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Role of Innate Host Defenses in Susceptibility to Early Onset Neonatal Sepsis

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Abstract

Neonatal sepsis continues to take a devastating toll globally. Although adequate to protect against invasive infection in most newborns, the distinct function of neonatal innate host defense coupled with impairments in adaptive immune responses, increases the likelihood of acquiring infection early in life with subsequent rapid dissemination and death. Unique differences exist between neonates and older populations with respect to the capacity, quantity, and quality of innate host responses to pathogens. Recent characterization of the age-dependent maturation of neonatal innate immune function has identified novel translational approaches that may lead to improved diagnostic, prophylactic and therapeutic modalities.

Keywords

Neonate; early onset sepsis; innate immunity; antimicrobial proteins and peptides; neutrophil; monocyte; acute phase response; complement; pattern-recognition receptors; TLR

Introduction

Infection claims the lives of nearly three thousand neonates worldwide every day¹. Preterm newborns are the most affected, as they exhibit the highest sepsis-related morbidity and mortality among pediatric patients². The distinct neonatal immune system, although adequate to protect against infection in most neonates, contributes to the newborn's enhanced susceptibility to infection³⁻⁶. The innate immune system represents the first line of prevention against microbial invasion and of defense once infection has occurred. Initial immunomodulatory efforts directed at improving neonatal sepsis survival through enhancement of innate immune function (also reviewed in Chapter XXXX of this issue), including IVIg, GM-CSF, and G-CSF, have thus far not yielded major benefit³. Dramatic progress in molecular characterization of innate immunity has been made over the past decade, paving the way to new prophylactic and therapeutic approaches. While the roles of innate

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immunity in adult sepsis have been examined in great detail⁷⁻¹², the distinct neonatal innate immune response remains incompletely characterized. In the context of increasing incidence of prematurity and the potential for neurodevelopmental impact in sepsis survivors¹³, there is an unmet medical need for novel approaches to prevention and therapy.

Early onset sepsis

Early onset neonatal sepsis (EONS) typically occurs during the first 24 hours of life, and is a fulminant multisystem infection acquired by vertical transmission from the mother¹⁴. Maternal factors that increase the risk of EONS include preterm labor and delivery, colonization with group B streptococcus (GBS), prolonged rupture of membranes, chorioamnionitis, and intra-partum fever¹⁵. The neonate can present with signs of respiratory distress including apnea, temperature instability, hypotension, bradycardia, tachycardia, lethargy or irritability, and abdominal distension or feeding intolerance requiring prompt evaluation and appropriate treatment from the physician. Once overtly symptomatic, mortality is unacceptably high¹⁶.

The incidence of EONS in *term* neonates is 1-2/1000 live births with a mortality of ~3%^{17, 18}. Responsible pathogens are dominated by two individual bacteria, GBS (41%) and *Escherichia coli* (17%)¹⁷. GBS emerged as the leading pathogen of EONS in the 1970s with case fatality rates as high as 55%¹⁹. A significant decrease in EONS due to GBS occurred, especially in neonates \geq 34 weeks gestation, following the national guidelines for the use of intra-partum antibiotic prophylaxis (IAP) to prevent neonatal GBS infection^{20, 21}. IAP has decreased overall GBS rates, but it is also associated with an increase in the incidence of EONS due to *Escherichia coli*¹⁶, and an increase in newborn exposure to antibiotics that may have adverse consequences²².

Preterm neonates, especially the very low birth weight (VLBW) neonate, suffer attack rates >10 times higher than those born at term with associated mortality in over one-third²³. Morbidities include increased respiratory distress, chronic lung disease (CLD), white-matter damage, neurodevelopmental impairment, and increased risk of death, especially with Gram-negative infections^{23, 24}. Compared to term neonates, VLBW neonates demonstrate an increased percentage of EONS caused by Gram-negative pathogens²⁵. EONS associated with Gram-negative infection is more likely to result in death within 72 hours of birth (29%) than Gram-positive infection (6%)²⁵. Extremely low birth weight neonates (<1000g, ELBW) are even more vulnerable as EONS accounts for over 50% of deaths that occur within the first 48 hours of life²⁶.

An adaptive immune response, including the selection and amplification of specific clones of lymphocytes (B-cells and T-cells) that results in immunologic memory, generally requires 5-7 days to develop. Moreover, the neonatal adaptive immune response is functionally distinct from the adult response at multiple levels⁵. As a result, the neonate is thought to largely depend on the function of innate immunity for protection from infection during the first days of life. The innate response, defined as that which is present at birth prior to microbial exposure, consists of a preformed immune response mediated by barriers, sentinel immune cells, pathogen recognition systems, inflammatory response proteins, host defense proteins and peptides, as well as passively acquired immunoglobulin from the mother.

An overview of the current state of knowledge regarding innate immunity of the newborn must take into consideration the research approaches that have thus far been employed to study this area. *In vitro* studies have largely focused on cord blood, though in some cases similar patterns and/or age-dependent maturation/normalization have been documented in newborn peripheral blood. *In vivo* studies of non-human vertebrates have largely focused on newborn mice. The neonatal mouse model has two potential limitations: (a) mice are not humans, and the innate immune system is particularly divergent²⁷, and (b) the post-natal age at which the mice are

studied is a matter of variation and debate. Although all approaches have limitations, they provide valuable information towards characterizing *in vivo* function. Herein, we will discuss neonatal innate host defense systems and their relationship to susceptibility to and progression of EONS.

Innate host defense systems

Maternal Innate defenses

In utero infection is a significant risk factor for the development of EONS. In particular, ~80% of preterm deliveries at less than 30 weeks gestation have evidence of intrauterine infection²⁸. Ascending infection induces maternal immune responses *in utero* that may also influence mobilization of fetal neutrophils (polymorphonuclear cells-PMNs) found in infected amniotic fluid²⁹ and the development of the fetal and neonatal inflammatory response³⁰. Immune responses to intra-amniotic infection likely begin with Toll-Like receptors (TLRs) expressed on maternal trophoblast cells²⁸ [Figure1]. Human trophoblast cells express all ten TLRs and, upon stimulation, produce inflammatory cytokines found in infected amniotic fluid^{28, 31}. In particular, elevated amniotic fluid IL-6 concentrations are commonly found during intra-amniotic infection³¹ and are associated with rapid parturition³² and acceleration of fetal lung maturation³³. In addition to the TLR-mediated innate immune sensing capabilities of trophoblasts, the amnion, chorion, placenta, amniotic fluid, cervical mucosa, and vagina are replete with antimicrobial proteins and peptides (APPs) that possess key host defense functions and are up-regulated with infection³⁴⁻³⁶. Thus, ascending bacterial infection that underlies EONS triggers early innate immune activation of both maternal and fetal tissues that mobilize host defense effectors to the amniotic fluid.

Epithelial and Mucosal Barriers

Host barriers provide the first means of protection from microbial invasion. There are two critical barrier regions: the mucosa (respiratory and intestinal) and the skin. While *in utero*, the fetus is protected within the normally sterile environment provided by the amnion and amniotic fluid that is replete with APPs⁴. At birth, vernix enhances skin barrier function for the late-preterm and term neonate but is largely absent in preterm neonates born before 28 weeks gestation. Vernix is a complex material comprised of water (80.5%), lipids (10.3%), and proteins (9.1%) produced by fetal sebaceous glands during the last trimester³⁷. The vernix provides a barrier to water loss, improves temperature control, and serves as a shield containing antioxidants and innate immune factors such as APPs³⁸. Furthermore, vernix is important for maintenance of the pH balance of the skin and thus sets the stage for appropriate colonization with commensal organisms instead of pathogens³⁸.

