Immunology of the Preterm Neonate: *How Robust?*

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Learning Objectives

• Outline the major components of the neonatal immune system

• Review the developmental deficiencies of the innate and adaptive immune system in the preterm neonate

• Understand the unique adaptive mechanisms that allow the neonate to mount an immune response despite all the known developmental constraints
Hematopoietic stem cells

Myeloid progenitors
- Neutrophil
- Monocyte
- Eosinophil
- Basophil

Lymphoid progenitors
Hematopoietic stem cells

Myeloid progenitors
- Neutrophil
- Monocyte
- Eosinophil
- Basophil

Lymphoid progenitors
- Plasmacytoid DCs
Hematopoietic stem cells

Myeloid progenitors

T-cell

T-helper cells

T$_H$1

T$_H$2

T$_H$17

T-reg cells

Cytotoxic T-cells

γδ T-cells

NKT-cells

Lymphoid progenitors

Peripheral

Thymus-derived

Invariant NKT cells

Diverse NKT cells
Hematopoietic stem cells

- Myeloid progenitors
  - T-cell
- Lymphoid progenitors
  - B-cell

Innate lymphoid cells

NK cells

Plasma cells

ILCs

ILC1    ILC2   ILC3
Preterm and term neonates have a limited NSP, which may be exhausted during sepsis.

Adult rats have an NSP of $6 \times 10^9$ cells/kg. There are $0.9 \times 10^9$ cells/kg at 19 days’ gestation and $1.2 \times 10^9$ cells/kg at term (=21 days).
How to Define Neonatal Neutropenia?

ANC <2 standard deviations below mean value for age or alternatively, <5th percentile for an age-defined population

Seen in 6-8% of all NICU patients; frequent in premature infants

(Manroe, 1977; Mouzinho, 1994; Schmutz, 2008)
DECREASED NEUTROPHIL PRODUCTION
Infants of hypertensive mothers
Donors of twin-twin transfusions
Neonates with Rh hemolytic disease
Neutrophils

- Neutrophil function includes emigration from bloodstream, phagocytosis, and microbial killing

- Neutrophils from both term and preterm neonates adhere poorly to endothelium. Neonatal neutrophils have less selectin and $\beta_2$-integrin (Mac-1/CD11b) expression. Trans-endothelial migration, which is dependent on cell deformability, is comparable to adults.

- Neonatal neutrophils show impaired chemotaxis. Term neutrophils show normal chemotaxis at 2 weeks. Late preterms achieve normal function at term PCA. VLBW neutrophils begin to mature at 2-3 weeks after birth and progress very slowly.

- Neutrophils from preterm neonates phagocytose slowly and ingest fewer bacteria; corrects by term PCA.

- Preterm neutrophils display poor respiratory burst and impaired killing of Staphylococcus aureus or E. coli. Improves by 2 months postnatal. Term neonates are normal in this regard.
$y = 0.0128x^4 - 0.6907x^3 + 9.9681x^2 - 9.0189x + 1076.2$
Does Monocyte Efflux to the Inflamed Intestine during NEC Cause Monocytopenia?
Feeding intolerance with no progression to NEC

Bell stage II

Bell stage III
### Diagnostic value of decreased peripheral blood AMCs as a test for NEC

<table>
<thead>
<tr>
<th></th>
<th>No NEC</th>
<th>NEC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;20% drop in AMC</td>
<td>54</td>
<td>41</td>
<td>95</td>
</tr>
<tr>
<td>Increased or ≤ 20% drop in AMC</td>
<td>137</td>
<td>19</td>
<td>156</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td>60</td>
<td>251</td>
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</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Value (95% CI)</th>
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<tbody>
<tr>
<td>Prevalence</td>
<td>0.24 (0.19–0.30)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.70 (0.57–0.81)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.71 (0.64–0.77)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.43 (0.33–0.54)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.88 (0.81–0.92)</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>2.42 (1.82–3.21)</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>0.44 (0.30–0.64)</td>
</tr>
</tbody>
</table>

Abbreviations: AMC, absolute monocyte count; CI, confidence interval; NEC, necrotizing enterocolitis.
Monocytes

Preterm monocytes show slightly impaired chemotaxis. However, activated monocytes show normal trafficking and adhesion molecule expression.

Preterm monocytes can kill pathogens (*Staphylococcus aureus, S. epidermidis, Escherichia coli,* and *Candida albicans*) comparable to adult monocytes.

During sepsis, neonatal monocytes express TLRs and cytokines at levels similar to adults. Monocytes from VLBW neonates may be slightly impaired than term infants.

Skewed pattern of cytokine expression with low levels of Th1-polarizing cytokines such as IL12p70 and interferon-α, and more of the anti-inflammatory cytokine IL-10.
Hematopoietic stem cells

Myeloid progenitors
- Neutrophil
- Monocyte
- Eosinophil
- Basophil

CD14\textsuperscript{hi} inflammatory Monocyte
- Macrophage
- Myeloid DCs

CD14\textsuperscript{low} CD16\textsuperscript{+} Monocyte
- Plasmacytoid DCs

Lymphoid progenitors
Cord blood contains fewer DCs (0.5%) than adult blood (1%).

