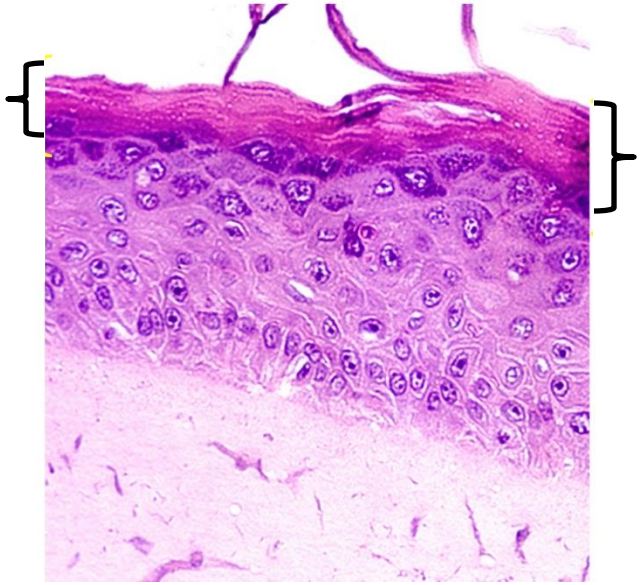


Barriers:
Skin

- 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
- Maturation accelerated in preterm infants
  - Up to 4 weeks to achieve barrier function



Barriers: Mucosa

- **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)

Inflammation: Big Picture

Barriers

Inflammatory challenge

Inflammation **PRECEDES** clinical signs

Dolor, Rubor, Calor, Tumor
Local or systemic clinical manifestations AND loss of function

Acute phase response

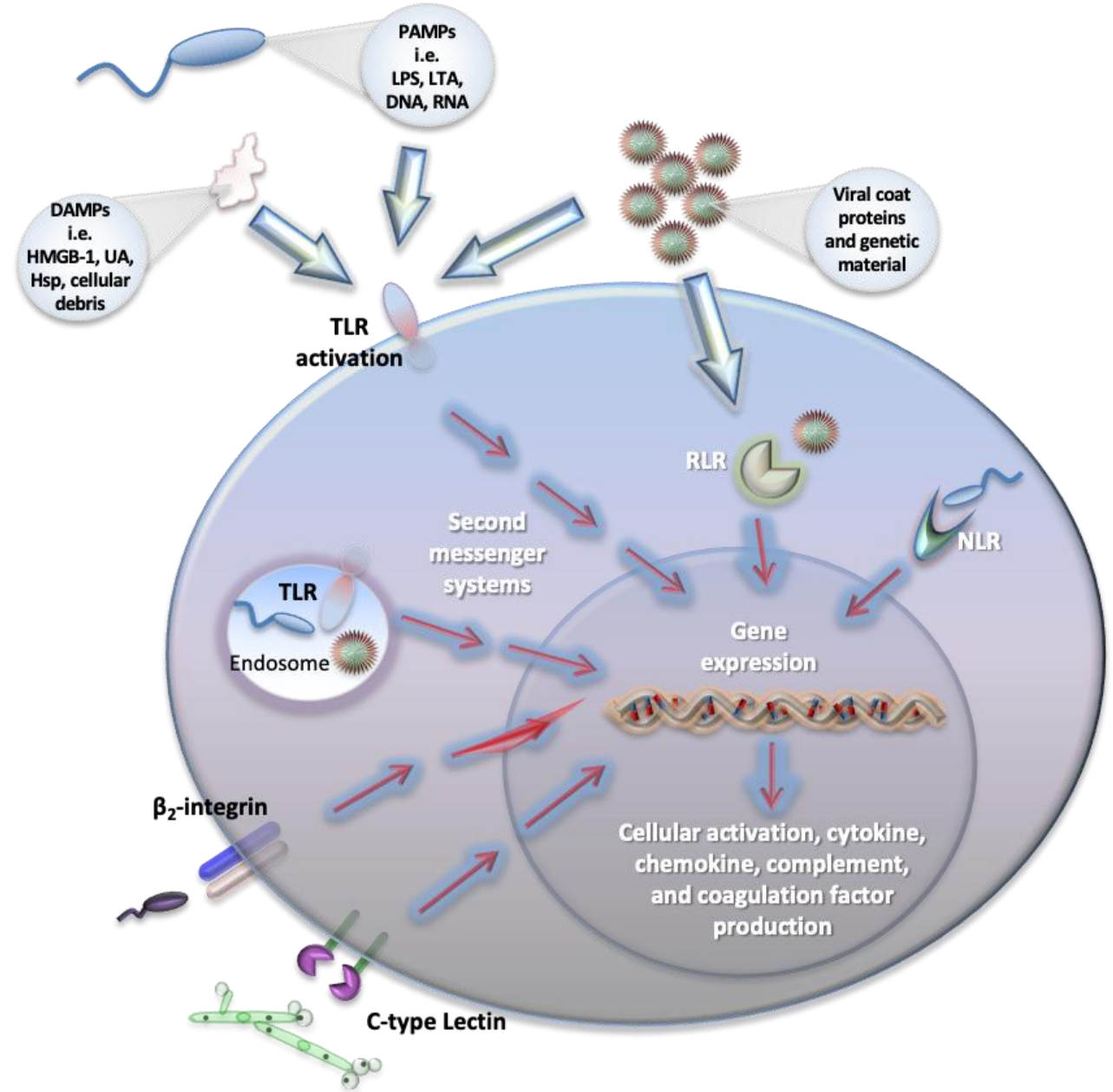
Resolution of inflammation

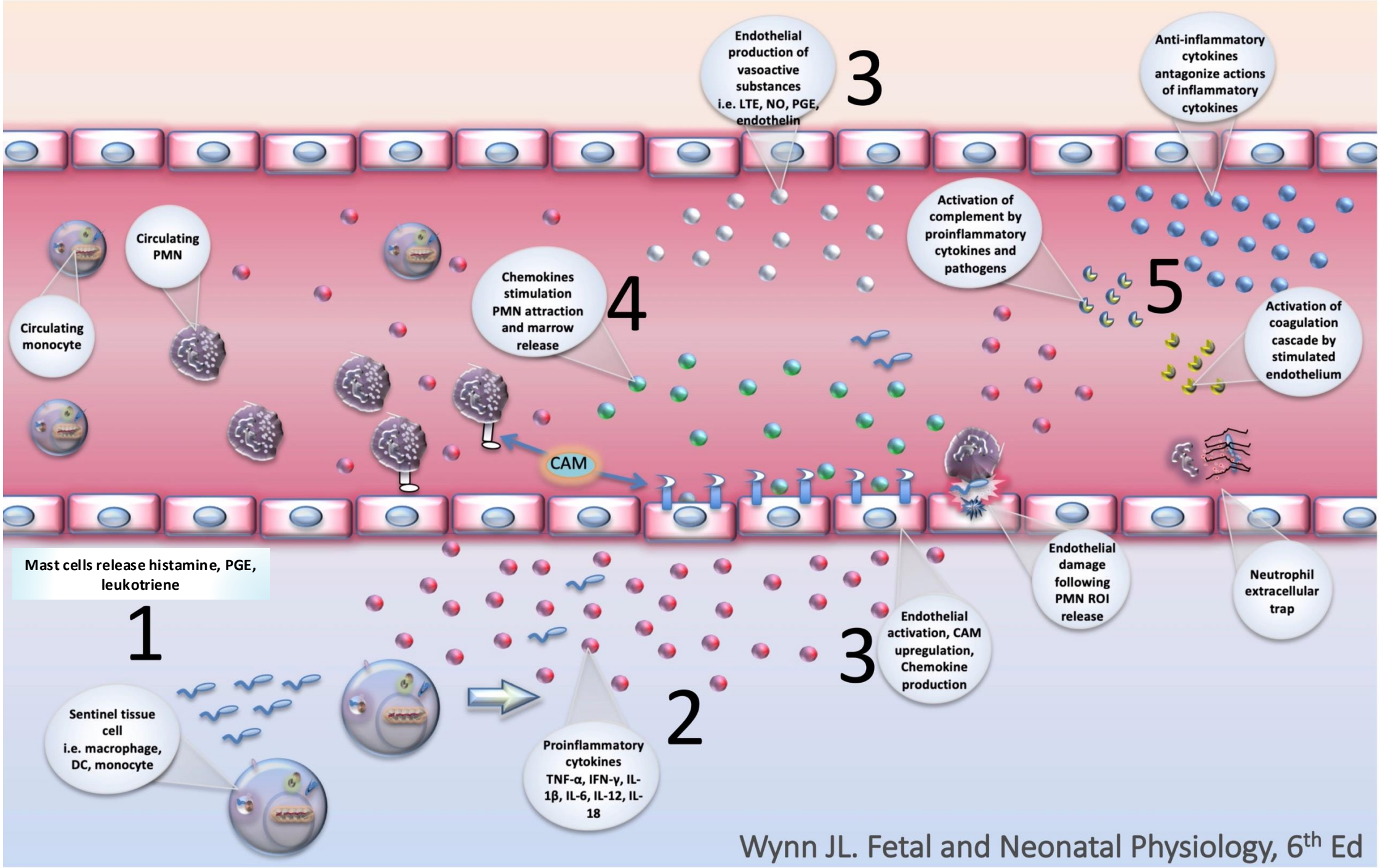
Resolution of clinical signs

Inflammation: Detail

- 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)
  - RLRs, NLRs




Endothelial production of vasoactive substances i.e. LTE, NO, PGE, endothelin
3
Anti-inflammatory cytokines antagonize actions of inflammatory cytokines
Activation of complement by proinflammatory cytokines and pathogens
5
Activation of coagulation cascade by stimulated endothelium
Circulating PMN
Circulating monocyte
Chemokines stimulation PMN attraction and marrow release
4
CAM
Mast cells release histamine, PGE, leukotriene
1
Sentinel tissue cell i.e. macrophage, DC, monocyte
Proinflammatory cytokines TNF-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18
2
3
Endothelial activation, CAM upregulation, Chemokine production
Endothelial damage following PMN ROI release
Neutrophil extracellular trap
Wynn JL. Fetal and Neonatal Physiology, 6th Ed

Inflammatory responses and mediators

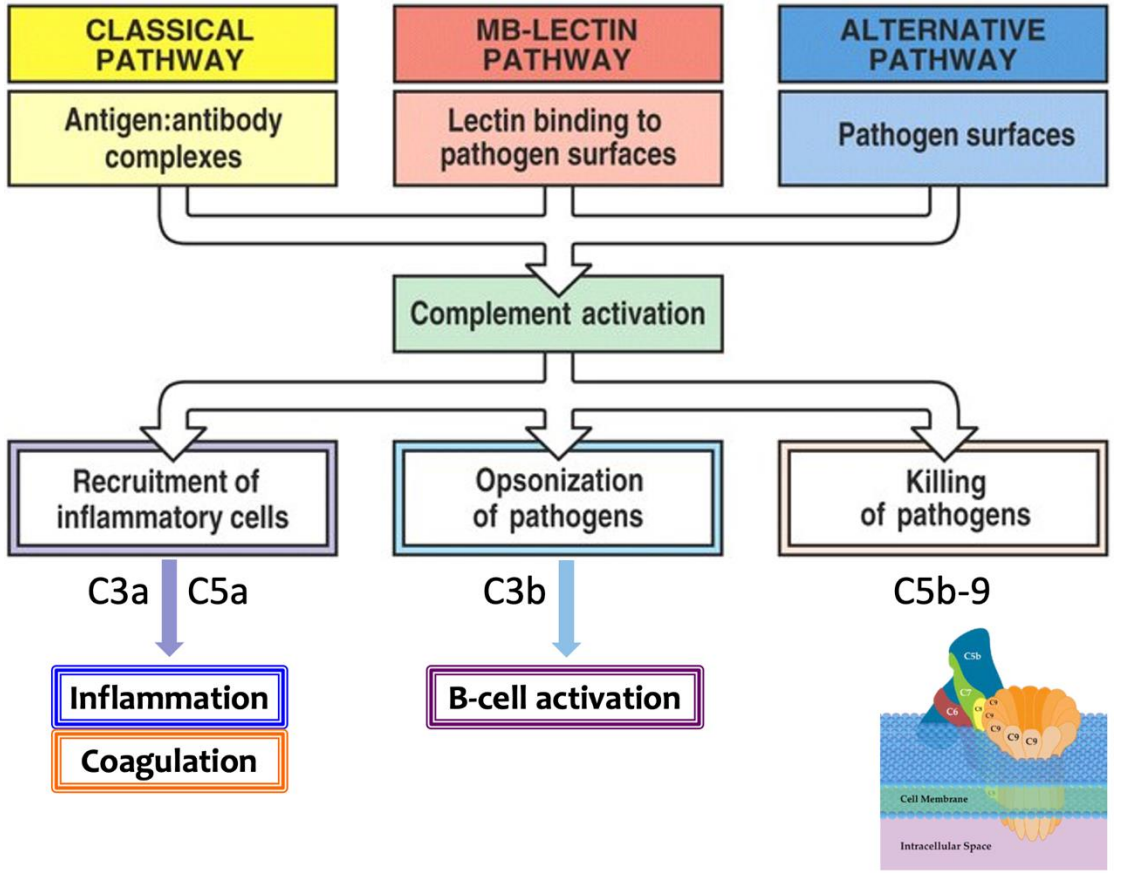
- **Fever** → IL-1, TNF-a, prostaglandins
- **Vasodilation** → Histamine, prostaglandin, nitric oxide, bradykinin
- **Chemotaxis** → Chemokines (IL-8), complement, eicosanoids (prostaglandins, leukotrienes)
- **Leukocyte adhesion** →Cytokines (IL-1, TNF-a), complement, eicosanoids (prostaglandins, leukotrienes), selectins
- **Increased vascular permeability** → Histamine, complement, bradykinin, leukotrienes, nitric oxide
- **Tissue necrosis** → Neutrophil granules, free radicals
- **Platelet aggregation** → Eicosanoids (prostaglandins, leukotrienes)
- **Opsonization** → IgG, complement C3b, CRP

Soluble components and opsonins

- **C3b**
  - Most critical bacterial opsonin
- **Fibronectin**
  - Enhance leukocyte adherence and migration
  - Levels are decreased in premature infants
- **C-reactive protein**
  - Produced by 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