The outermost layer of the skin, the stratum corneum, prevents microbial invasion, maintains temperature, and reduces the risk of dehydration through prevention of transcutaneous water loss³⁹. The immature and incompletely developed stratum corneum of preterm newborns takes at least 1-2 weeks after birth to become fully functional⁴⁰ and may take up to 8 weeks to develop in the extremely preterm neonate significantly increasing the risk for barrier dysfunction⁴¹. Neonates have an increased density of hair follicles as compared to adults, which provides a larger reservoir for skin commensal organisms such as *Staphylococcus epidermidis*^{42, 43}. Additionally, the risk for a microbial breach of the cutaneous barrier rises in the presence of intravenous catheters essential for critical care. The response of the dermal innate immune system to host commensal organisms is exemplified by the common newborn rash erythema toxicum. Recent evidence suggests that this common newborn condition is an immune-mediated manifestation of the bacterial colonization of the skin^{42, 43}. Because the skin is arid, it is a more formidable barrier for microbial invasion than that of the moist mucosal surfaces. However in extremely preterm infants, humidification systems are used to decrease insensible

water loss and these may delay cornification of the skin and thus predispose to microbial growth.

Mucosal barriers contain multiple components that serve to prevent infection including acidic pH, mucus, cilia, destructive enzymes, APPs, opsonins such as surfactant proteins, sentinel immune cells such as macrophages, dendritic cells (DC), PMN, and T cells, as well as commensal organisms⁴⁴. After birth, the gut is quickly colonized and contains a significant repository of microorganisms⁴⁵. Although not believed to contribute significantly to the development of EONS, intestinal barrier integrity is paramount for prevention of spread of microorganisms out of the intestinal compartment and likely plays a role in the development of necrotizing enterocolitis⁴⁶. Factors known to disrupt the neonatal intestinal barrier are antibiotic treatment, hypoxia, or remote infection as has been recently reviewed^{3, 47}.

Ascending infection, with or without chorioamnionitis, or acquisition of microbes during passage through the birth canal represent the common origins of EONS¹⁴. The portal of pathogen entry into the neonate is primarily through the respiratory tract. *In utero*, amniotic fluid and pulmonary APPs, surfactant proteins A and D, alveolar macrophages, PMNs, and trophoblast-based TLRs serve as the first line of defense [Figure 1]. After birth, respiratory mucosal function can be impaired by surfactant deficiency, altered mucus production, intubation and mechanical ventilation that is associated with decreased mucociliary clearance, and airway irritation. The surface and submucosal gland epithelium of the conducting airways is a constitutive primary participant in innate immunity through the production of mucus and mucociliary clearance of pathogens and debris⁴⁸. Premature neonates have relatively more goblet cells than more mature neonates leading to a decrease in mucociliary clearance. Additional insult to the neonatal respiratory system may result from physical damage to lung parenchyma via atelectrauma, barotrauma, or chemical injury (e.g. oxygen or aspiration). Intubation is also associated with the progressive accumulation of colonizing bacteria and bacterial endotoxin in respiratory fluids with concomitant mobilization to the airway of endotoxin-modulating host defense proteins⁴⁹. Neonates with surfactant deficiency lack host defense proteins with valuable immune function such as surfactant proteins A and D that are absent in commercially available exogenous surfactants due to destruction during preparation⁵⁰. There is an age-dependent maturation in the ability of respiratory epithelium to elaborate APPs (cathelicidin [LL-37] and β -defensins) such that respiratory epithelium of preterm newborns mounts a deficient antimicrobial peptide response⁵¹. These deficiencies as well as those related to cellular function in combination with invasive procedures lead to a reduction in respiratory barrier function that increases the risk for early infection.

Pathogen recognition systems

The development of an immune response by local immune sentinel cells, including macrophages, endothelium, epithelium, PMNs, and DCs, is dependent on the identification of invading pathogens or the presence of tissue damage that result in elaboration of exogenous or endogenous danger signals. Evolution of pattern recognition receptors (PRRs) capable of recognizing damage/danger associated molecular patterns (DAMPs) [cytokines, intracellular proteins, and/or mediators released by dying or damaged cells] in addition to pathogen associated molecular patterns (PAMPs) [e.g., bacterial cell wall components, flagellin, nucleic acids] allows for specificity in the innate immune response. PRRs are present on the cell surface, within intracellular vesicles, and in the cytoplasm of multiple cell types. Examples include the TLRs, nucleotide-binding oligomerization domain (NOD)-like receptors, retinoic acid-inducible protein I (RIG-I)-like receptors, and integrins^{52, 53}. As described below, with respect to multiple microbial stimuli, engagement of neonatal PRRs on neonatal blood leukocytes often leads to a pattern of response that is distinct from that in older individuals

with impaired neonatal production of pro-inflammatory/ T_H1 -polarizing cytokines that may increase the risk for development and progression of infection.

Activation of TLRs by microbial or synthetic agonists results in downstream production of cytokines, chemokines, and complement and coagulation proteins, as well as initiation of antimicrobial effector mechanisms including enhanced phagocytic function⁵⁴. There are 10 TLRs in humans that respond to specific molecular triggers^{54, 55}. Molecular identification of particular pathogens and subsequent signaling can occur via simultaneous stimulation of multiple TLRs, allowing for tremendous diversity in specific pathogen detection and response^{55, 56}. As TLRs play an essential role in recognition and response to pathogens, alterations in their expression, structure, and signaling pathways can impair host defense and increase host vulnerability to infection⁵³.

Basal expression and cellular distribution of TLRs is similar in monocytes derived from term umbilical cord blood and peripheral blood monocytes obtained from adults⁵⁷. Cord blood monocyte TLR4 expression has been found to increase with gestational age⁵⁸. In a study of patients with EONS, peripheral blood cells from both preterm and full-term neonates demonstrated up-regulation of TLR2 and TLR4 mRNA during Gram-positive and Gram-negative bacteremia, respectively⁵⁹.

Hypomorphic mutations or decreased expression of proteins that enhance TLR activation may increase the risk for progression of early neonatal infection⁶⁰⁻⁶². Examples of ancillary proteins needed for optimal TLR-mediated pathogen recognition include lipopolysaccharide-binding protein (LBP) that extracts LPS monomers from Gram-negative bacteria and delivers them to the endotoxin receptor (CD14/MD2/TLR4) on the monocyte surface and CD14, both of which are normally up-regulated during neonatal sepsis and are required for LPS-mediated signaling through TLR4⁶³⁻⁶⁵.

In addition to TLRs and their co-receptors, other intracellular signaling mechanisms are important for the detection of pathogens. Examples of cytosolic receptors include the nucleotide oligomerization domain leucine-rich repeat containing family or NOD-like receptors (NLRs), which detect peptidoglycan in the cytosol, as well as the retinoic-acid-inducible protein I (RIG-I)-like receptors (RLRs) which sense double-stranded RNA of viral origin and induce production of type I interferons (IFNs)⁵³. Intracellular bacteria, including *Listeria monocytogenes*, can be recognized by NLRs⁶⁶. Polymorphisms in NLR domains are associated with dysregulated inflammatory pathology including Neonatal-Onset Multisystem Inflammatory Disease (cryopyrin)⁶⁷, but NLR alleles have not yet demonstrated any correlation with sepsis susceptibility⁶⁸. Much remains to be learned about the functional expression of NLRs in neonates.