Neonatal myeloid DCs are immature (lower CD83, CD86 expression), produce less IL-12 and IFN-γ but more IL-10 than adult DCs.

Neonatal DCs also perform poorly as accessory cells for T-cell mitogenic responses.
T-lymphocytes

Most T-cells carry a TCR composed of $\alpha$ and $\beta$ chains, but in about 5% of all T-cells, the TCR is comprised of $\gamma$ and $\delta$ chains.

Each TCR protein chain contains an immunoglobulin-like extracellular region with a variable (V) domain at its N-terminal and a constant (C) domain at the C-terminal end. TCR diversity results from recombination of variable (V), diversity (D) and joining (J) gene segments in individual T-cells.

Term infants have more T-cells in peripheral blood than adults. Neonates have CD4/CD8 ratios of 5:1, which decline to adult values (2:1) by 4 years of age.

Preterm infants have lower CD4$^+$ and CD8$^+$ counts than term.

80% T-cells in cord blood have a naïve CD45RA phenotype, compared to <50% of circulating T-cells in adults. Memory CD45RO T-cells reach adult levels in adolescence.
Hematopoietic stem cells

Myeloid progenitors

Lymphoid progenitors

T-cell

- T-helper cells
  - $T_H^{1}$
  - $T_H^{2}$
  - $T_H^{17}$

- Thymus-derived

- Peripheral

- Invariant NKT cells

- Diverse NKT cells
T-helper lymphocytes

Naïve CD4+ cells differentiate into three effector T-helper (Th) subsets: Th1, Th2, and Th17

Th1 cells defend against intracellular pathogens and virus-infected cells; produce IL-2, IFN-γ, TNF, IL-13, and GM-CSF

Neonates have low Th1 function: produce less IL-12 and IFN-γ, and express less CD154 (CD40 ligand) than adults.

Th2 cells participate in allergic reactions; produce IL-4, IL-5, IL-9, IL-10, IL-12 and IL-13

Th17 cells protect against infections, activate neutrophils and macrophages; produce IL-17A, IL17-F, IL-21, IL-22 and IL-26. Cord blood T-cells show limited capacity to produce IL-17
T-regulatory lymphocytes

Derived from naïve CD4+ cells; suppress immune responses by expressing IL-10, TGF-β, cytotoxic molecules, and modulators of cAMP

Two populations: thymus-derived (tTregs) and peripherally-derived Tregs (pTregs)

In the fetus/preterm infant, T-regs are tolerogenic and promote self-tolerance

During midgestation, CD4+CD25+ Fox P3+ Tregs constitute 20% of all CD4+ cells in lymphoid tissues. Represent <5% of CD4+ cells in cord blood from term infants and in the adult blood

Preterm and term T-regs are less functional than adults: limit contact between DCs and effector T-cells, causing decreased DC immunogenicity and impaired T-cell activation
CD8\(^+\) T cells can differentiate into cytotoxic T-lymphocytes. Protect against intracellular pathogens – cause cytotoxicity by releasing pore-forming mediators (perforin/granzyme) or by activating fas-mediated apoptosis.

Neonatal CTLs are less efficient than in adults. Circulating \(\alpha\)FP and prostaglandins may inhibit CTL activity in neonates.

Perforin facilitates Granzyme B entry into cells where it promotes cell death.
γδ T-cells respond to non-peptide microbial metabolites, show cytotoxicity, and produce interferon-γ and TNF. γδ IELs protect epithelial tissues from injury.

Constitute 10% of circulating T-cells during midgestation but the number declines to about 3% at term, in skin and mucosa.

In the intestine, 30% of IELs, 10% of mucosal lymphoid tissue lymphocytes, and 5% of LPLs have the γδ TCR.
NKT cells express TCR-αβ chains and NK cell markers. Classified into two main subsets, that is, type I or invariant NKT cells and type II or diverse NKT cells.

Invariant NKT cells have limited TCR diversity and recognize lipid antigens such as α-galactosylceramides in the context of the MHC-like molecule CD1d. This works similar to PRRs, but for microbial lipids. I-NKT cells secrete anti- and pro-inflammatory cytokines and activate adaptive immune responses.

Diverse NKT cells express a variety of TCRs and may recognize lipid antigens with cross-reactivity between mammalian and microbial phospholipids, possibly representing the adaptive immune arm for lipid antigens.
Hematopoietic stem cells

- Myeloid progenitors
- Lymphoid progenitors

  - T-cell
  - B-cell
B-lymphocytes

Preterm and term neonates have more circulating B-cells than adults. B-cell counts peak at 3 months and then decline to adult levels by 6 years of age.