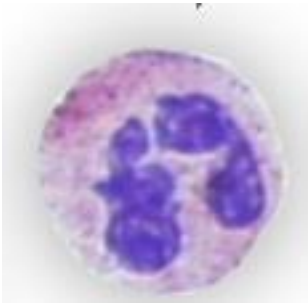
Neonatal Complement System

- Complement
  - **Recruits leukocytes**
  - **Opsonizes and kills pathogens**
- 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



Neonatal Neutrophils

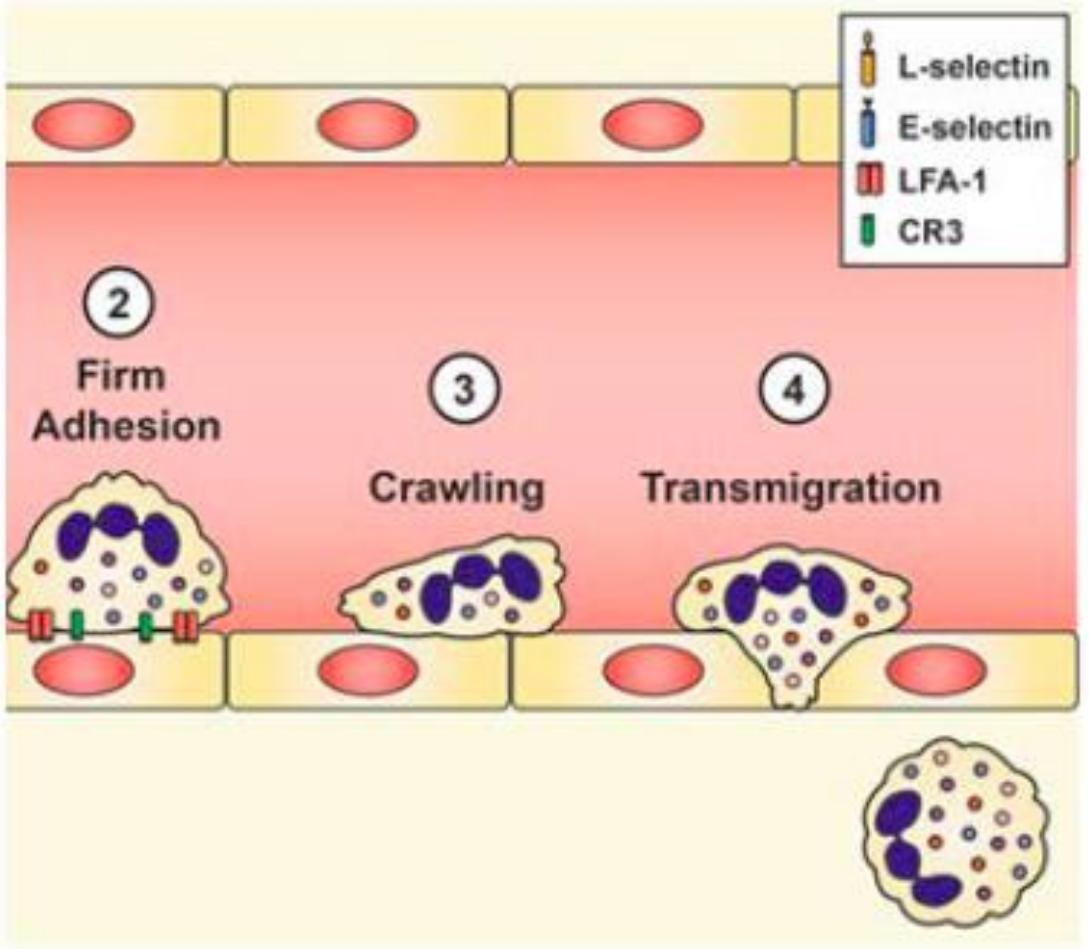
- Neonatal PMN morphology is similar to those from children and adults
- Phagocytic and microbicidal activity of PMNs from healthy neonates comparable to adults
  - Poor phagocytic and killing (respiratory burst) in sick and/or preterm neonates
    - Via reactive oxygen metabolite production by NADPH oxidase
  - Neutrophil function reduced with prematurity, gram negative sepsis, indomethacin, and intrapartum magnesium



- Numerous *in vitro* functional deficiencies
  - Chemotaxis
  - Adhesion →Decreased L-selectin and beta-2 integrin
  - Granule content →Acid and activities of digestive, enzymatic, or microbicidal components from granules

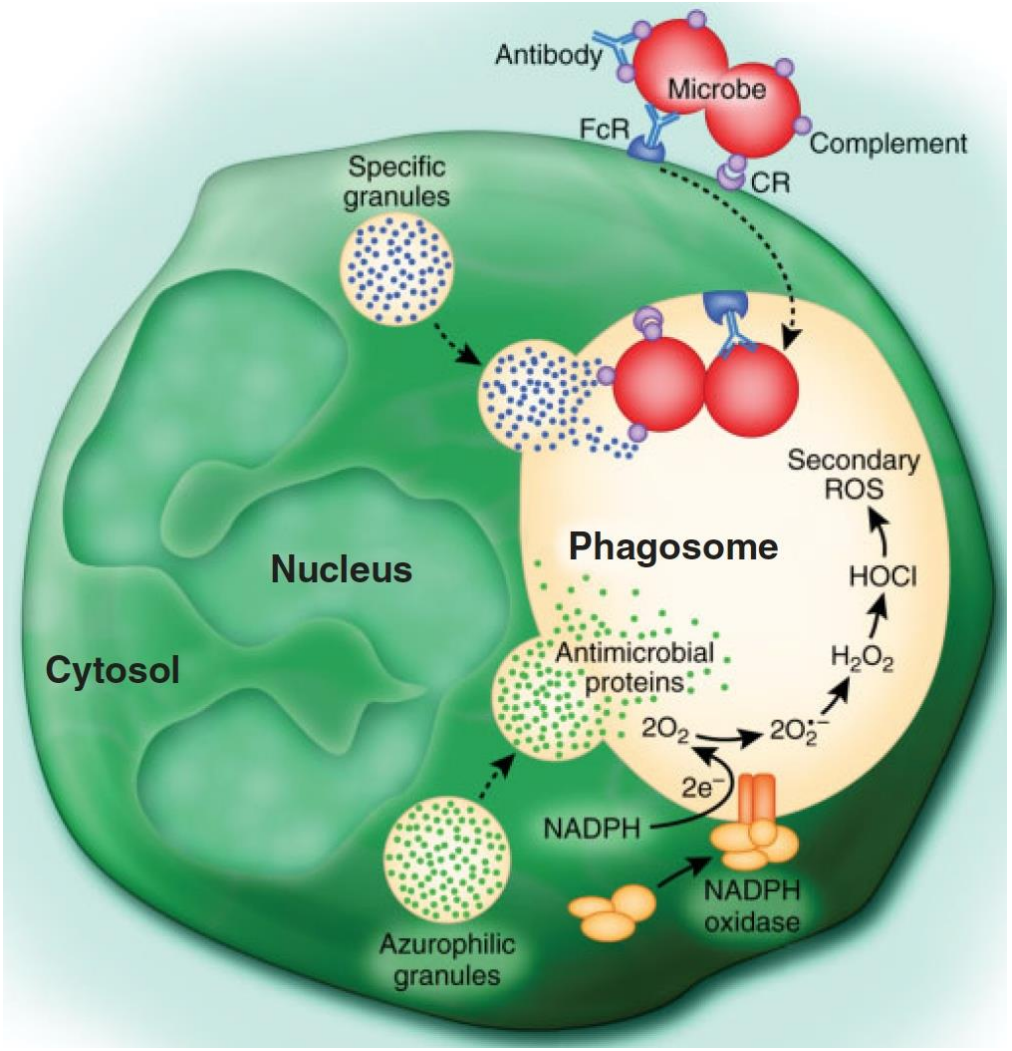
PMN Chemotaxis and Diapedesis

- Reduced basal chemotaxis and random migration
  - ~1/2 speed of adults
  - Worse in post-op and septic neonates
  - Serum chemokine levels are normal
  - Impaired signaling down stream of chemokine-receptor binding
  - Small improvement following labor
- Decreased L-selectin and β2 integrin
  - Cell adhesion molecules for rolling and diapedesis
- Deformation reduced
  - Worse in band forms

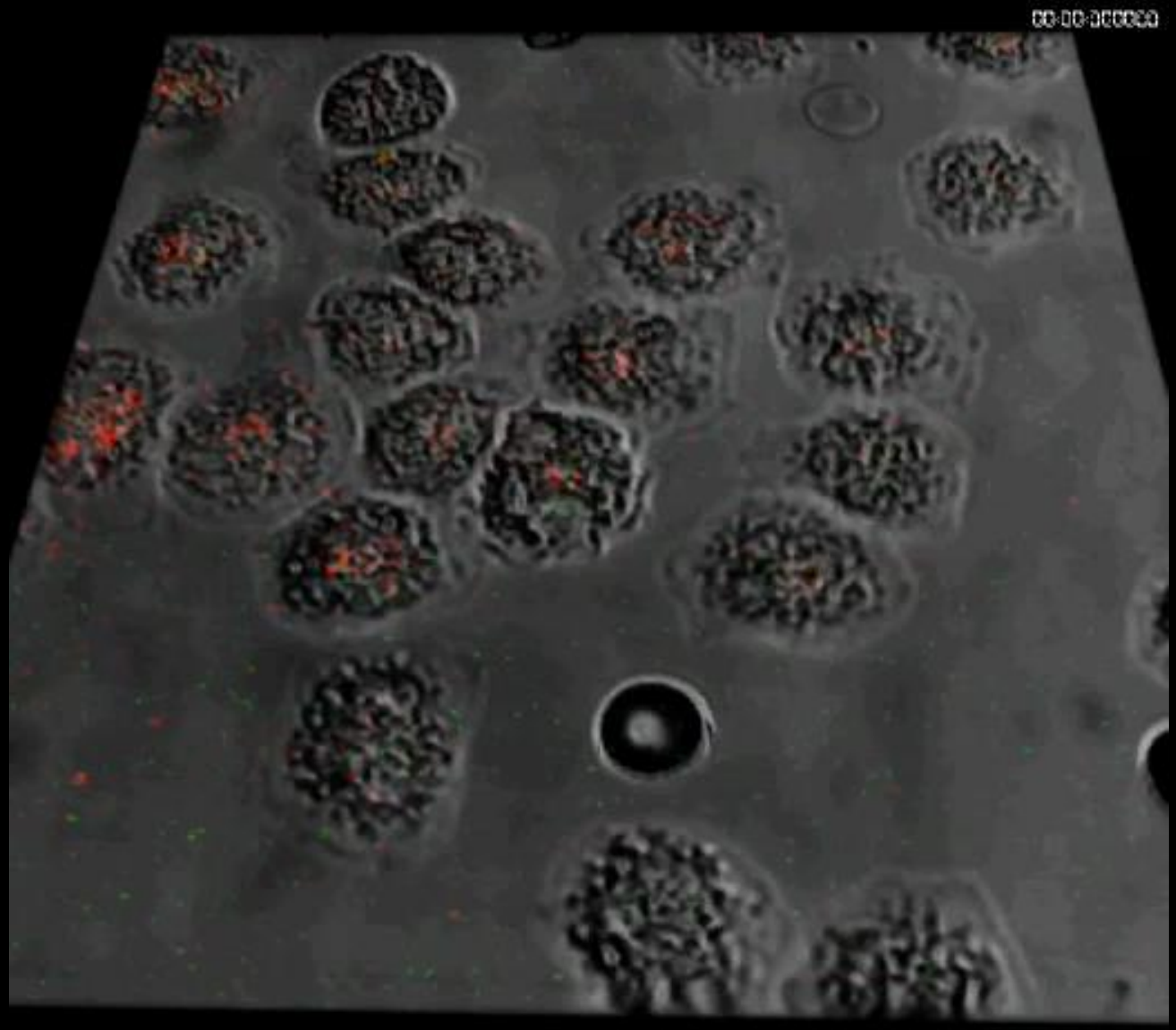


Pathogen killing

- **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**

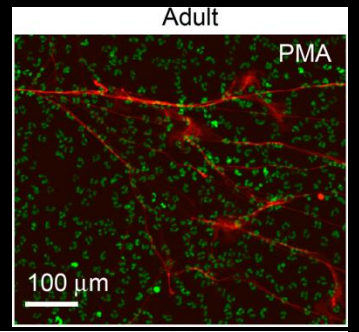


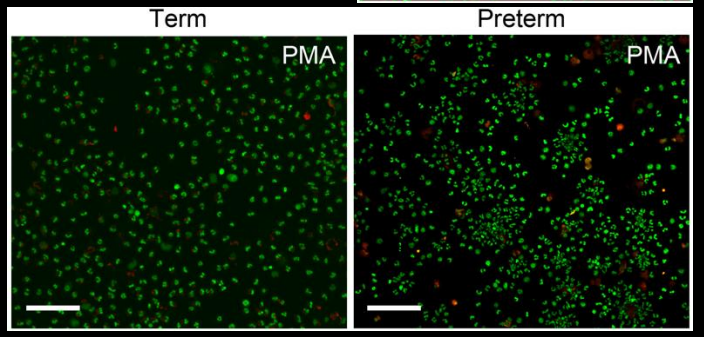
Neutrophil NETs: Kamikaze



Poor extracellular trap formation (term and preterm)