Recognition of a PAMP or DAMP by PRRs activates a complex series of intracellular cascades that trigger gene activation⁵³. Polymorphisms or mutations in TLRs and downstream signaling molecules such as MyD88 (Myeloid differentiation factor 88), NEMO (NF- κ B essential modulator), and IRAK-4 (IL-1-receptor-associated kinase 4) are associated with increased risk for infection in adults⁶⁹⁻⁷⁴ and in children⁷⁵⁻⁷⁸. Children with IRAK-4 deficiency manifest decreasing susceptibility to pyogenic infection with age, suggesting that the TLR pathway is of greatest importance early in life, in line with findings in murine models^{79, 80}. In contrast to adults, specific characterization of neonatal PRR intracellular signaling intermediates, their regulation, and response to infection, have been incompletely characterized⁸¹. Of note, newborn umbilical cord PMNs were found to have lower MyD88 expression and reduced p38 phosphorylation following *ex vivo* stimulation with endotoxin, potentially contributing to diminished responses⁸². Decreased MyD88, IRF5, and p38 phosphorylation was noted in endotoxin-stimulated monocytes isolated from both umbilical cord and peripheral venous

blood that exhibited gestational age-specific diminution^{83, 84}. Deficient expression of TLR second messenger proteins in preterm newborns likely contributes to the increased risk of infection as compared to more mature neonates.

Another important neonatal PRR is the β_2 -integrin complement receptor 3 (CR3) that functions as a pathogen sensor on the surface of phagocytes in addition to its role in binding complement. CR3 (also known as MAC-1 and CD11b/CD18) binds LPS, in cooperation with or independent of CD14, as well as other microbial surface components and triggers up-regulation of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production⁸⁵. Engagement of CR3 on PMN activates both reactive oxygen intermediate (ROI) production and phagocytosis. Accordingly, decreased expression of L-selectin and CR3 on stimulated neonatal PMN impairs neonatal PMN and monocyte activation and accumulation at sites of inflammation^{86, 87}. This decreased expression persists for at least the first month of life in *term* infants possibly contributing to an increased risk for infection⁸⁸. The expression of CR3 (CD11b) may be reduced further in preterm neonates as compared to term neonates⁸⁹. In umbilical cord blood from neonates less than 30 weeks gestation, PMN CR3 content was similar to levels found in those with type 1 leukocyte adhesion deficiency (failure to express CD18)^{86, 87}. Thus, decreased leukocyte CR3 surface expression increases the likelihood of suboptimal pathogen detection and cellular activation, particularly in the preterm neonate.

Innate cellular immunity

Antigen presenting cells (APCs)—After compromising barriers, invading pathogens come into contact with sentinel immune cells including monocytes, macrophages, and DC (see Figure 1, chapter xxxxxx). Following detection of microorganisms via the PRRs, these APCs amplify cellular recruitment through production of inflammatory mediators (complement, cytokines, coagulation factors, and extracellular matrix proteins⁹⁰), phagocytose and kill pathogens, and present foreign antigens to cells of the adaptive immune system. Initial pathogen detection and innate response by APCs are critical for the development of an effective host immune response.

Responses following stimulation of PRRs in neonatal blood monocytes are generally reduced as compared to adults^{57, 91}. In response to many stimuli, including most TLR agonists, human neonatal cord blood mononuclear cells tested *in vitro* exhibit a marked polarization, with impaired production of T_H1 -polarizing cytokines such as TNF- α , IFN- γ , and IL-12p70 and increased production of T_H2/T_H17 and anti-inflammatory cytokines such as IL-6, IL-10, IL-17, and IL-23^{4, 92}. Elevated concentrations in neonatal cord blood mononuclear cells of intracellular cAMP, a secondary messenger that inhibits production of T_H1 -polarizing cytokines, appears to contribute to this skewed cytokine pattern⁹³. Proposed benefits of this skew are immune tolerance to avoid potentially harmful allo-immune reactions between fetus and mother, avoidance of excessive inflammation upon initial microbial colonization, and induction of epithelial APPs to avoid microbial penetration and infection^{4, 94}. However, a consequence of impairment in T_H1 -polarizing cytokine production by neonatal mononuclear cells is a reduced ability to defend against infection with microorganisms; particularly intracellular pathogens such as *Listeria spp.*⁹⁵, *Mycobacteria spp.*⁹⁶, and Herpes simplex virus⁹⁷.

Presentation of foreign epitopes by antigen presenting cells (APC) including monocytes, macrophages, and DC to cells of the adaptive immune system is less efficient in neonates as compared to adults. Neonatal monocytes and DCs demonstrate impaired expression of MHC-II^{98, 99} and of co-stimulatory molecules including CD40 and CD86¹⁰⁰ resulting in reduced antigen presentation to naïve CD4⁺ T cells. Neonatal DCs require increased stimulation for activation¹⁰¹ and demonstrate poor stimulation of T-cell proliferation as well as a predilection for the induction of immune tolerance via impaired LPS-induced IL-12p70 production¹⁰². Still,

neonatal cord blood DCs stimulated with LPS can effectively induce cytotoxic lymphocyte responses¹⁰³. A reduction in APC function impairs development of effective adaptive immunity, placing further burden on innate immune function for pathogen clearance.

In addition to altered cytokine production and reduced antigen presentation, monocyte phagocytic and chemotactic function are also reduced during neonatal sepsis relative to adults and neonatal baseline values^{104, 105}. The number of peripheral monocytes decreases during sepsis (between 60-120 hours after presentation), likely secondary to extravasation and differentiation into macrophages and DCs. Located just below epithelial borders, macrophages encounter pathogens immediately after entry. Like monocytes, macrophages play an important role in the amplification of the immune response through the production of cytokines and chemokines, phagocytosis and killing, and antigen presentation to naïve CD4⁺ T cells. Neonatal macrophages are poorly responsive to several TLR agonists¹⁰⁶ and IFN- γ ⁹⁴. During infection, neonatal macrophages exhibit decreased production of reactive nitrogen intermediates¹⁰⁷.

In summary, neonatal APCs, including monocytes, macrophages, and DCs, exhibit deficits in pathogen recognition, activation following stimulation, phagocytic function, bactericidal function, and amplification of the immune response that may increase the risk for the development and progression of EONS. Of note, however, certain stimuli, such as *GBS*, *Mycobacterium bovis* (Bacille Calmette Guerin), and TLR8 agonists can effectively activate neonatal APCs *in vitro*¹⁰⁸⁻¹¹⁰. The molecular rules that govern which stimuli do or do not effectively activate human neonatal APCs is a topic of active research with important translational implications for neonatal adjuvant development.

Neutrophils—Neutrophils or polymorphonuclear leukocytes (PMN) are the primary mediators of neonatal innate cellular defense. Antimicrobial mechanisms employed by PMNs have been reviewed in detail¹¹¹. Many quantitative and qualitative PMN deficits have been described for neonates as compared to adults^{94, 112}. PMN are activated and increased in number with onset of early during sepsis in term and preterm neonates^{113, 114}. Mature neonatal bone marrow PMN reserves are rapidly depleted during infection¹¹⁵, which results in a release of immature “band” forms (described as a left shift); the proportion of which can be used to aid in assessing the probability of sepsis¹¹⁶. Neonates who develop sepsis-associated neutropenia are more likely to have Gram-negative sepsis and exhibit a higher mortality^{114, 117, 118}. Conflicting reports exist regarding the risk of EONS in VLBW newborns with neutropenia associated with other causes such as maternal preeclampsia¹¹⁸⁻¹²¹. ELBW newborns experience the highest frequency of neutropenia among all neonatal groups. However, neutropenia among ELBW newborns is most often not secondary to sepsis. Furthermore, ELBW newborns with neutropenia do not suffer increased subsequent mortality due to sepsis as compared with non-neutropenic ELBW newborns¹²².