>90% B-cells in the fetus/neonate express CD5 and are called B-1 cells. Proportion drops to 75-80% during infancy and to the 25% adult levels by late adolescence. Express activation markers (CD25) and a CD11b⁺ sIgM^{high} sIgD^{low} phenotype.

Localize in spleen, peritoneum. Broad polyspecific specificities, and the restricted immunoglobulin repertoire suggests a role in the innate, rather than in adaptive immunity.

Respond to T-cell-independent, carbohydrate antigens (unlike follicular B-2 cells that respond to protein antigens).
B-lymphocytes

Preterm infants produce antibodies, but may not respond to all antigens in a vaccine, may remain limited to IgM with delayed isotype switch, and may produce antibodies of low affinity.

Postnatal age is a better predictor of antibody response than gestation. Both preterm and term infants respond weakly to diphtheria toxoid in the 1st week and better at 1-2 months of age. Premature infants respond poorly to the hepatitis B vaccine in early infancy but are comparable to term infants in later infancy.
B-lymphocytes

Most immunoglobulins in cord blood are derived from maternal IgG (particularly IgG1 and IgG3)

Preterm infants have lower IgG levels. Term infants have serum IgG levels (1000 mg%) similar or higher than maternal serum

Immunoglobulin levels drop to 300-500 mg% at 3-5 months, when the infant starts producing more. This nadir is reached earlier and is lower in preterm infants
Hematopoietic stem cells

- Myeloid progenitors
  - T-cell

- Lymphoid progenitors
  - NK cells
  - Non-cytotoxic ILCs
    - ILC1
    - ILC2
    - ILC3
Natural Killer Cells

Neonatal NK cells are immature (low CD8 and CD57, ICAM-1, and CD161)

Show less cytolytic activity than in adults
Innate Lymphoid Cells

Non-cytotoxic ILCs are derived from the common lymphoid progenitor but do not exhibit antigen specificity

ILC1s express Th1-associated cytokines such as IFN-γ and TNF to protect against intracellular bacteria and parasites

ILC2s express Th2 cytokines (including IL-4, IL-5, IL-9 and IL-13). Detectable in cord blood, and are implicated in intestinal inflammation in gastroschisis

ILC3s express Th17 cytokines IL-17A, IL-17F, IL-22, GM-CSF, and TNF, to promote antibacterial immunity and inflammation. Abundant in gut mucosa and may be involved in enhancing IgA production and in shaping the local microbiome
Neutrophil defects, T-cell defects, severe combined immunodeficiency, and bone marrow failure syndromes can present in neonatal period.

T-cell defects may include DiGeorge syndrome, hyper-IgM syndrome, and ZAP-70 tyrosine kinase defects.

SCID include Purine nucleoside phosphorylase deficiency (viral infections, severe varicella, GVHD), cartilage hair hypoplasia, IL2Rα defects, X-SCID (Jak3) and AR SCID (ADA, RAG1/2, IL7Ra).

B-cell defects present only after maternally-derived antibody levels drop during infancy.

Transient hypogammaglobinemia of infancy may be considered after 6 months; low basal but normal stimulated Ig responses.
• The neonate and the young infant depend mainly on innate immunity for host defense. With limited antigenic exposure, the adaptive immune arm is still in development.

• Neutropenia is seen frequently during neonatal period. Neonatal neutrophils show functional deficiencies in movement, phagocytosis, and microbial killing.

• With the exception of a few deficiencies, neonatal monocytes and macrophages are functionally comparable to their counterparts from adult subjects.

• Neonatal T- and B-cells are still developing. Several adaptive mechanisms, such as the B-1 cells that can function without assistance from T-cells, and the production of immunoglobulins with polyspecific antigen binding, are unique to the neonate and partially mitigate the deficiencies in adaptive immunity.

• The innate lymphoid cells are a recently-described, exciting new subset in innate immunity that are likely to play a major role during the neonatal period and early infancy.


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Severe combined immunodeficiency (SCID)

4 groups according to T/B/NK cells

**T- B- NK-**
- Low CD3+/CD19+/CD56+
- ADA deficiency

**T- B+ NK-**
- Low CD3+/CD56+, CD19+ normal
- Most common SCID - Common gamma chain
- JAK3 deficiency

**T- B+ NK+**
- Low CD3+, normal CD19+/CD56+
- IL-7Ra (CD127)
- IL-2Ra
- CD45
- CD3 chain defect

**T- B- NK+**
- Low CD3+/CD19+, normal CD56+
- Omenn syndrome - RAG, Artemis
- Cernunnos
- Ligase 4 defect
- Nijmegen breakage
Screening for SCID

T-cell receptor excision circles (TRECs): Circular DNA by-products of TCR gene rearrangement joined by RAGs

A particularly frequent intermediate TREC in humans can be assayed from peripheral blood DNA by qPCR

SCID patients with low T-cells lack TRECs, which can be checked from dried blood spots
NEC is marked by a macrophage-rich infiltrate.
Specificity

AMC, A = 0.76

Normalized AMC = 0.8

NEC from Other Causes of Feeding Intolerance