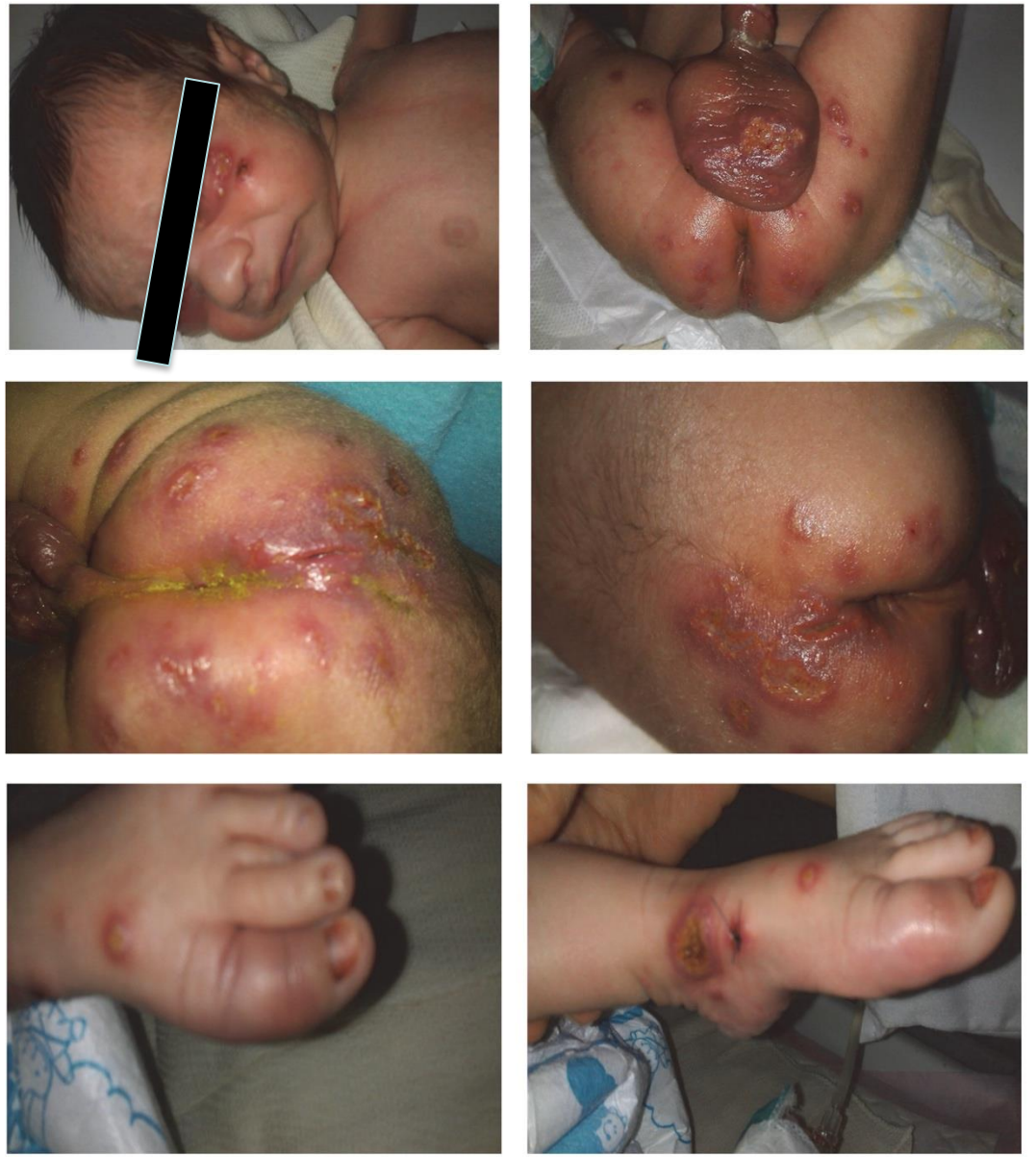
Yost CC, et.al. Blood. 2009.
Marcos V et al. Blood. 2009.





Case #1

- 17 day-old Neonate presents with vesiculopapular rash around eyes, anus, diaper area, feet; dactylitis of toe
- Born at term to parents (cousins), no complications, home in 2 days
- No fever, no poor feeding, no resp distress, otherwise non-toxic



Case #1

- Broad spectrum antimicrobial treatment started
- CXR normal
- Skin lesions positive for Staph aureus – neg for HSV
- Skin biopsy showed necrotizing granulomatous tissue reaction
- Bone scan showed osteo in multiple locations

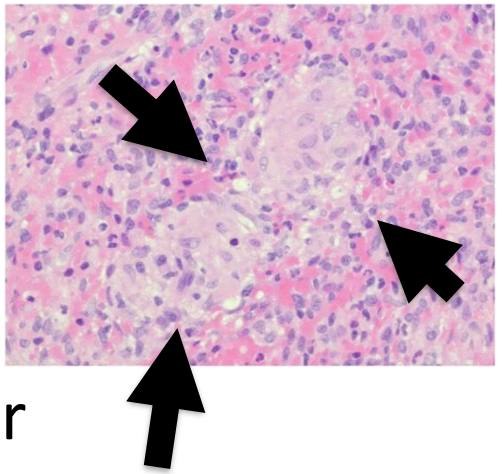
Table 1: Results of the routine laboratory tests.

Parameter	Before treatment	After treatment	Units
WBC	15.2	11.3	K/μL
Neut	55	41	%
Lymph	31.6	32.8	%
Mono	11.9	21.9	%
Eos	1.6	4	%
RBC	4.23	3.75	M/μL
Hgb	13.3	11.5	g/dL
Platelet	112	582	K/μL
CRP	56.2	22	mg/L

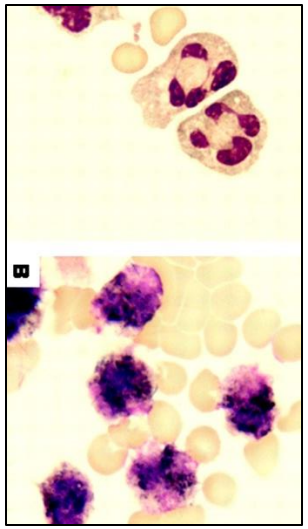
Table 2: Results of the lumbar puncture.

Parameter	Value
Protein	45 mg/dL
Glucose	57 mg/dL
WBC	1/μL
RBC	700/μL
Smear	Negative
Culture	Negative
PCR for HSV	Negative

Chronic Granulomatous Disease



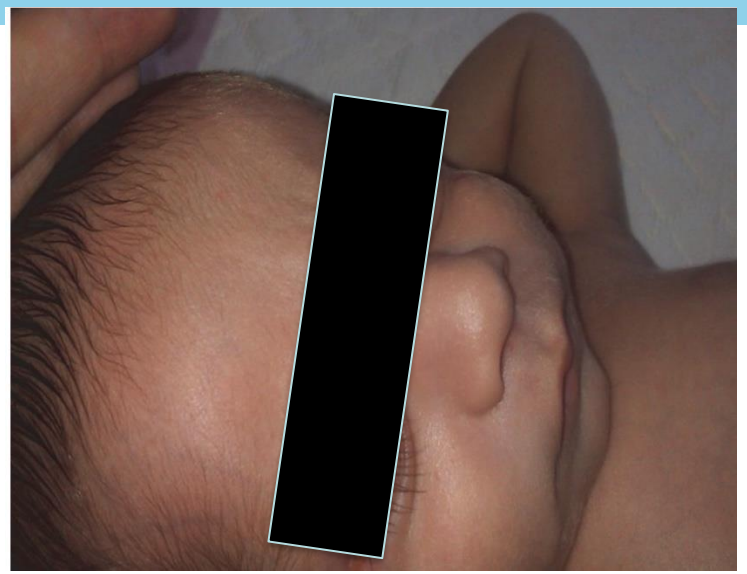
- 1 in 200,000
- Deficiency or absent NADPH oxidase function in phagocytic cells
- 80-90% MALE- usually X-linked recessive
- Severe, recurrent infections with catalase-positive bacteria or fungi (*Staphylococcus aureus, Burkholderia cepacia, Serratia marcenscens, Nocardia spp, and Aspergillus spp*)
- Diagnosis made by flow cytometry (DHR)
- Old way NBT→remains colorless
  - Demonstration of absent or severely deficient respiratory burst activity in phagocytes

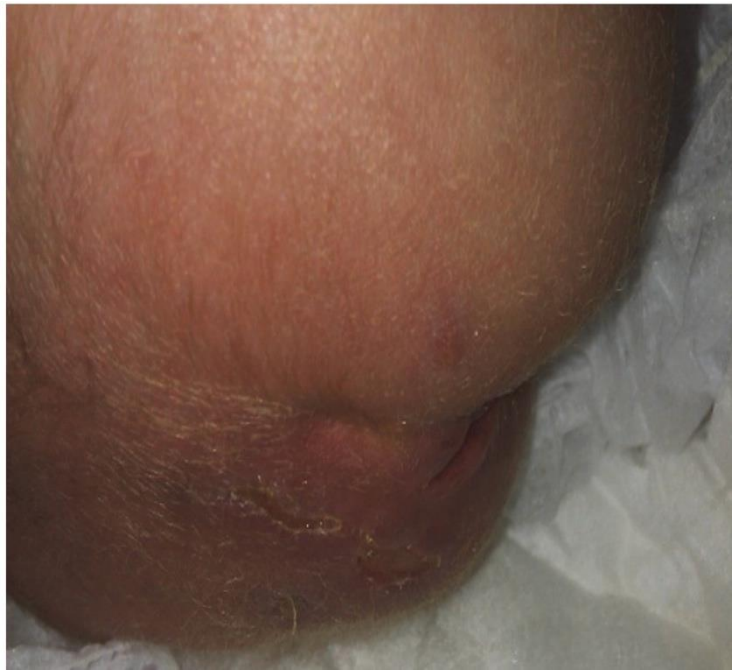


- Treatments include antibacterial/antifungal prophylaxis, interferon gamma and HSCT

Case #1

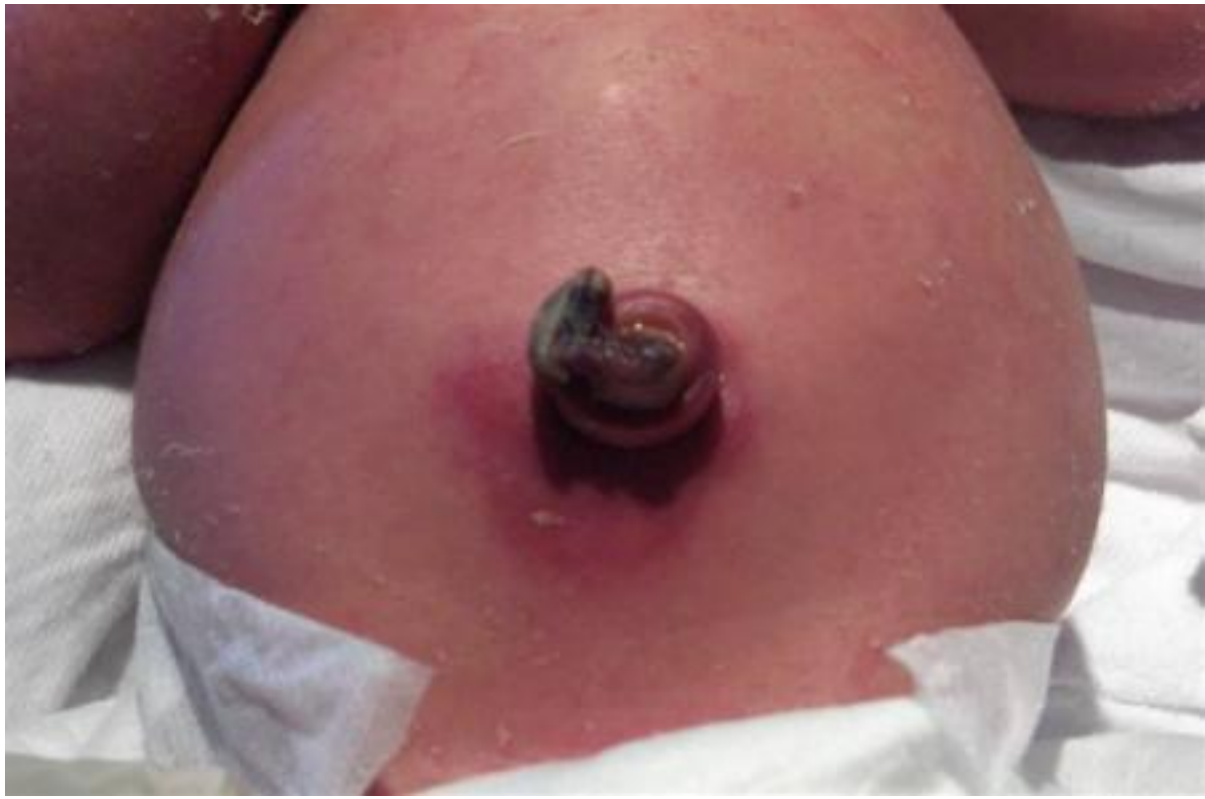
- 6 weeks antibiotics
- Skin lesions improved
- CGD dx made DHR
- Discharged home on antibacterial and antifungal prophylaxis





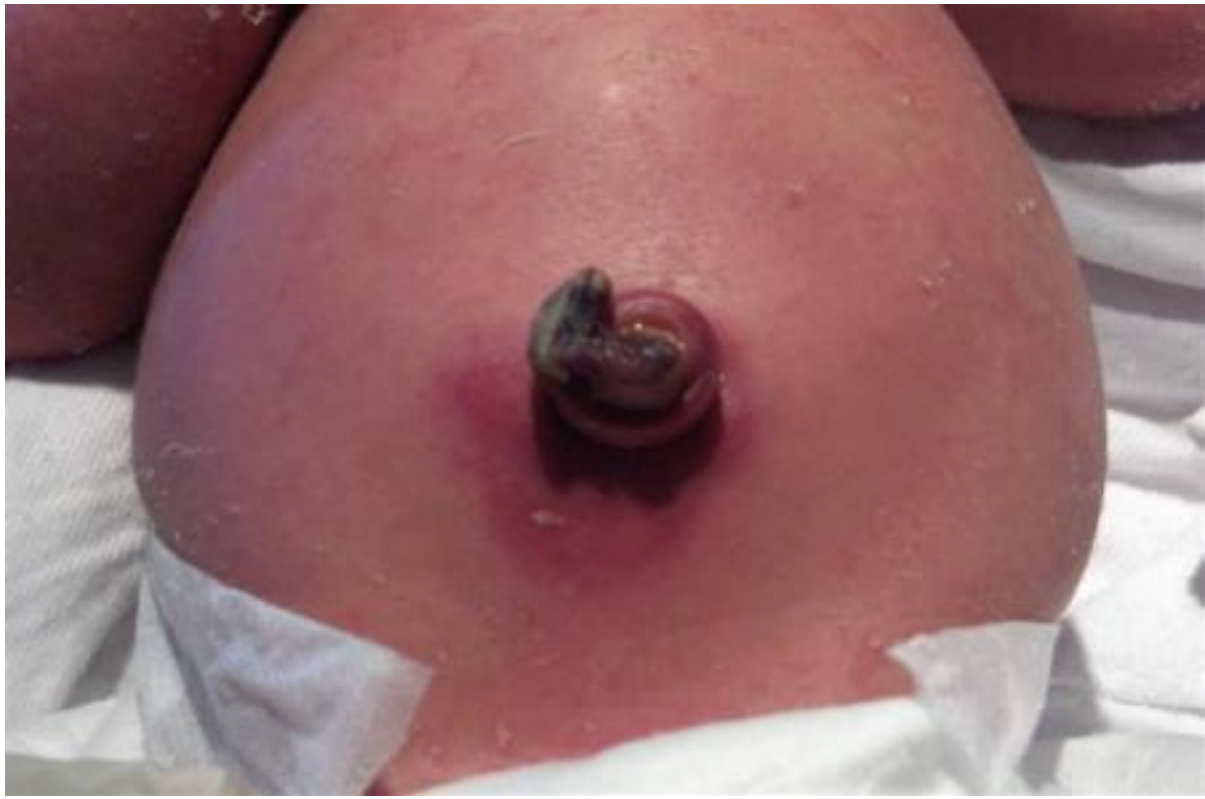
Case #2

- Term neonate, DOL 22 presents with umbilical redness (cord still intact), respiratory distress, tachycardia, fever to 38.2, poor feeding
- Mom 22 year-old, uneventful pregnancy and vaginal delivery, home in 24 hours, consanguinity
- Evaluation included standard tests/treatment for sepsis
- CBC revealed WBCs at 48k with a neutrophil predominance
- Abdomen exam reveals no pus visible around cord stump