In addition to the quantitative PMN deficits, multiple qualitative defects of neonatal PMN function are noted. The process of PMN recruitment and extravasation to sites of inflammation is less efficient in neonates contributes to their increased susceptibility to early infection. Specifically, PMNs from compared to older age groups and likely preterm and term neonates exhibit reduced basal chemotaxis and random migration¹²³, that is exacerbated in septic and post-operative newborns¹²⁴. Impaired signaling downstream of chemokine-receptor binding may be partially responsible for this finding¹²⁵. The propensity towards very high production of IL-6, a cytokine with anti-inflammatory properties that reduces PMN migration to inflammatory sites, may also contribute to the inability of newborns to mount an adequate PMN response in the context of sepsis^{92, 126}. In septic neonates, bacterial evasion mechanisms may also contribute to poor chemotaxis¹²⁷. Emerging data suggests that IL-8 priming of PMNs that occurs during labor significantly improves PMN chemotaxis over that seen with caesarean

delivery and even adult controls¹²⁸. Modifications in PRRs following labor may also contribute to the improvement in cellular chemotaxis. TLR4 is up-regulated on monocytes and PMN from term neonates after labor and PMN migration is improved following exposure to a TLR4 agonist¹²⁹⁻¹³¹. Decreased neonatal expression or upregulation of surface adhesion molecules on PMNs (LFA-1[preterm neonates], CR3[term and preterm neonates], L-selectin[term and preterm neonates]) during sepsis limits rolling and subsequent diapedesis^{86, 87}. Basal PMN deformation is reduced, and this impairment is further exacerbated in the immature band forms found commonly during infection, which reduces the ability to migrate between endothelial cells to sites of infection¹³². Aggregation defects lead to vascular accumulation of newborn PMNs following stimulation and contribute to decreased extravasation, rapid depletion of bone marrow reserves, vascular crowding¹³², and an increased likelihood of microvascular ischemia^{112, 133}.

Similar basal degranulation capabilities are present in PMNs from premature and term neonates, but content differs compared to adults. Compared to other innate immune cells, PMNs represent the most abundant and reliable source of APPs. PMNs of term neonates contain similar amounts of myeloperoxidase and defensins, but reduced lactoferrin, elastase, and bactericidal/permeability-increasing protein (BPI) relative to adult PMNs^{134, 135}. Neonatal PMN respiratory burst activity is also distinct as compared to adult PMN function. For example, hydroxyl radical production by term PMNs is reduced, particularly under stressed or septic conditions^{112, 134}. In contrast, superoxide production may actually exceed that of adult PMNs, but is suppressed during sepsis, contributing to poor microbicidal activity¹³⁶⁻¹³⁸.

When the target is fully opsonized, *ex vivo* phagocytosis of bacteria by PMN from late-preterm and term neonates is equivalent to adult controls whereas that from VLBW function remains depressed^{112, 139, 140}. Neonatal PMN exhibit delayed apoptosis, sustained capacity for activation (CD11b up-regulation) and cytotoxic function (ROI production), as well as extracellular release of destructive enzymes or ROI¹¹². These mechanisms contribute to local tissue and endothelial damage with resultant increases in inflammatory cytokine production¹⁴¹⁻¹⁴⁴. Novel PMN bactericidal mechanisms have been recently identified in studies of adult cells. TLR4-mediated activation of platelets by Gram-negative bacterial endotoxin or lipopolysaccharide (LPS) induces platelet-PMN binding with subsequent extracellular release of neutrophil extracellular traps (NETs) that contain DNA¹⁴⁵. NETs also contain APP and hydrolytic enzymes that result in bacterial killing even after PMN death^{146, 147}. Formation of NETs following stimulation was absent in preterm (≤ 30 weeks) and nearly absent in term neonates¹⁴⁸. Overall, the constellation of neonatal PMN functional deficits may increase the risk for development and rapid dissemination of infection.

Mast Cells—Mast cells may possess diverse immune functions including production of vasoactive substances, cytokines, phagocytosis of pathogens, and antigen presentation¹⁴⁹. These capabilities suggest the potential for mast cells to make a significant contribution to neonatal innate host defenses. Mast cells likely participate in the initial response to pathogens and may contribute to the neonate's relative predilection for immune tolerance through the actions of histamine. Of note, neonatal mast cells secrete significantly more histamine following stimulation as compared to adults¹⁵⁰. Production of histamine is well known to facilitate vasodilation, but also is capable of modification of subsequent immune responses. Specifically, neonatal mast cell histamine production alters DC cytokine production (increased IL-10 and decreased IL-12) and subsequent T cell polarizing activity (T_H2 phenotype)¹⁵¹. Recently mast cell involvement was demonstrated in the common newborn rash, erythema toxicum, where mast cell recruitment, degranulation, and tryptase expression was noted¹⁵².

Inflammatory response proteins

Complement—Non-cellular elements of the innate immune response include inflammatory response proteins such as complement, acute phase reactants, cytokines, chemokines, coagulation proteins, and vasoactive substances. Actions of complement include opsonization and killing of pathogens, alteration of vascular tone to facilitate recruitment and activation of leukocytes, initiation of the coagulation cascade, and proinflammatory cytokine production⁷. Neonates exhibit gestational age-related decreases in complement proteins, assays of hemolytic function, and complement-mediated opsonic capabilities as compared to adults [Figure 2a]¹⁵³⁻¹⁵⁶. In particular, neonates have very low levels of C9¹⁵⁷, which is critical for the formation of the membrane attack complex (MAC) and is associated with increased susceptibility to *Neisseria* infection in older populations. Neonatal *Neisseria* infections are most commonly ophthalmologic and rarely become systemic despite this deficiency. Moreover, human neonatal cord blood complement levels are sufficient to enhance GBS-induced TNF production *in vitro*, (via alternative complement pathway activation and engagement of monocyte CR3)¹⁰⁸⁻¹¹⁰. Nevertheless, reduced complement levels may contribute to the rapid proliferation of bacteria when assayed in human cord blood *in vitro*¹⁵⁸.

During bacterial infection the alternative complement pathway is the primary route of complement activation in both preterm and term neonates^{159, 160}. Significant increases in alternative pathway components have been found in the plasma from newborns with sepsis including Factor B, C3a desArg, C3bBbP (C3 convertase), and sC5b-9 (MAC), with C3a desArg reaching levels seen in infected adults¹⁵⁹. In these studies, markers of classical pathway activation were not elevated during sepsis and suggest that antibody-mediated complement activation does not play a significant role in neonatal sepsis even in term neonates that have received ample passive immunization via placental antibody transfer¹⁶⁰. As mannose-binding lectin (MBL) is capable of activating the alternative pathway, and decreased MBL levels are associated with an increased risk of sepsis during the first month of life (term and preterm), it is likely that MBL plays an important role in complement activation and thus innate host defense [Figure 2a]^{161, 162}.

Multiple aspects of innate cellular recruitment, activation, and function are attributed to the actions of complement-receptor binding¹⁶³⁻¹⁶⁶. For example, neonatal leukocyte expression of complement receptors (CR1, CR3) increases during sepsis resulting in enhanced pathogen recognition, phagocytosis, and production of ROI, as well as enhanced endothelial adhesion, rolling, and migration. Additional effects of complement protein-receptor binding include aggregation of platelets and endothelial activation (production of chemokines, cytokines, vasoactive substances). Blunted up-regulation of CR3 and deficiencies of C5aR on neonatal PMNs following stimulation likely contributes to poor complement-mediated chemotaxis and transmigration compared to adult PMNs^{167, 168}.

