HHS Public Access

Author manuscript

Arch Dis Child Fetal Neonatal Ed. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Arch Dis Child Fetal Neonatal Ed. 2018 July ; 103(4): F391–F394. doi:10.1136/archdischild-2017-313595.

Why are preterm newborns at increased risk of infection?

Amélie Collins[a], **Jörn-Hendrik Weitkamp**[b], and **James L. Wynn**[c,d]

[a]Department of Pediatrics, Division of Neonatology, Columbia University, New York City, New York

[b]Department of Pediatrics, Division of Neonatology, Vanderbilt University, Nashville, Tennessee

[c]Department of Pediatrics, Division of Neonatology, University of Florida, Gainesville, Florida

[d]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, Florida

Abstract

One in ten newborns will be born before completion of 36 weeks gestation (premature birth). Infection and sepsis in preterm infants remains a significant clinical problem that represents a substantial financial burden on the health care system. Many factors predispose premature infants for having the greatest risk of developing and succumbing to infection as compared to all other age groups across the age spectrum. It is clear that the immune system of preterm infants exhibits distinct, rather than simply deficient, function as compared to more mature and older humans and that the immune function in preterm infants contributes to infection risk. While no single review can cover all aspects of immune function in this population, we will discuss key aspects of preterm neonatal innate and adaptive immune function that place them at high risk for developing infections and sepsis, as well as sepsis-associated morbidity and mortality.

Introduction

Early-life infection is a significant cause of global morbidity and mortality. Long-term, often life-long, neurodevelopmental impairment increases the burden on health care systems. Distinct immune function in preterm infants is a significant contributor to infection risk. Here, we will highlight key aspects of preterm neonatal immune function that place them at high risk for developing infections and sepsis.

Innate immunity

The first line of innate defense against infection is the physical barrier (skin and mucosa) that prevents or delays the entry of pathogens. During the third trimester, fetal sebaceous glands in the skin produce a lipid-rich material called vernix caseosa (“cheesy varnish”) that hydrates the skin, maintains the pH, and contains a defined subset of functionally active antimicrobial proteins and peptides (APPs)(1). APPs act locally and at a systemic level to

Correspondence to: James L. Wynn, MD, Associate Professor of Pediatrics, Department of Pediatrics, Division of Neonatology, University of Florida, 1600 SW Archer Road, PO Box 100296, Office: (352) 273-8980, Pager: (352) 413-5056, Fax: (352) 273-9054, james.wynn@peds.ufl.edu.

Financial Disclosure Statement for all authors: No author has any financial relationships that could be broadly relevant to the work.

defend against infection, with the predominant mechanism of action being disruption of cellular membranes(2). Premature infants often do not have vernix caseosa, because its production begins during the third trimester. There is a wide timeframe (weeks) for attainment of skin barrier function after birth, with more premature infants taking longer than those born closer to term. Preterm stratum corneum is thinner and contains higher levels of involucrin, albumin, and proinflammatory cytokines than term stratum corneum. The immaturity of the premature stratum corneum is exacerbated by the insults (vascular access devices and adhesives) inflicted as a part of life-saving intensive care. Reducing skin breeches and attention to maintenance and prompt removal of central venous lines are key to prevention of nosocomial infections.

The mucosal barrier (respiratory and gastrointestinal) plays a critical role in the protection of the host from microbial invasion with pathogenic organisms while benefitting from the necessary interaction with commensal organisms. The conducting airways contain secretory epithelial cells (e.g. mucus-producing goblet cells) as well as neuroendocrine cells, non-ciliated Clara cells and ciliated epithelial cells. These cells form the mucociliary escalator that moves particles, toxicants, mucus and trapped microbial content away from the alveoli. The relative abundance of goblet cells (peaks at midgestation) in conjunction with fewer ciliated cells results in decreased mucociliary clearance in premature infants compared to term infants. Diminished lung expression of pathogen-sensing molecules such as Toll-like receptor (TLR) 4 (cognate receptor for lipopolysaccharide) and TLR2 (receptor for peptidoglycan) correlates with the inability of neonatal animals to recruit neutrophils in response to TLR ligand challenge, also contributing to morbidity from pulmonary infections. Preterm infants exhibit reduced APPs in tracheal aspirates as compared to term infants. The surfactant proteins SP-A and SP-D produced by type II alveolar cells belong to the collectin family of innate host defense proteins. These proteins, absent from commercial preparations of surfactant, bind a wide variety of PAMPs, and increase clearance of pathogens by enhancing opsonization and killing by alveolar macrophages. Exogenous surfactant therapy stimulates endogenous surfactant production, however, longitudinal studies of exogenous surfactant administration in premature infants with RDS have shown that it takes 48 – 72 hours for SP-A and SP-D levels to become similar to non-RDS controls(3). Taken together, these facets leave the preterm infant vulnerable to significant morbidity from early life pulmonary inflammation and infection.