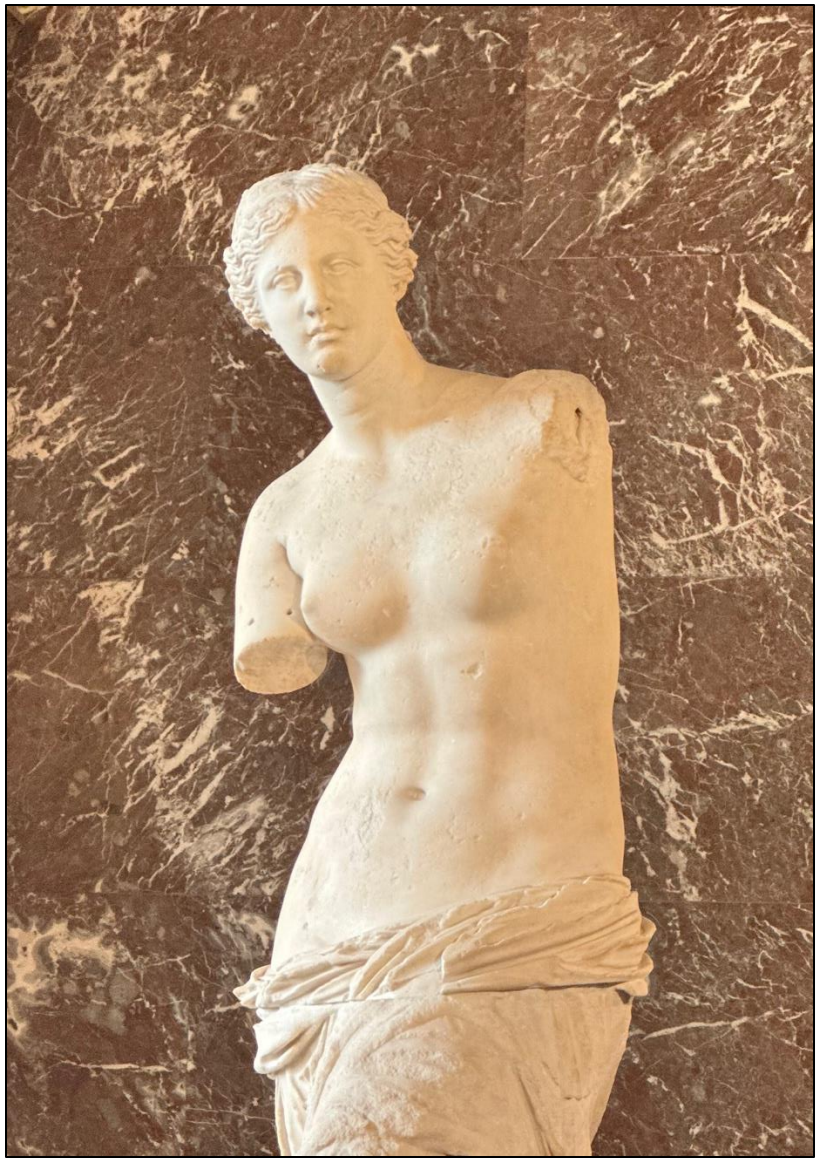
Case #2

- Baby is treated for several days with antibacterial drugs
- Flow cytometry for surface markers performed due to significant leukocytosis and delayed cord separation revealed reduced CD11b/CD18 expression on neutrophils



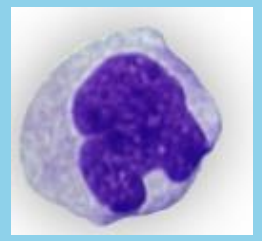
Leukocyte Adhesion Deficiency

- LAD-1 (predominant type)
  - Deficiency of leukocyte **integrins** (very low to absent surface CD11b/CD18 on PMNs)
  - Autosomal recessive
  - 1-2 per 1,000,000
- LAD-2
  - Deficiency of **selectin** function (normal CD11b/CD18, decreased CD15 on PMNs)
- Clinical presentation (LAD-1)
  - Recurrent infections
  - Delayed separation of umbilical stump
  - Leukocytosis
  - NO PUS!!

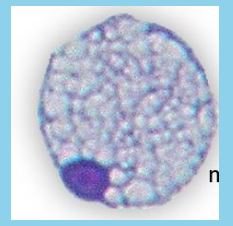


Other disorders of neutrophil dysfunction and production

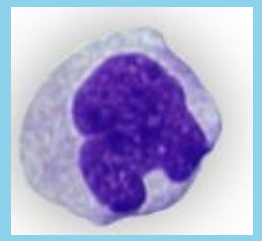
- Myeloperoxidase deficiency
  - Most common neutrophil deficiency (1 in 2-4K people), can be clinically silent
  - Recurrent candidal infections
- Chédiak-Higashi syndrome
  - Abnormal degranulation, recurrent infections, albinism, giant intracellular granules
- Hyperimmunoglobulin E
  - STAT3 mutation alters PMN chemotaxis
- G6PD
  - Decreased NADPH activity → decreased oxygen dependent killing
- Galactosemia
  - Inhibitory effects of galactose on PMNs
- Neutropenia
  - Glycogen Storage Disease 1B, Shwachman-Diamond syndrome, Kostmann syndrome, Reticular dysgenesis
- Trisomy 21
  - Depressed chemotactic activity



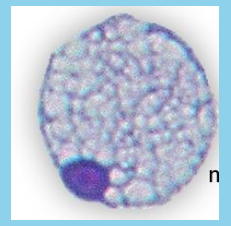
Monocytes & Macrophages



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



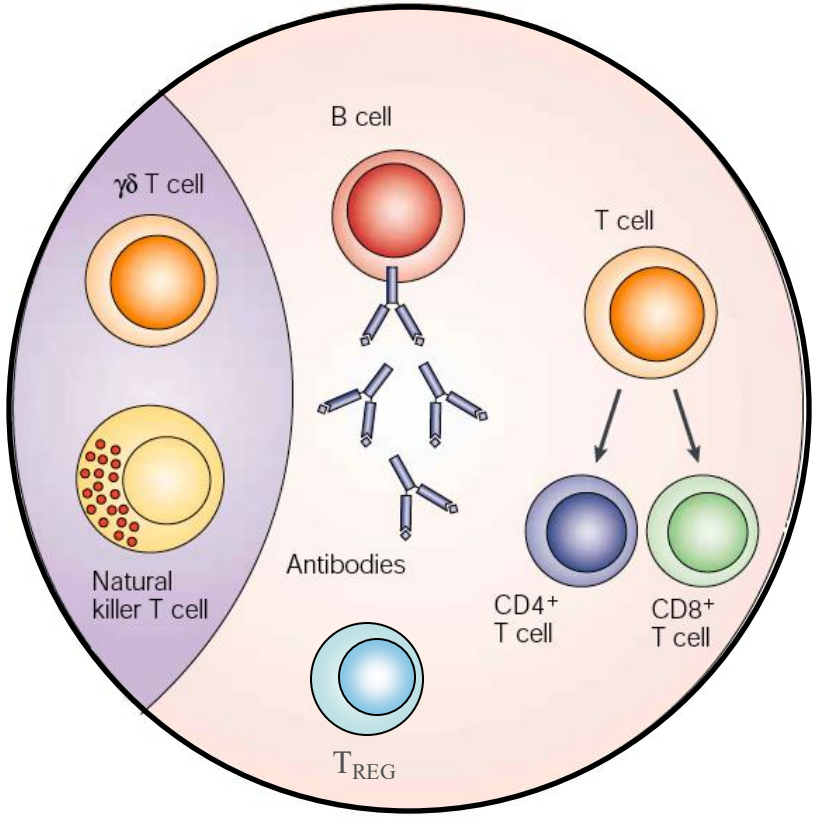
Monocytes & Macrophages



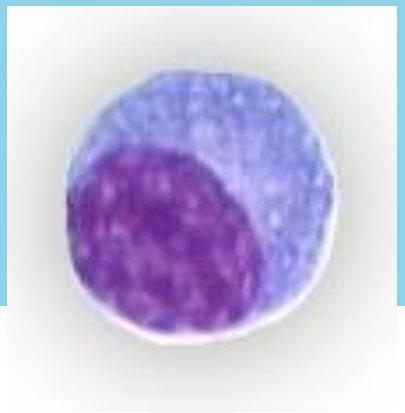
- Monocytes
  - Poor cytokine production and antigen presentation well into infancy (~12 months)
  - Low/absent number of monocytes in blood is common for first 5 days
  - Reduced co-stimulatory cell-surface molecules
  - decreased migration
- Macrophages
  - Poorly responsive to IFN-γ
    - decreased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

Adaptive Immune System

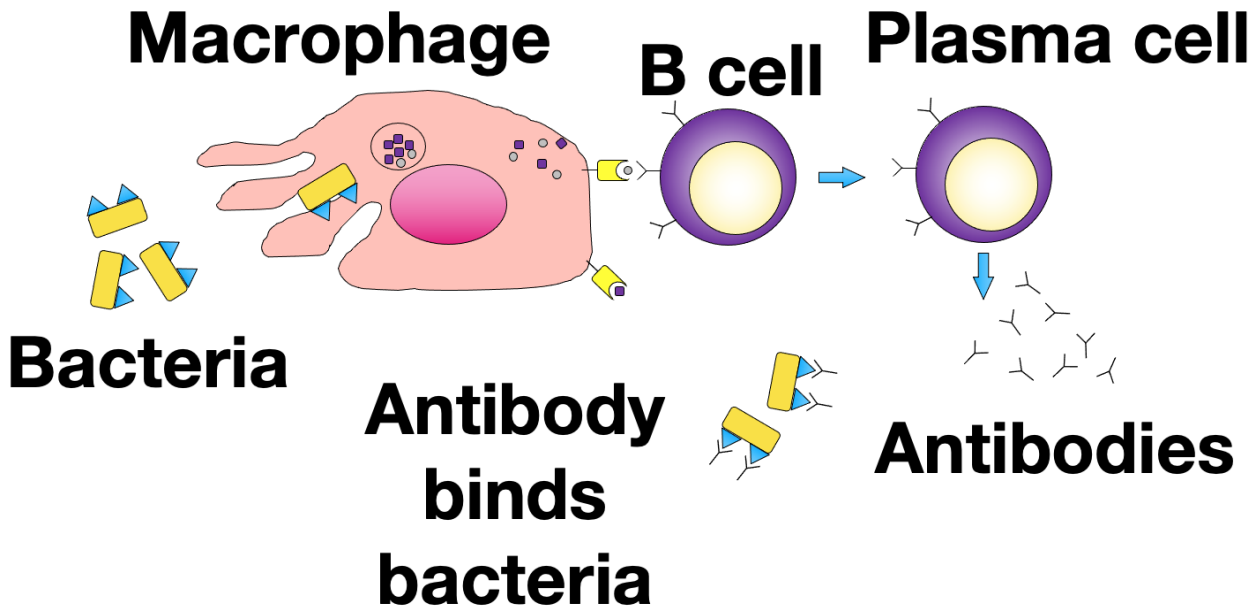
- Antigen-specific response
- **Takes 4-7 days to develop**
- Cell-mediated responses
  - –T cells
    - •Helper T cells ($CD4^+$)
    - •Cytotoxic T cells ($CD8^+$)
- Humoral responses
  - –B cells
    - Plasma cells
      - –Secrete immunoglobulin
- Immunoregulatory functions
  - $-T_{REG}$