Cytokines and chemokines—Activation and amplification of host immune cells during infection is mediated in part by production of cytokines and chemokines following stimulation of PRRs. Interleukin (IL)-1 β , IL-6, IL-12, IL-18, interferon gamma (IFN- γ), and tumor necrosis factor-alpha (TNF- α) are among the elevated pro-inflammatory cytokines commonly found during neonatal sepsis¹⁶⁹. Important aspects of the cytokine response following TLR stimulation in neonates are diminished. In particular, decreased production of T_H1-polarizing cytokines (IL-1 β , TNF- α , IFN- γ , and IL-12^{57, 170-172}) with enhanced production of T_H2-polarizing cytokines relative to adults likely contribute to neonatal susceptibility to infection^{8, 57, 79, 80, 83, 170}. Decreased neonatal cytokine production during infection as compared to adults is likely related to decreased intracellular mediators of TLR signaling⁸³. Plasma adenosine, an endogenous plasma metabolite that rises with hypoxia and stress, is elevated in newborn blood plasma and inhibits production of TNF- α by monocytes with

preservation of IL-6 synthesis⁹³. Adenosine binds the G-protein-coupled adenosine 3 receptor (A3R) expressed on neonatal leukocytes, thereby triggering intracellular accumulation of cAMP, a second messenger that inhibits production of T_H1-polarizing cytokines while preserving that of T_H2-polarizing cytokines, including IL-6^{4, 93}. The skewed polarization of neonatal cytokine responses against T_H1 polarizing cytokines may reduce the risk of immune reactions that could trigger preterm birth or excessive post-natal inflammation during colonization with commensal flora, but may also contribute to neonatal susceptibility to infection with intracellular pathogens.

Notable exceptions to the impairment in TLR-mediated neonatal production of T_H1-polarizing cytokines are agonists of TLR8. Synthetic TLR8 agonists, including imidazoquinolines and single-stranded RNAs induce marked neonatal production of TNF- α to adult levels revealing a signaling pathway that is fully functional at birth¹⁰⁹. These observations raise the possibility that TLR8 agonists may have unique efficacy as stand alone immune response modifiers in newborns and/or as neonatal vaccine adjuvants, a major unmet medical need¹⁷³.

In addition to altered signaling capacity and the effects of adenosine on cytokine production following TLR stimulation, alterations in the genes coding for cytokines and their receptors have the potential to contribute to the neonate's increased susceptibility to EONS. Following the discovery of specific cytokine or cytokine receptor polymorphisms and their association with an increased risk for infection in older populations, neonates have been evaluated with mixed results¹⁷⁴⁻¹⁸⁰. Large, population-based studies will be necessary to more completely characterize the impact of genetic variation in cytokines and their receptors on the risk for development of neonatal infection.

Development of an effective inflammatory response is necessary to successfully respond to an infectious challenge. However, uncontrolled proinflammatory responses may lead to host tissue injury through excessive cellular activation. A recent evaluation in mice given a large intraperitoneal dose (10mg/kg) of endotoxin revealed an increase (>3 fold) in the relative systemic inflammatory response (serum TNF- α , MCP-1, and IL-6) in neonatal mice as compared to adult mice¹⁸¹. Another murine evaluation reported reduced IL-6 (3 fold) and elevated TNF- α (3 fold) in 1 day old neonates compared to adults *in vivo* two hours after subcutaneous LPS exposure¹⁸². The finding of elevated endotoxin-induced serum TNF- α in neonatal mice relative to adult mice is in contrast to previous reports in humans^{57, 170-172} and mice^{79, 80}, may reflect specific features of the model or the timing of cytokine sampling, and will require further investigation.

Acute phase reactants—The production of inflammatory cytokines early during infection is associated with increased hepatic production of innate immune proteins known as acute phase reactants (APR). Predominantly induced by IL-6, the primary function of these components is to reduce host bacterial load through improved cellular recruitment, opsonin function, and direct antimicrobial activity. Examples include MBL, LBP, pentraxins (C-reactive protein [CRP] and serum amyloid A [SAA]), and fibronectin^{4, 63, 64, 169, 183-186}. Of note, APRs have been studied in neonates with sepsis primarily to assess for diagnostic utility, rather than immunologic function. In particular, elevated plasma concentrations of CRP and LBP are often associated with EONS^{64, 187}. Despite APR increases and the presence of maternally-derived immunoglobulin, neonates exhibit impaired opsonizing activity compared to adults which likely increases the risk for progression of infection¹⁸⁸.

The coagulation cascade is intimately tied to inflammation, including complement activation, and is important in preventing microbial spread beyond the local environment. A microvascular pro-coagulant state develops via stimulation of monocytes, PMNs, platelets, and endothelium resulting in expression of tissue factor^{189, 190}. Tissue factor-mediated activation of the

coagulation cascade results in activation of thrombin-antithrombin complex, plasminogen activator inhibitor, and plasmin-a2-antiplasmin complex¹⁹¹, as well as inactivation of protein S and depletion of anticoagulant proteins including antithrombin III and protein C^{192, 193}. Activated platelets may be consumed in clot formation and/or may also be removed from the circulation by the liver¹⁹⁴ potentially resulting in thrombocytopenia, particularly during Gram-negative and fungal infections¹⁹⁵⁻¹⁹⁷. Systemic activation of coagulation is associated with consumption of clotting factors and increased risk of bleeding, inflammation, and disseminated intravascular coagulation (DIC)¹⁹⁸. Inflammatory dysregulation and its effects on the coagulation system are demonstrated by the association of altered ratios of serum pro- and anti-inflammatory cytokines and the development of DIC in neonates¹⁷⁰.

Passive immunity—Neonates have passively acquired antibodies via placental transfer with a significant increase beginning around 20 weeks gestation. As a result, preterm neonates have lower IgG levels as compared to term neonates [Figure 2a]; particularly IgG1 and IgG2 subclasses¹⁹⁹. Examination of the impact of low immunoglobulin in preterm neonates 24-32 weeks gestational age (serum levels <400mg/dL total IgG at birth) revealed an increased risk for development of *late*-onset infection but not mortality compared to those with levels >400mg/dL after controlling for gestational age. There was a 15% increase in LONS risk in these infants for every 100mg/dL decrease in serum IgG below baseline that was *not* reduced with IVIg infusion^{200, 201}. In the child or adult with deficient serum IgG1 (<100mg/dL)^{202, 203}, there are also significant infectious consequences²⁰⁴. Interestingly, despite functional immune system limitations of both innate and adaptive immunity, most premature neonates do not develop overwhelming bacterial sepsis, even in the absence of significant levels of passively acquired immunoglobulins. Reliance on other means of innate immune defense likely provides the premature neonate with some degree of microbial control mechanisms.

Antimicrobial proteins and peptides

APPs represent the most phylogenetically ancient means of innate immune defense against microbial invasion. Present in nearly every organism including bacteria, plants, insects, non-mammalian vertebrates, and mammals, these small, often cationic peptides are capable of killing microbes of multiple types including viruses, bacteria, parasites, and fungi largely by disruption of the pathogen membrane²⁰⁵. Constitutive expression of APPs occurs in humans on barrier areas with consistent microbial exposure such as skin and mucosa [Figure 1]. Following microbial stimulation, both release of pre-formed APPs as well as inducible expression are thought to contribute to early host defense²⁰⁶. Importantly, there is no evidence to date for the development of microbial resistance to APPs that target fundamental components of the microbial cell wall. Some APPs can bind and neutralize microbial components such as endotoxin, precluding engagement with TLRs and other PRRs and thereby reducing inflammation.