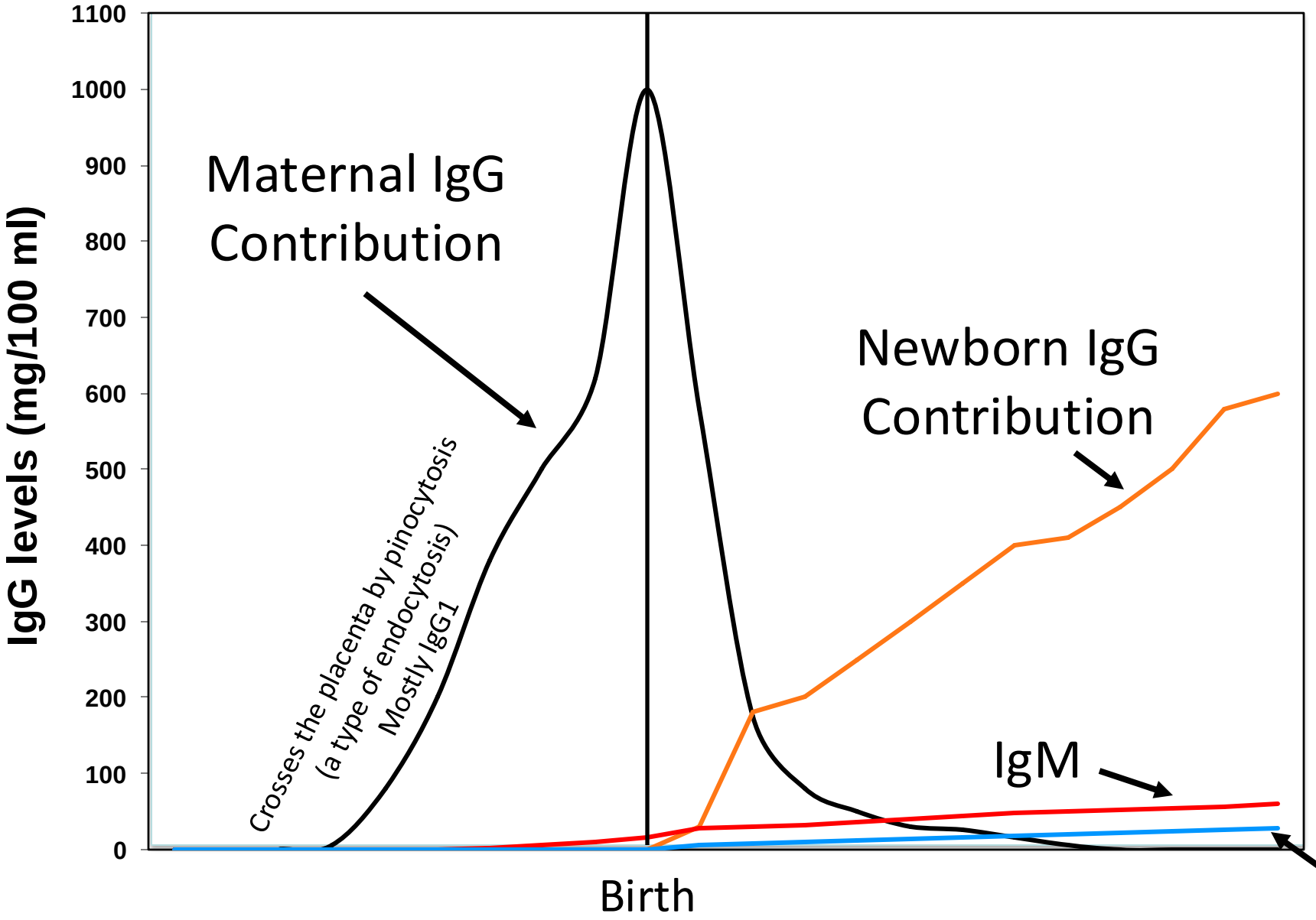
B cells



- 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



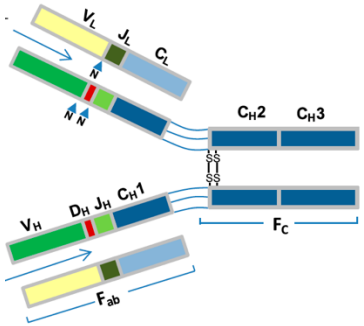
Immunoglobulin Levels With Age

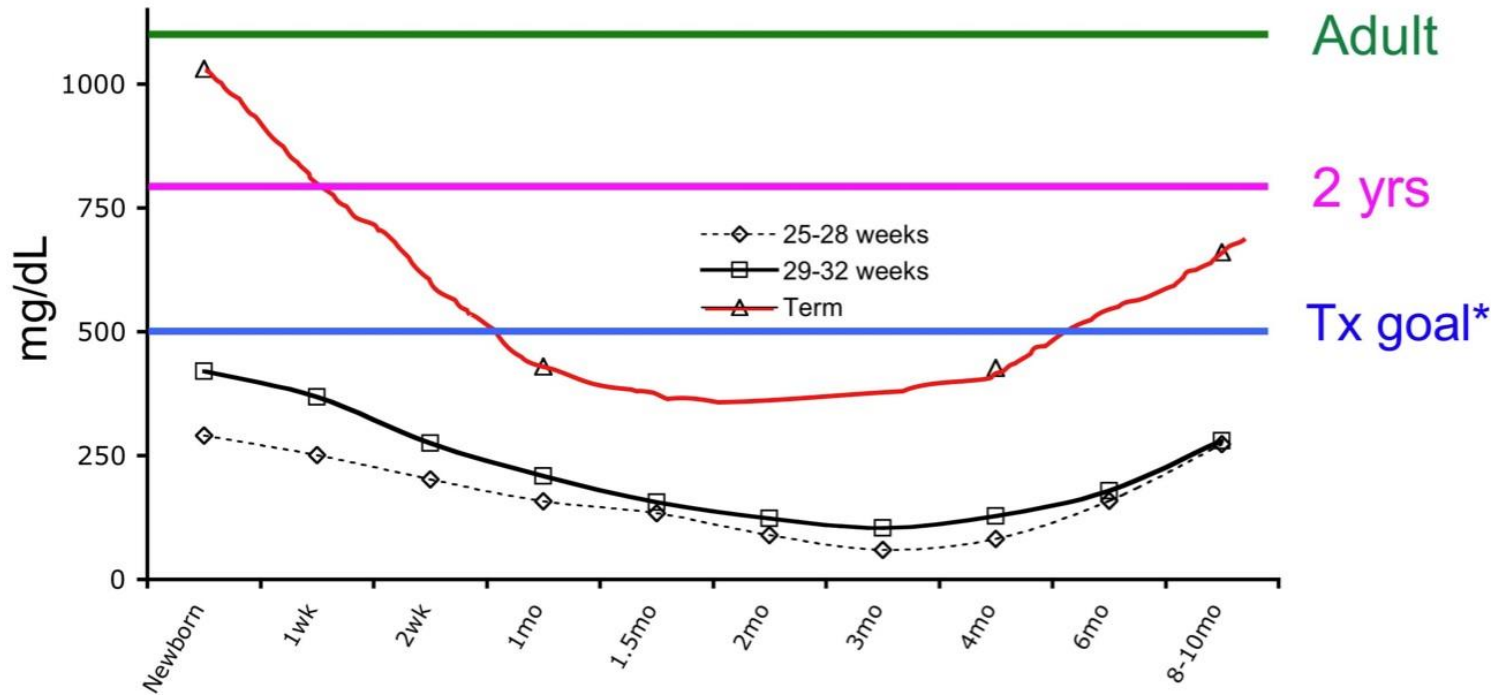


- Antibody made in germinal centers: these are absent in the fetus and do not develop until there is antigenic stimulation
- Fully differentiated plasma cells can secrete and produce IgG, IgM, and IgA by 20-weeks gestation
- First isotype produced after antigen stimulation is IgM

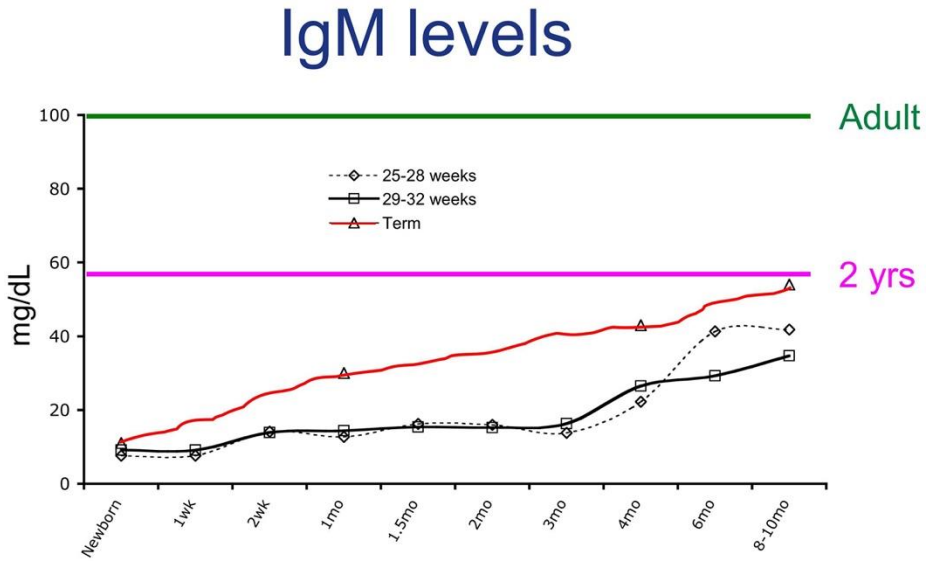
IgG Levels in Neonates

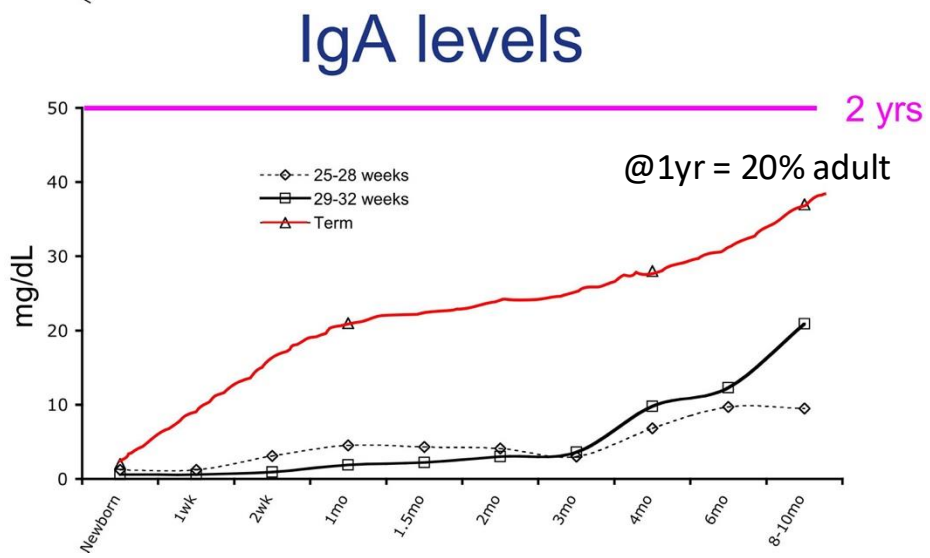
- Term infants may have IgG levels in excess of maternal IgG levels
- Growth restricted and prematurely born infants have lower levels
- ½ life of maternal antibody is 30 days
  - Maternal IgG still detected until 18 months life
- Nadir at 2-4 months of life



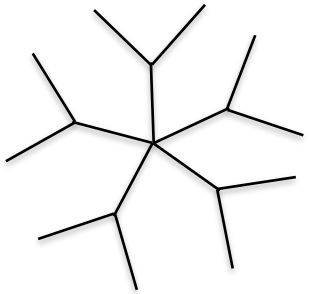


Other Igs





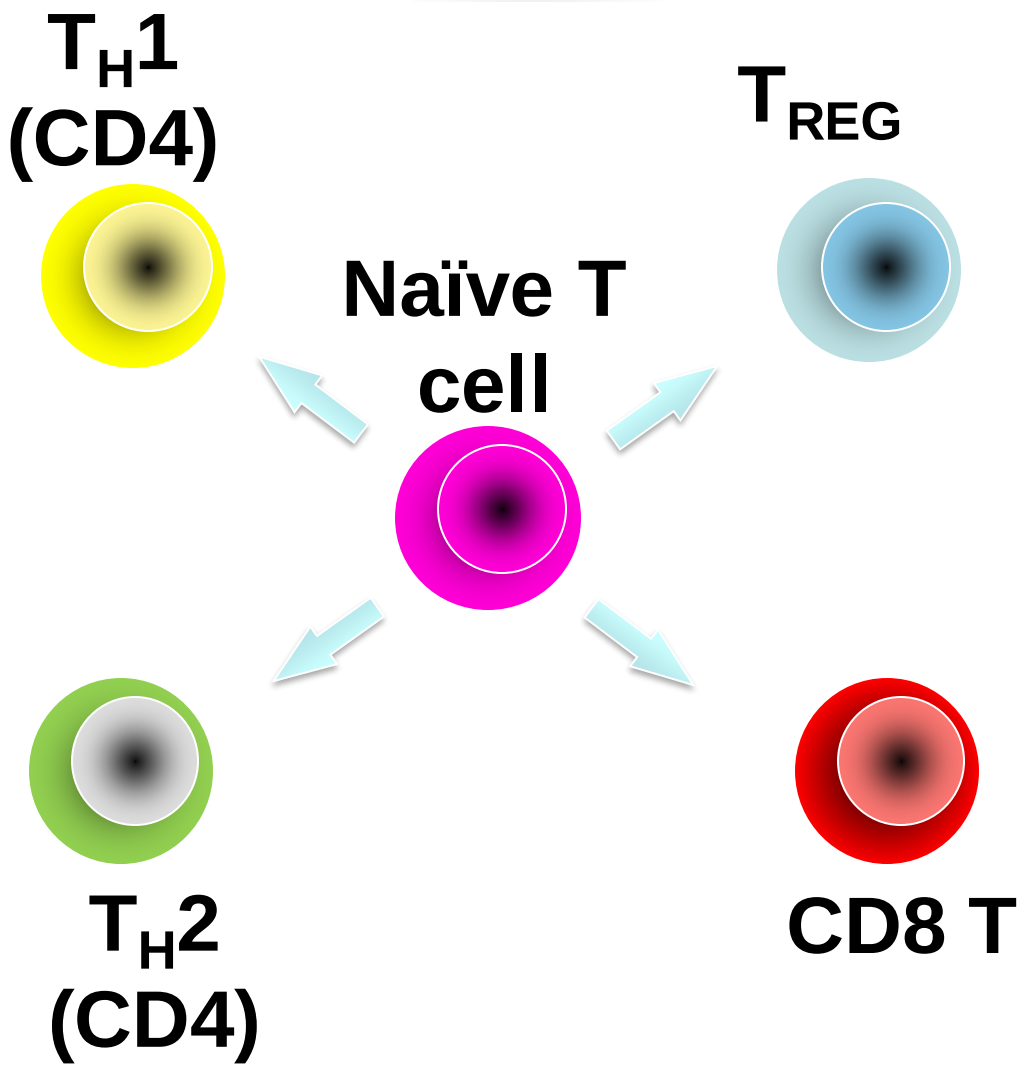
- IgM (pentamer) and IgA (dimer) levels are low at birth as they do not cross the placenta
- Infections with CMV, rubella, and Toxo can raise levels



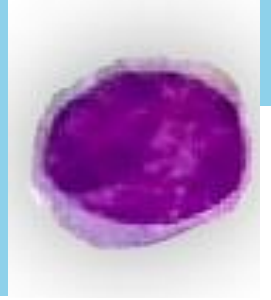
- Only source of IgA (secretory) is from colostrum
- IgE sensitizes mast cells
- IgD can dampen food allergen reactions by decreasing IgE-mediated mast cell degranulation

CD4 T Cells

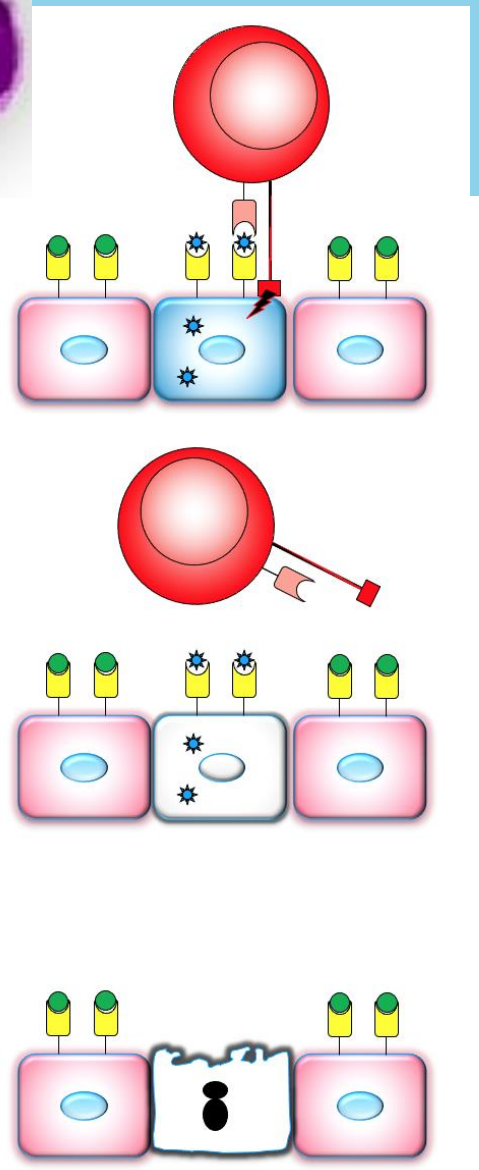
- "Helper" cell → Coordinate the immune system
- Stimulated by cytokines and antigen presenting cells
- Secrete cytokines
- Stimulate B cells to make immunoglobulin
- Activate macrophages
- Can function similarly to adults if appropriately stimulated



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

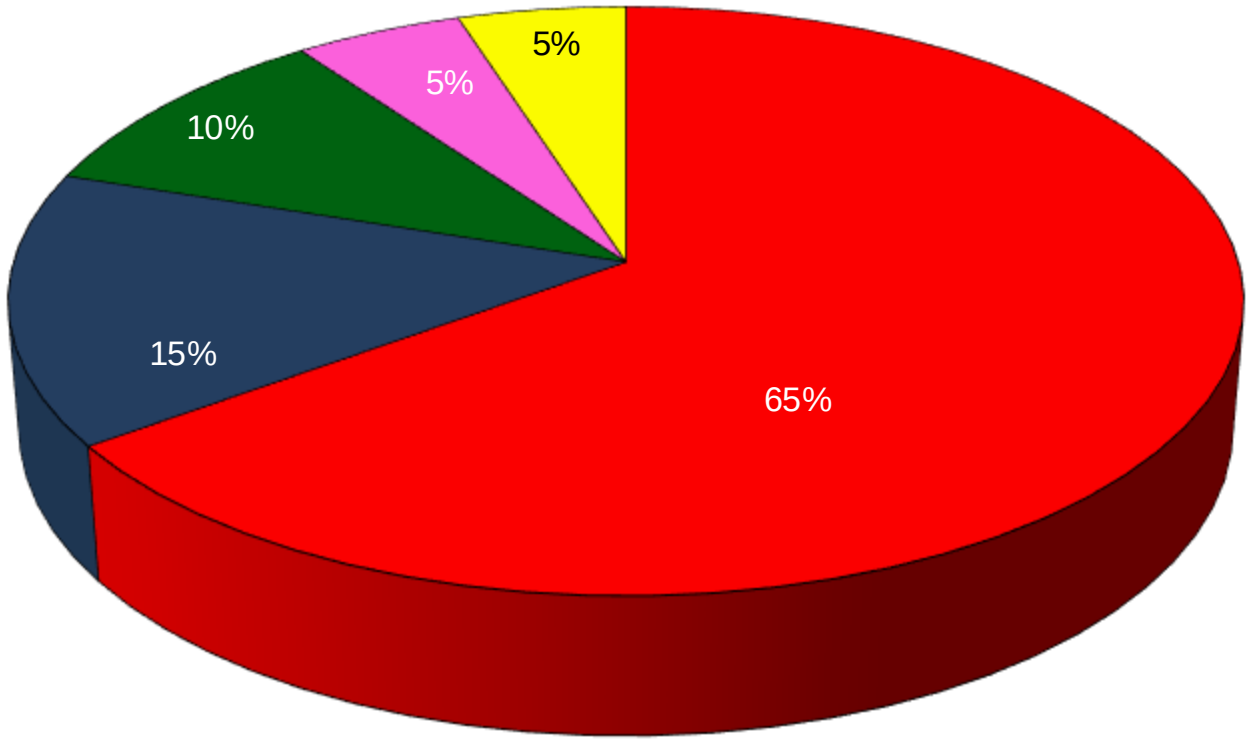


NEONATAL PRIMARY IMMUNODEFICIENCY

Over 400 genetic defects identified in human inborn errors of immunity

Often considered diagnoses of exclusion because of rarity

Primary immunodeficiency: estimated 1 in 1200 births



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
    - Exception is maternal common variable immunodeficiency
- 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

X-linked Agammaglobulinemia

- 3-6 per million males
- Profoundly diminished Ig levels of all isotypes
  - IgM levels are undetectable
- B cell-specific src-associated (Bruton's) 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
- Treatment: q3-4 week IVIg and appropriate antimicrobial treatment
  - Keep IgG levels > 500 mg/dl

Neonatal Primary Immunodeficiency

- Other PIDs may present in the NICU but without immunologic or PID-related signs
  - 22q11 →hypocalcemia, interrupted aortic arch
  - Wiscott-Aldrich: x-linked triad of thrombocytopenia, eczema, increased viral infections →bleeding after circumcision due to platelet dysfunction
- Other PIDs that may be present but not often dx in NICU
  - XLA
  - IgA deficiency
  - CVID
  - Ataxia telangiectasia
  - Complement defects
  - Autoinflammatory syndromes
  - Chédiak-Higashi
  - Cartilage-hair-hypoplasia (skeletal dysplasia; SCID or CID)

Defects in Cell-Mediated Immunity

- Chromosome 22 q11.2 deletion (DiGeorge) syndrome
- Wiskott-Aldrich Syndrome
- Perinatal HIV-1 infection
- SCID
- Ataxia telangiectasia (DNA repair disorder- severe neurodegenerative disorder with variable immunodeficiency)- may have low TRECs
- IPEX syndrome (T regulatory cell disorder, Immunodysreguation, Polyendocrinopathy, Enteropathy)

Severe Combined Immunodeficiency (SCID)

- 1 per 54,0000-58,000
- 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
  - Uncommon now with irradiated blood products
  - 20-30% risk of maternal cell-mediated GVHD (placental transfer of maternal T cells)

SCID

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

Ten abnormal genes in human SCID

Cytokine-receptor genes

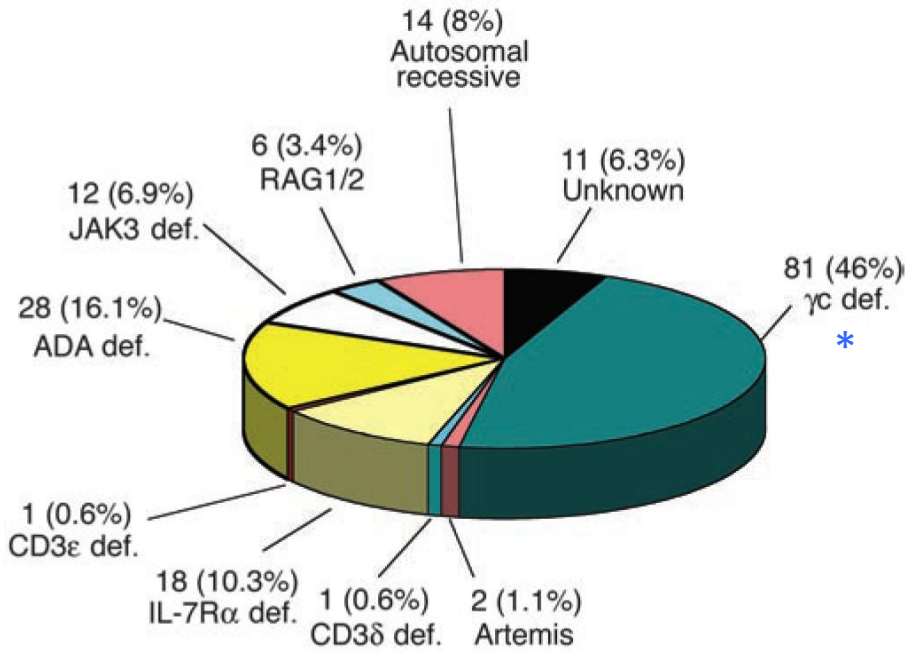
IL-2RG *
JAK3
IL-7Rα

Antigen-receptor genes

RAG1
RAG2
Artemis
CD3δ
CD3ε

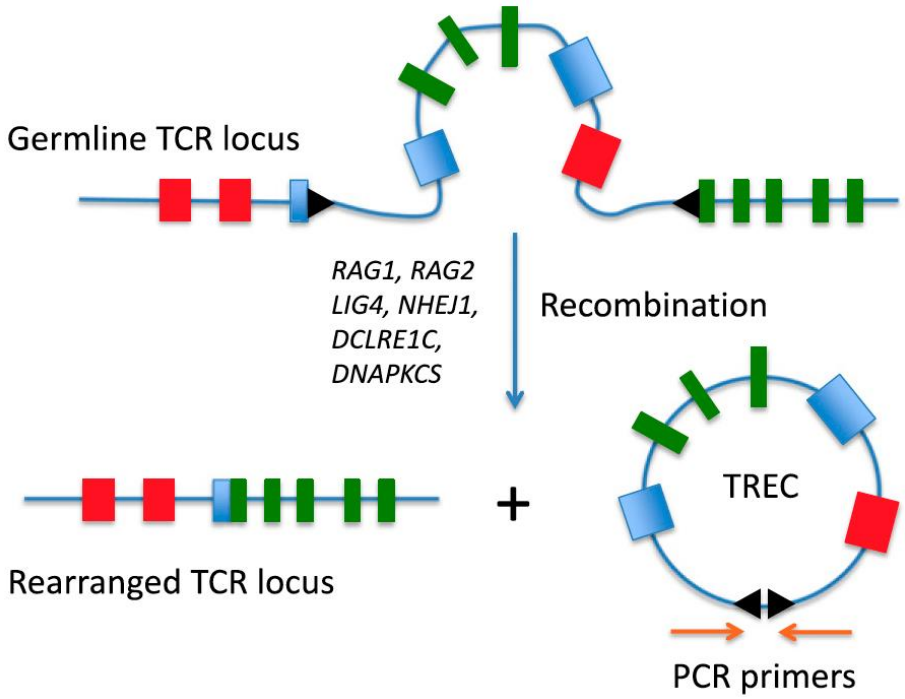
Other genes

ADA
CD45



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



NEONATAL PRIMARY IMMUNODEFICIENCY

Additional maternal for consideration

PID Dx tools

- Screening – TRECs
- Diagnostic testing
  - Clinician suspicion
  - CBC with diff
  - Flow cytometry
    - Cell surface markers and enumeration
    - ROS
  - Ig subsets
  - Functional assays
  - Genetic testing

Chromosome 22q11.2 Deletion Syndrome

- 1 per 4000
- Absent thymic tissue leads to $CD3^+$ (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

Wiskott-Aldrich Syndrome

- 1-10 per million boys; more rare in girls
- 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

Clinical features of immunodeficiency

Delayed umbilical cord separation

Infection – particularly repeated

Failure to thrive

Chronic diarrhea

Symptomatic infection due to live vaccines (eg, rotavirus, Bacille Calmette-Guérin [BCG], oral polio)

Heart or lung disease

Congenital asplenia

Mucosal abnormalities such as thrush, mouth sores, and ulcerations

Petechiae, melena, bleeding

Cutaneous rashes, pigmentary abnormalities

Syndromic appearance (abnormal facies or habitus) including facial or craniofacial anomalies

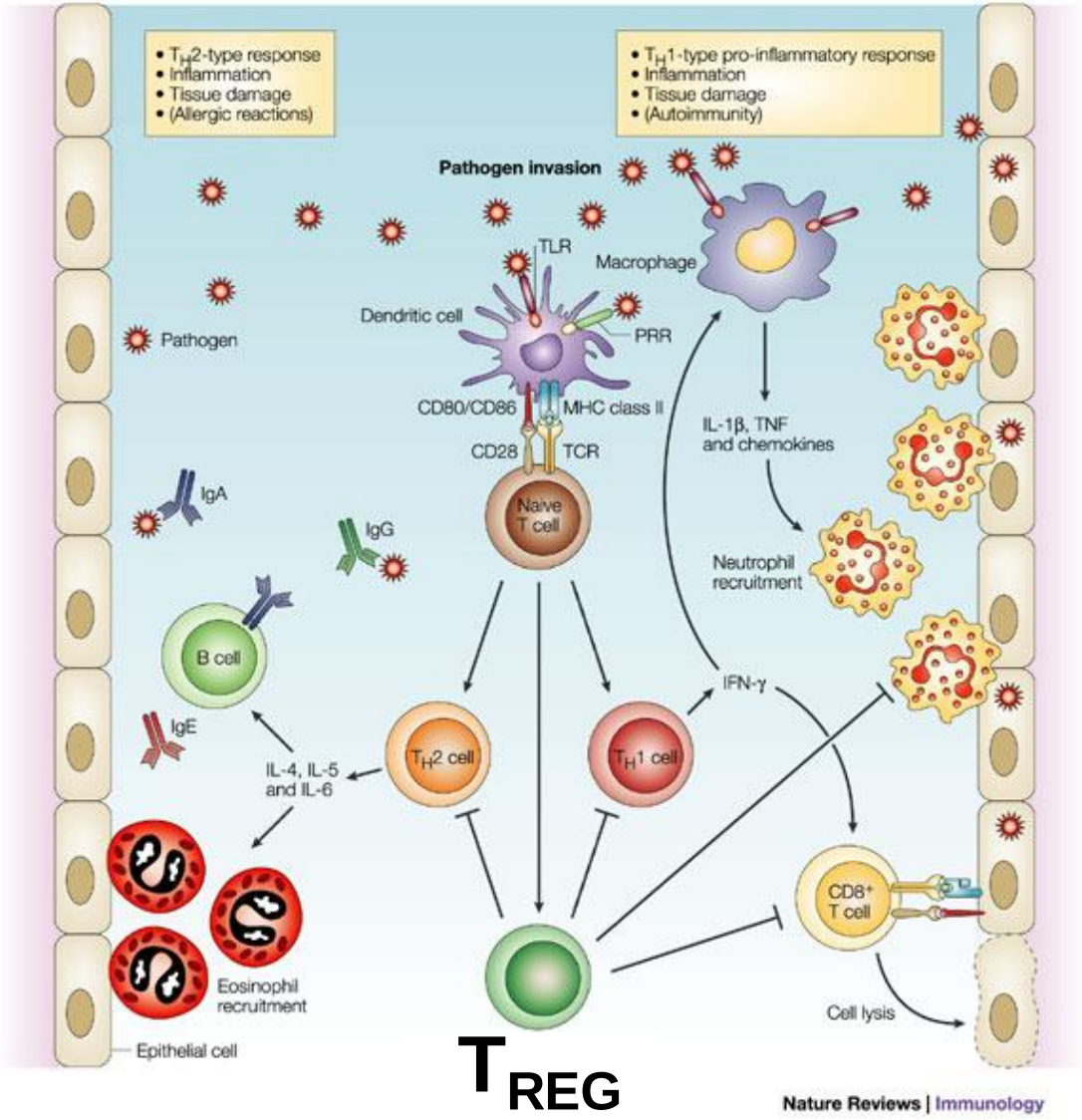
Lymphadenopathy and/or hepatosplenomegaly