Bactericidal/permeability-increasing protein (BPI) is a 55kDa protein present present in the respiratory tract, PMN primary granules, and blood plasma [Figure 1]. BPI exerts selective cytotoxic, anti-endotoxic, and opsonic activity against Gram-negative bacteria¹⁵⁸. Lactoferrin is the major whey protein in mammalian milk (in particularly high concentrations in colostrum) and is important in innate immune host defenses. An 80kDa protein, lactoferrin is also present in tears and saliva and has antimicrobial activity both via binding iron (depriving microorganisms of this key nutrient) and by direct membrane perturbing activity. Lysozyme is present in tears, tracheal aspirates, skin, and in PMN primary and secondary granules and contributes to degradation of peptidoglycan of bacterial cell walls. Secretory phospholipase 2 (sPLA2) can destroy Gram-positive bacteria through hydrolysis of their membrane lipids²⁰⁷. PMN elastase is a serine protease released by activated PMNs with microbicidal function and is believed to play a role in the inflammatory damage seen with PMN recruitment, particularly

in the lung^{89, 208}. Cathelicidin and the defensins are other APPs that possess antimicrobial properties²⁰⁹. LL-37, the only known human cathelicidin, is present in the amniotic fluid, vernix, skin, saliva, respiratory tract, and leukocytes (PMNs, B cells, T cells, monocytes, and macrophages) [Figure 2B]. α -defensins are cysteine-rich 4 kDa peptides expressed in the amniotic fluid, vernix, spleen, cornea, thymus, paneth cells, and leukocytes (PMNs, monocytes, macrophages, and lymphocytes). β -defensins are found in skin, GI tract (salivary gland, tonsil, gastric antrum, stomach, liver, pancreas, small intestine, colon), reproductive organs and urinary tract (placenta, uterus, testes, kidney), respiratory tract, breast milk and mammary gland, and thymus. In addition to microbicidal action, APPs possess a wide range of immunomodulatory functions on multiple cell types from both the innate and adaptive immune system^{206, 210, 211}. Immunomodulatory effects on macrophages, DC, monocytes, mast cells, PMNs, and epithelia attributed to APPs include altered cytokine and chemokine production, improved cellular chemotaxis and recruitment, improved cell function (maturation, activation, phagocytosis, ROI production), enhancement of wound healing (neovascularization, mitogenesis), and decreased apoptosis.

Plasma and intracellular contributions—The cytosolic granules of PMN are rich in APPs that can be released upon stimulation into the extracellular space including α -defensins, lactoferrin, lysozyme, LL-37, sPLA₂, and BPI. Following release into the phagolysosome following phagocytosis or extracellularly into the surrounding tissue or blood, APPs contribute to microbial killing and binding of bacterial toxins such as LPS. Many APPs (LL-37, α/β -defensins, and BPI) can potentially reduce the intensity of the inflammatory response associated with the presence of bacterial toxins. Recently, gestational age-related decreases in the cord blood concentration of several APPs (LL-37, BPI, Calprotectin, sPLA₂, α -defensins) were described in comparison to maternal serum levels [Figure 2b]²¹². Plasma APP deficiencies may contribute to the increased risk of infection associated with prematurity, and their absence may increase the risk of excessive levels of bacterial toxins. Up-regulation of APPs (defensins) occurs in blood of infected adults²¹³ and children (defensins, lactoferrin)²¹⁴. The effect of sepsis on the production of plasma APPs in neonates has not been investigated in detail. PMNs from term neonates produce similar concentrations of defensins but reduced BPI and elastase as compared to adults^{112, 134, 135}. While term neonates demonstrate up-regulation of plasma BPI during infection, premature neonates showed a decreased ability to mobilize BPI upon stimulation²¹⁵, which may contribute to their risk for infection with Gram-negative bacteria. Polymorphisms in BPI increase the risk for Gram-negative sepsis in children, but the impact of these polymorphisms in neonates is unknown²¹⁶.

Skin—APPs present in amniotic fluid, vernix, and on newborn skin including lysozyme, LL-37, ubiquitin, α/β -defensins, and psoriasin have antimicrobial efficacy against common neonatal pathogens such as *Staphylococcus aureus*, *E. coli*, *Klebsiella spp*, and GBS²¹⁷⁻²¹⁹. Expression of LL-37 and β -defensin 2 are elevated on neonatal skin as compared to adult skin²²⁰. A role for β -defensins in defense against vertical HIV transmission has been suggested by a recent single-nucleotide polymorphism study²²¹. The common benign newborn skin lesions of erythema toxicum are associated with induction of LL-37 production⁴² demonstrating up-regulation following colonization of the skin with commensal flora including coagulase-negative Staphylococci^{42, 43}. The increased concentration of APPs on neonatal skin as compared to adults and their up-regulation in response to colonization likely serves to improve immune protection for the fragile skin barrier [Figure 1]^{217, 222}.

Airway—Airway protection is conferred *in utero* in part via amniotic APPs. In the absence of microbial invasion of the amniotic cavity, levels of calprotectin and BPI exhibit gestational age-dependent expression with much lower concentrations of amniotic fluid BPI levels in mid-trimester (14-18 week) fetuses²²³. Significant increases (>10 fold) in amniotic fluid APPs (BPI,

α -defensins, and calprotectin) occur in the presence of intra-amniotic microbial infection and preterm labor²²³ [Figure 1].

β -defensins 1-3, LL-37, lactoferrin, lysozyme, and BPI have been measured in neonatal tracheal aspirates. Reduced basal β -defensin concentrations in tracheal aspirates from preterm neonates may contribute to the risk of early pulmonary infection⁵¹. Mobilization of APPs is evident by increases in airway fluid concentrations of BPI, LL-37, and β -defensins that occur with mechanical ventilation, pneumonia, or systemic infection^{49, 206, 224}. Reduced lactoferrin and lysozyme concentrations have been described in tracheal aspirates of neonates with BPD and may contribute to chronic cellular inflammation due to poor clearance of bacteria²²⁵.

GI tract—APPs are present in meconium and are constitutively secreted by GI epithelium (predominantly Paneth cells). Up-regulation of APPs occurs upon stimulation in neonatal intestine following microbial colonization²²⁶ and with the development of necrotizing enterocolitis (NEC)²²⁷. Premature neonates have fewer Paneth cells and express less APPs as compared to more mature neonates that may increase their risk for poor intestinal barrier function or the development of NEC²⁰⁶. Human milk is an important post-natal source of APPs, including lactoferrin, defensins (α and β), and LL-37²²⁸ that inhibit the growth of bacteria and may be inactivated by human milk fortifier²²⁹. No newborn formula preparation contains human-derived, bovine-derived, or synthetic APPs at this time. Although unlikely to have a significant impact on EONS because onset precedes significant oral intake, human milk-derived APPs likely participate in the reduction of late-onset infection seen in neonates fed human milk²³⁰.

Efforts to reduce EONS mortality and enhance innate defenses

Successful obstetric interventions directed at reducing the incidence and impact of EONS have included antimicrobial treatment of mothers with GBS colonization²³¹, premature rupture of membranes^{232, 233}, preterm premature rupture of membranes^{234, 235}, and intra-amniotic infection²³⁶. For a complete review on attempts to enhance neonatal immunity, the reader is referred to prior publications^{3, 237} and to chapter XXX in this issue on neonatal adjuvants for prophylaxis and treatment of sepsis.

Activated protein C (aPC) administration demonstrated a modest reduction in mortality when given to adults with sepsis²³⁸. However, despite reduced plasma levels of aPC in septic neonates²³⁹, evaluations of aPC in children and infants with sepsis revealed no difference in mortality as compared to placebo but identified an increase in the risk for bleeding in infants < 60 days of age²⁴⁰.

Based on suboptimal neonatal PMN function, the presence of limited bone marrow PMN reserves, and the poor outcomes associated with septic neutropenia, cytokine therapy and granulocyte transfusions have been evaluated as prophylaxis to reduce the development of sepsis and as treatment to enhance neonatal immunity and sepsis survival. However, despite being well tolerated and increasing the number of effective circulating PMNs, granulocyte transfusions do not improve sepsis survival²⁴¹. However, treatment with colony stimulating factor therapy (G-CSF, GM-CSF) in a subgroup (97 neonates) with documented culture-positive sepsis (largely due to Gram-negatives and GBS) and neutropenia (ANC<1700/ μ L) significantly reduced the risk of death (RR 0.34 [95% CI 0.12, 0.92]). Therefore colony stimulating factors may be beneficial under these specific circumstances although further studies focused on this sub-population and outcome are needed^{242, 243}.