Autoimmunity

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/IgE
- Decreased CD4 cells, normal number CD8

T regulatory cells

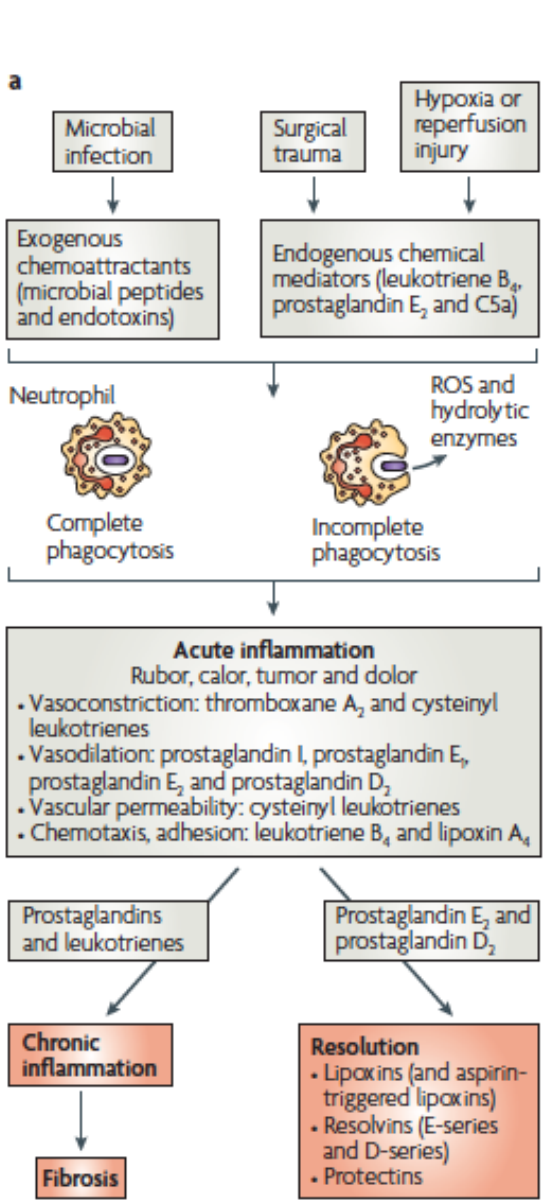


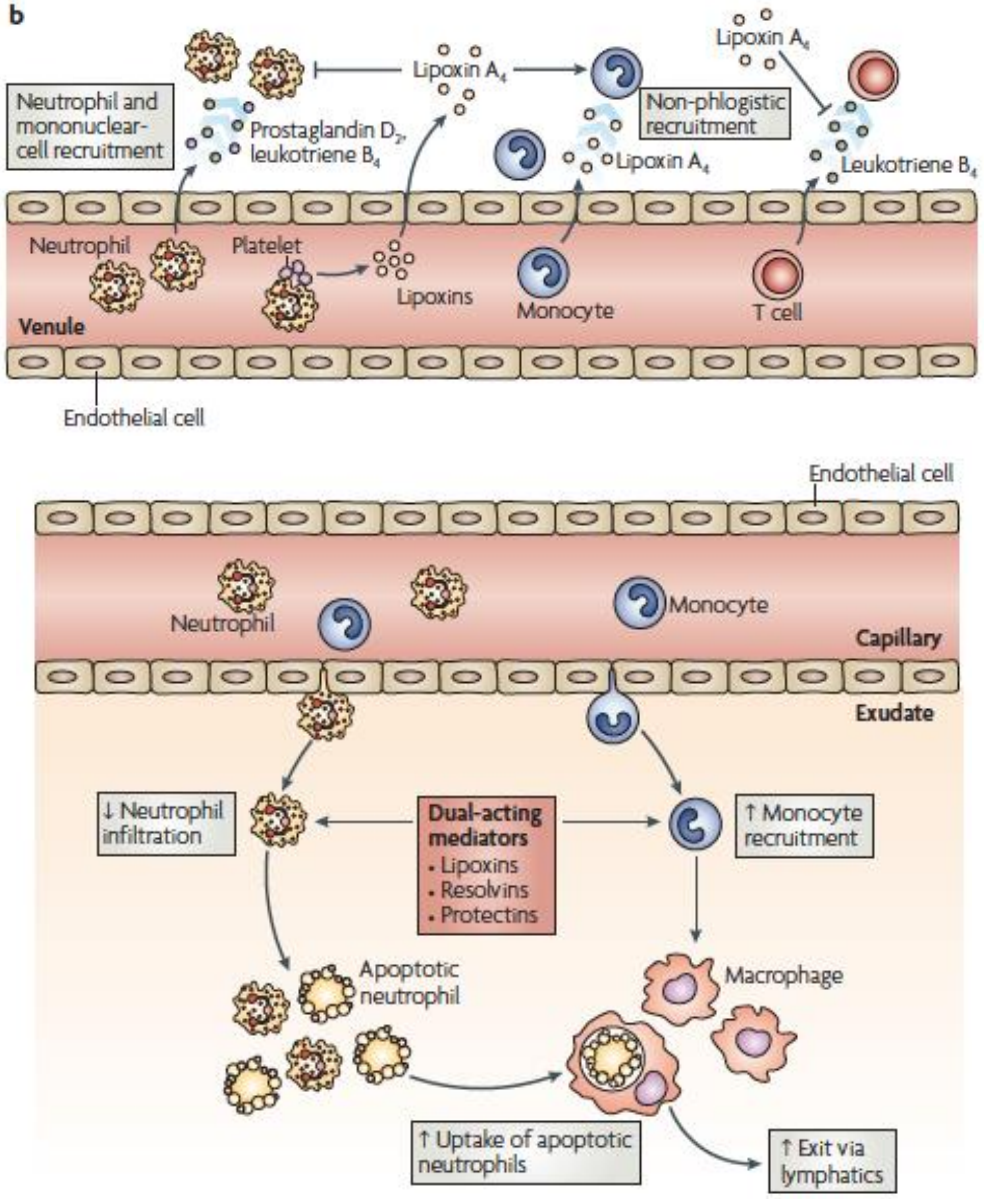
- **High proportion of T_{REG} particularly in premature**
- **Numbers decrease to adult levels by 3-6 years of age**
- **Necessary for prevention of in utero rejection**
- **May down modulate APC function**

IPEX syndrome

- Immunodysreguation, Polyendocrinopathy, Enteropathy
  - VERY rare→But presents in neonatal period
- Mutation in forkhead box protein 3 located on the X chromosome
- Impaired T_{REG} suppressor function
- Decreased IL-2 and IFN-γ production
- Severe watery diarrhea, FTT, dermatitis, type I DM
- Increased IgE levels and eosinophilia