Future directions and translational opportunities

Biomarkers—Recent discoveries in neonatal immunology have brought to light some new diagnostic opportunities. New proteomic approaches can enhance detection of subclinical intra-amniotic infection, chorioamnionitis, and EONS through identification of specific inflammatory factors including IL-6 and select defensins and have helped to more completely characterize the immune response to *in utero* infection^{244, 245}. Multiple biomarkers of inflammation including cytokines (IL-1 β , IL-6, IL-8 [chemokine], IL-10, IL-18), APRs (CRP, procalcitonin, SAA, LBP), cell surface markers and molecules (E-selectin, P-selectin, VCAM-1, CD11b, CD64, HLA-DR, CD69), receptors (IL-2 soluble receptor, urokinase plasminogen-activated receptor), and enzymes (neutrophil elastase, urokinase plasminogen activator) have been explored to enhance diagnostic accuracy^{169, 187, 208, 246}. A review of existing and novel diagnostic laboratory tests to aid in identification of septic neonates is covered in Chapters XXXX yyyy and zzzzzz within this issue.

Novel anti-infectives—The characterization of the properties of APPs have prompted significant biopharmaceutical development and investigation²⁴⁷. None of these therapies have been examined or are approved for use in neonates at present but hold promise for improvement of innate immune function. For example, development and use of skin preparations that contain APPs (synthetic or natural) may provide fragile neonatal skin with improved barrier function and capacity to protect against early microbial invasion with a resultant decrease in infectious risk. Administration of recombinant BPI congeners (e.g., rBPI₂₁) with endotoxin neutralizing activity may reduce the deleterious actions of LPS via neutralization, reduce pro-inflammatory cytokines, and improve bacterial clearance via direct microbicidal activity¹⁵⁸. Other APPs may also have potential to reduce sepsis mortality when administered systemically, and are under evaluation²⁴⁸. Pretreatment with LL-37 was recently evaluated in neonatal rats given systemic LPS²⁴⁹. Low dose systemic pretreatment with LL-37 reduced inflammation (decreased CRP levels) and mortality compared to sham but higher doses led to increased mortality. As with all new investigational agents, safety concerns will need to be thoroughly investigated in preclinical neonatal animal models prior to systemic administration to human neonates. Recently, administration of enteral bovine lactoferrin to preterm human newborns was associated with a reduction in the incidence of late-onset sepsis (bacterial and fungal)²⁵⁰.

Timely abrogation of the inflammatory response helps to prevent the spread of inflammation beyond the local environment to a systemic level where dangerous consequences can occur¹². Simultaneous increases in anti-inflammatory cytokines such as IL-4, IL-10, IL-11, and IL-13 occur during sepsis and counter the actions of the pro-inflammatory cytokines^{169, 251-256}. Cytokine and receptor antagonists also prevent ligand-receptor coupling and reduce the effects of inflammatory cytokines. Examples of these regulatory molecules elevated during sepsis include TNFR2, IL-6sR, sIL2, and IL-1ra^{197, 253, 257}. Of note, despite apparent benefit in preclinical adult models of sepsis, anti-cytokine therapies aimed at reducing proinflammatory cytokines have been evaluated in infected human adults with minimal benefit and potential harm, likely reflecting the importance of TNF and other inflammatory cytokines to host defense²⁵⁸. In neonates, reduction of excessive or sustained inflammation could theoretically decrease the frequency and intensity of devastating post-sepsis sequelae including chronic lung disease²⁵⁹, retinopathy of prematurity²⁶⁰, and white matter damage¹³.

Pulmonary infection and or intubation and subsequent ventilator-associated lung injury increase the likelihood of respiratory mucosal damage and loss of barrier integrity. Of note, intubated preterm newborns demonstrate accumulation of endotoxin in airway fluids corresponding to duration of intubation, raising the possibility that endotoxin contributes to pulmonary inflammation and its consequences⁴⁹. In this context, improvement of pulmonary mucosal immune function through the administration of anti-infective proteins such as

surfactant proteins A and D, β -defensins, or endotoxin antagonists may help to reduce local and systemic inflammation associated with pneumonia. Administration of recombinant surfactant protein D reduced pulmonary and systemic inflammation following administration of intra-tracheal LPS in a preterm animal model²⁶¹. Caution is indicated as recent data showed that some APPs may bind SP-D, reducing its benefit, and may increase the risk for infection²⁶².

Immune adjuvants—Sepsis survival benefit following innate immune priming via specific TLR agonists has been demonstrated in a neonatal preclinical model⁸⁰. Priming with select TLR agonists resulted in improved cellular recruitment and function as well as decreased bacteremia and significantly improved sepsis survival over sham-primed septic neonates. Further evaluation, including assessment of safety, will be necessary before such positive immunomodulation reaches phase I trials in humans. Of note, vaccination with BCG, a live attenuated strain of *Mycobacterium bovis* with TLR2/4 agonist activity, was associated with an improvement in survival in low birth weight neonates (not related to tuberculosis infection) compared to those that did not receive the vaccine and may represent an existing example of the benefits and safety of innate immune priming²⁶³.

Summary

Neonatal EONS continues to take a devastating global toll. The innate immune system is distinct at birth and although usually adequate, does leave the newborn at increased risk of infection. Unique differences exist between newborns and older individuals with respect to capacity, quantity, and quality of innate host responses to pathogens. Characterization of the age-dependent maturation of neonatal innate immune function has identified novel translational approaches that may lead to improved diagnostic, prophylactic and therapeutic approaches.

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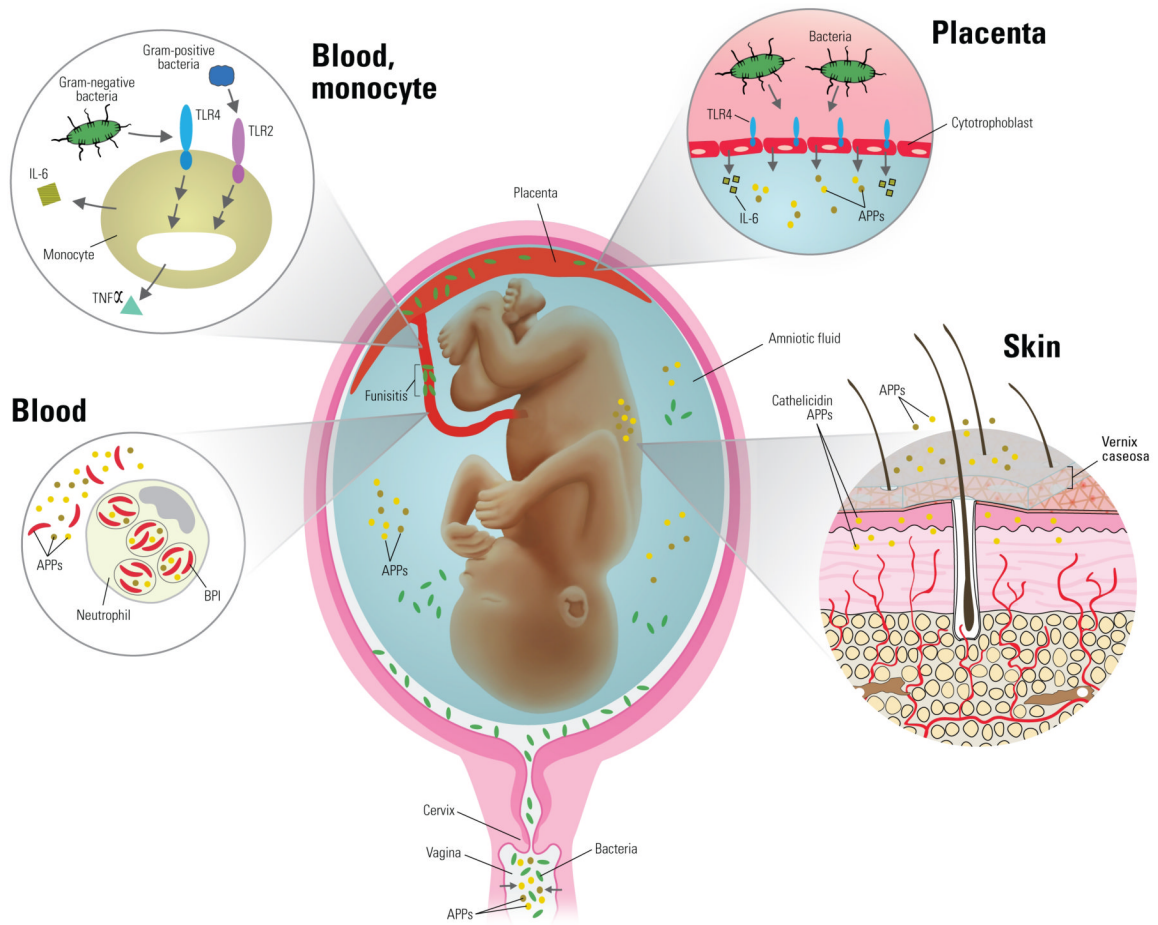