PGE and leukotrienes

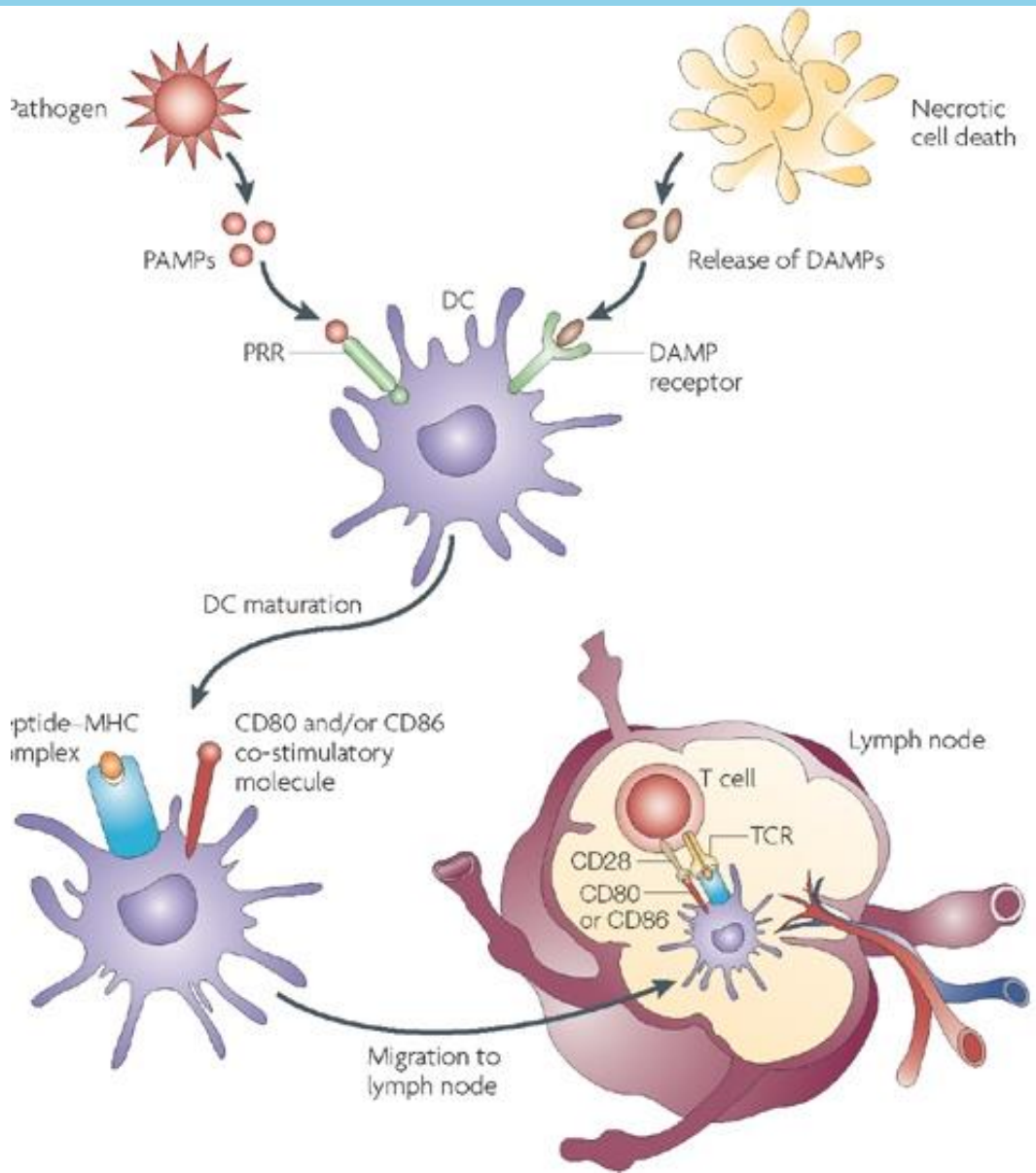




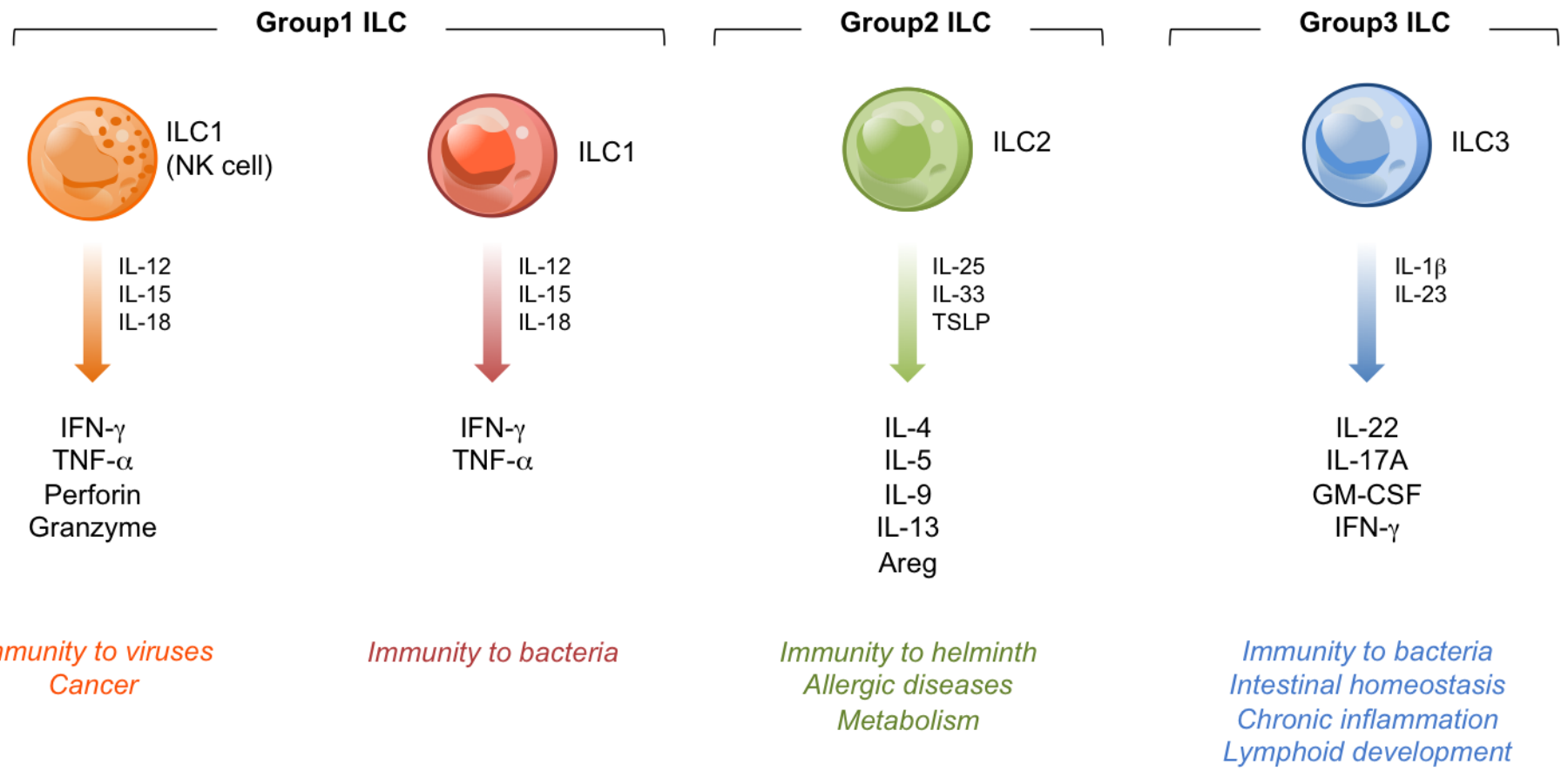
Dendritic cells

- Professional antigen presenting cells that activate T cells

- Neonatal DCs require greater stimulus for activation

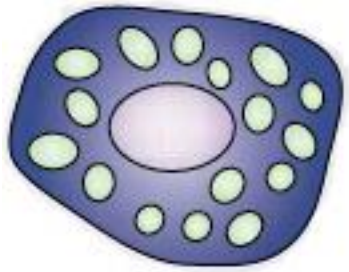


Innate lymphoid cells



Eosinophils and Mast cells

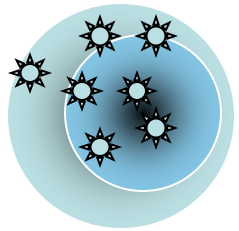
- Produce cytokines, phagocytose Ag-Ab complexes, produce HDP/cytotoxic molecules, vasodilatory substances
- Expansion associated with sepsis in the very premature
- Associated with erythema toxicum
- Extracellular trap production?
- Role in immune response is incompletely defined

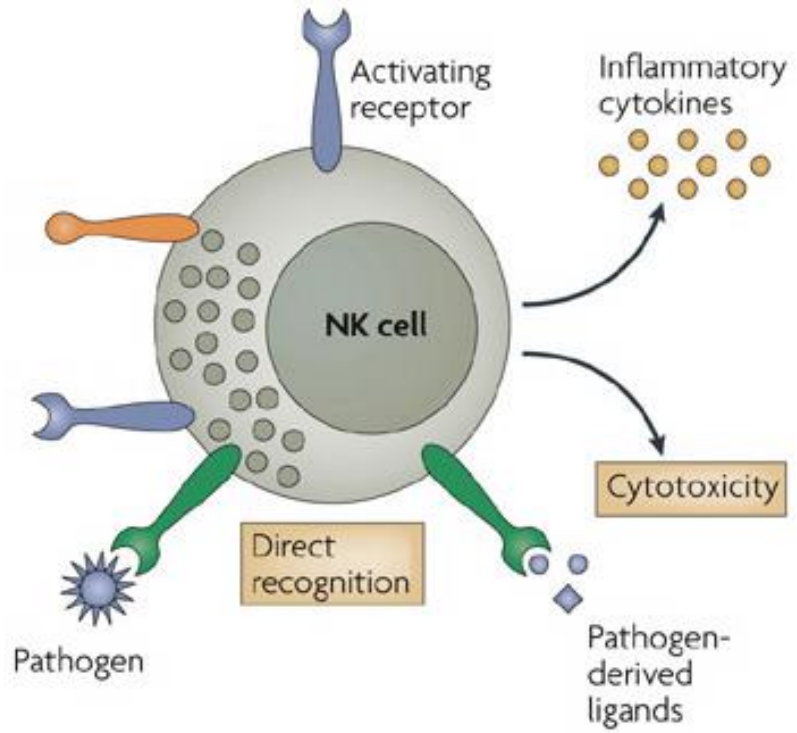


- Produce histamines, cytokines, phagocytose pathogens, and participate in Ag presentation
- Associated with erythema toxicum
- Extracellular trap production?
- Role in immune response is incompletely defined

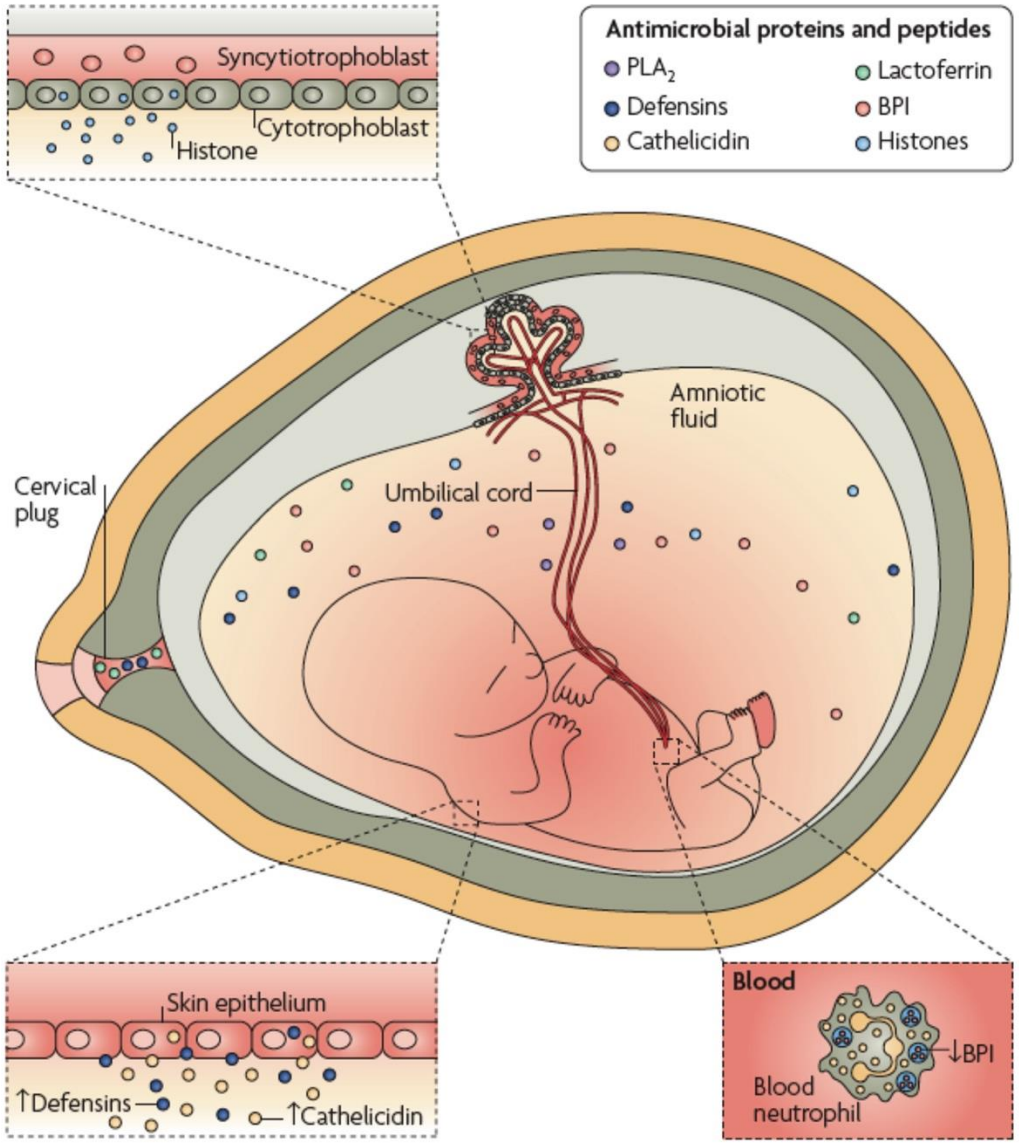
Natural Killer Cells

- Play a role in the response to viral pathogens
- Direct cellular killing (of infected cells) and cytokine production
- 2x adult numbers at birth
- Impaired antiviral cytokine responses (perforin, IFN-γ, TNF-α)
  - **Li J et al. Cell Mol Immunol. 2013**
- Cytotoxic function is reduced at birth
  - **Georgeson GD, et al. Eur J Pediatr. 2001 Aug;160(8):478-82.**
- Cytolytic activity normalizes between 6-12 months
  - NK "competency" after 1 month
    - **Remington and Klein, Infectious Diseases of Fetus and Newborn. 6th ed.**

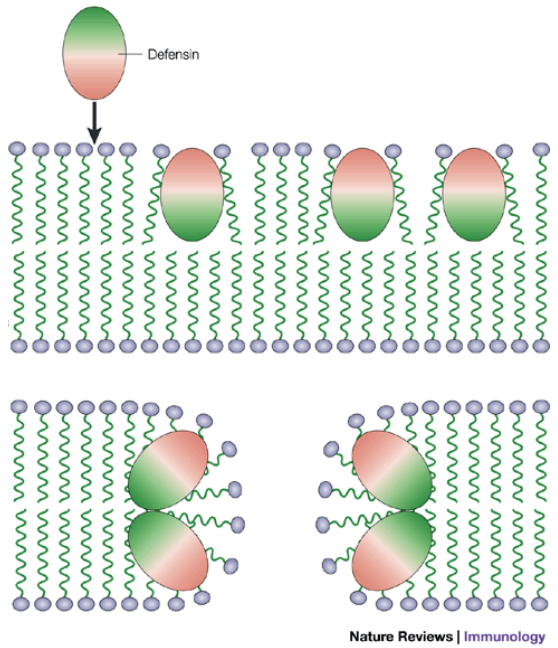




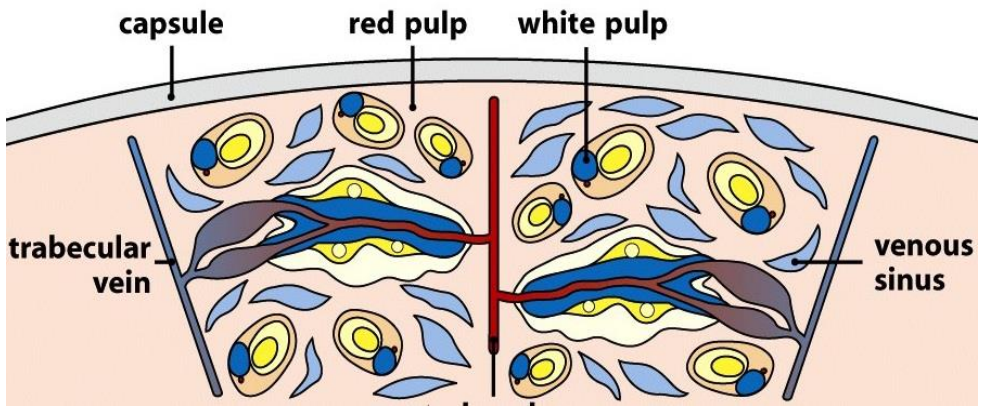
Natural Killer Cells

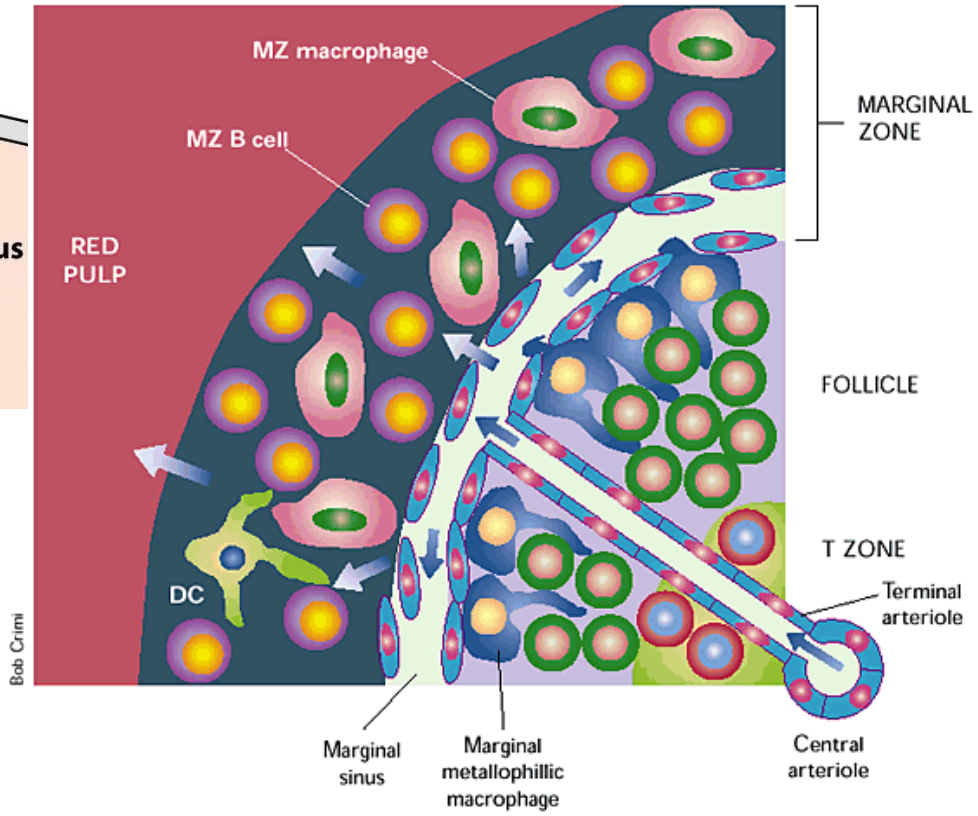


- Phylogenetically most ancient
  - "Beginning" of the immune system
  - Bacteria, plants, insects, non-mammalian vertebrates
- Antimicrobial activity against bacteria, viruses, fungi, parasites



Splenic function





- Assist in synthesis of antibodies against carbohydrate antigens
- Clear old red cells and microorganisms from blood
  - Not equivalent to adults until follicular development occurs