Figure 1. Role of innate immunity in responses to in utero infection

Early onset sepsis is typically caused by an ascending maternal lower genital tract infection. Antimicrobial proteins and peptides (APPs) within the vagina and amniotic fluid help to reduce bacterial burden and are up-regulated during infection. Pathogen detection begins with placental trophoblast cell Toll-like receptors (TLR) that up-regulate APP and inflammatory cytokine production. Neonatal monocytes are simultaneously stimulated via TLR-mediated pathogen detection with subsequent cytokine production that in turn activates innate immune function (neutrophils, macrophages) and induces the hepatic production of acute phase reactants. Innate immunity within the skin and vernix (APPs) facilitates appropriate commensal microbial colonization associated with erythema toxicum.

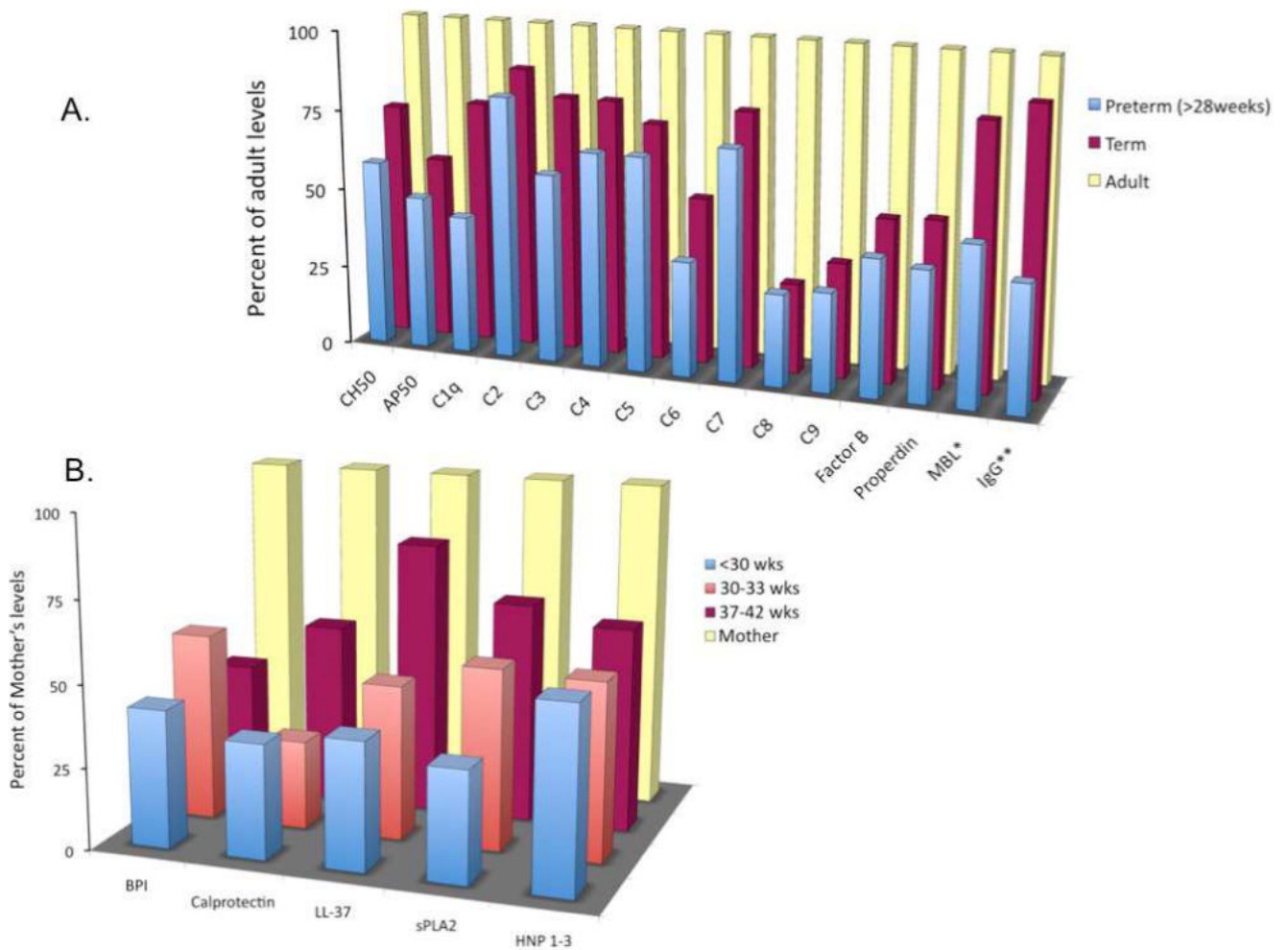


Figure 2. Comparison of neonatal and adult levels of opsonins and antimicrobial proteins and peptides

A. Complement functional assays and complement proteins, mannose-binding lectin, and IgG concentrations in preterm neonates, term neonates, and adults. (Reproduced with permission from Lewis DB and Wilson CB. "Developmental Immunology and Role of Host Defenses in Fetal and Neonatal Susceptibility to Infection. Pgs 87-210, In "Infectious Diseases of the Fetus and Newborn Infant": 2006 Elsevier Saunders, Philadelphia. Remington, Klein, Wilson, and Baker.) *- Reproduced with permission from Lau YL, et al. Mannose-binding protein in preterm infants: developmental profile and clinical significance. *Clin Exp Immunol* 102:649, 1995, **-Reproduced with permission from Fanaroff AA, et al. Incidence, presenting features, risk factors and significance of late onset septicemia in very low birth weight infants. *The National Institute of Child Health and Human Development Neonatal Research Network. Pediatr Infect Dis J* 17:593, 1998. **B.** Serum antimicrobial protein and peptide levels in preterm neonates, term neonates, and maternal levels. (Reproduced with permission from Strunk T, et al. Reduced levels of antimicrobial proteins and peptides in human cord blood plasma. *Arch Dis Child Fetal Neonatal Ed* 94:F230, 2009.). MBL-mannose-binding lectin, BPI-bactericidal/permeability-increasing protein, sPLA2-Secretory phospholipase 2, HNP-human neutrophil peptide.