Multiple factors including human milk contribute to the barrier function of the gastrointestinal mucosa. Peristalsis, gastric acidity, luminal mucus secreted by goblet cells in the crypts, the intestinal epithelial cell layer, immunoglobulin (Ig) A, the underlying lamina propria that contains a diversity of innate immune cell types, and the intestinal microbiome are active participants in host defense. Decreased motility in preterm infants secondary to incomplete innervation of GI motor complexes increases the dwell time for intraluminal bacteria, promoting bacterial overgrowth and increasing the opportunities for translocation through the epithelial layer. H2 blockers, which are associated with sepsis and necrotizing enterocolitis (NEC), reduce gastric acidity that normally reduces the number of pathogens that reach the distal GI tract. Intestinal permeability in the premature gut is increased as a result of dysregulation of tight junction proteins, which are preserved by small molecules such as butyrate and glutamine. As in the respiratory tract, specialized goblet cells in the

intestine secrete mucins that contribute to the mucus layer, which also contains APPs secreted by Paneth cells. Some APPs are constitutively expressed (e.g. defensins and lysozyme), others are induced in response to microbial products (e.g. Reg3g and angiogenins). Infection risk is increased secondary to reductions in lamina propria lymphocytes, secretory IgA, intestinal epithelium repair capacity, mucus and APP production as compared to adults. Intestinal TLRs that recognize pathogen-associated molecular patterns (PAMPs), and their associated signaling pathways such as NF-kB and heat shock proteins (HSPs), are developmentally regulated(4), resulting in a highly immune-reactive intestine that likely contribute to the development of sepsis and necrotizing enterocolitis.

The microbiome shapes the innate immune system and is necessary for effective barrier function. Significant differences in the composition of the microorganisms that comprise the GI microbiome have been found based on mode of delivery, breastfeeding vs formula feeding, and exposure to antibiotics in the neonatal period. Premature infants have an intestinal microbiome that contains fewer *Bacteroidaceae* and more *Lactobacillaceae* than term infants, explained in part by developmental immaturity of the glycosyltransferase enzyme system that regulates glycosylation of IEC receptors bound by colonizing bacteria(5). Empiric antibiotic therapy in the first postnatal days correlates with a microbiome that has lower microbial diversity, and prolonged early empiric antibiotic therapy is associated with increased risk of sepsis, NEC, and death(6).

Preterm infants may experience limited enteral feedings and or exposure to breastmilk that collectively increase the risk for infection. Breastmilk contains many bioactive molecules that provide innate immune function to the newborn including antimicrobial peptides (cathelicidin, lactoferrin), lysozyme, and secretory immunoglobulin A (sIgA), which confer immunity to enteric pathogens. Milk oligosaccharides that are digested to short chain fatty acids in the colon promote the growth of probiotic commensal organisms and stimulate plasma cells to produce sIgA, anti-inflammatory cytokines (IL-10, TGFβ), and growth factors such as EGF. The importance of human milk in neonatal intestinal host defense is evidenced by the reduction in sepsis, NEC, and death in preterm infants fed breast milk, both mother’s own milk and donor breast milk, compared to formula(7).

Inflammatory response elements

Once the barriers have been breached, pathogens gain access to local tissues and have the potential to enter the bloodstream, leading to widespread dissemination, a systemic inflammatory response, and sepsis. Inflammatory response elements of the innate immune system are critical to help activate local innate immune cells, as well as contain and destroy the pathogen. Examples of these elements include cytokines, chemokines, acute phase reactants, APPs, and the complement system.

Cord blood levels of AMPs such as bactericidal/permeability increasing protein (BPI), cathelicidin (LL-37), secretory phospholipase A2 (sPLA2), and human β-defensin 2 (HBD2) are significantly decreased in preterm compared to term infants, while others (calprotectin, human neutrophil defensins 1-3, HNP1-3) are decreased in cord blood of neonates of all gestational ages compared to adult(8).

The complement system encompasses three pathways for pathogen recognition that converge on the complement component C3 and lead to a common terminal lytic pathway. Complement cascade factors also play a significant role in priming the adaptive immune system, promoting inflammation, and activating the clotting cascade. Serum complement activity is decreased in term newborns compared to adult, and further diminished in preterm infants(9). This decreased activity has largely been attributed to global decreases in complement effectors and inhibitors, with preterm levels ranging from 10-80% of adult levels and remaining low until up to 1 year of age(9). Notably, C7 is one of the few complement factors not synthesized in the liver (it is predominantly synthesized by neutrophils), and its levels even in preterm infants are close to those in adults. Lytic activity of neonatal neutrophils is augmented when co-cultured with adult serum while lytic activity of adult neutrophils is diminished in the presence of neonatal serum(10). The lectin pathway of complement functions in the absence of antibodies by recognizing conserved carbohydrate structures on pathogens leading to opsonization and phagocytosis, making it an important pathway in antibody-deficient neonates. The best characterized molecule that activates the lectin pathway is mannose-binding lectin (MBL). Common polymorphisms exist at the MBL locus, leading to reduced circulating MBL levels (present in about 1/3 of the population). Low MBL levels are associated with pneumonia and sepsis in premature infants(11). Inflammatory response proteins serve to activate the innate immune system and to opsonize pathogens for clearance by innate immune effector cells. Importantly, the process of pathogen clearance requires far more than just opsonizing antibody or complement. The combination of ineffective *production* of these factors and orchestration of both inflammatory response factors with the cellular innate immune system is a major contributor to infectious risk in the preterm infant.

Cellular innate immunity

Neutrophils are phagocytic cells that play a crucial role in controlling microbial infections. The range of neutrophil counts considered normal in preterm and term infants over the first few days of life is very wide(12). Neonates have limited neutrophil storage pools which increases the risk of neutropenia with significant infection. Unfortunately use of recombinant colony-stimulating factor (G-CSF or GM-CSF) as either prophylaxis or treatment of sepsis has not been effective in reducing mortality in preterm neonates(13). Upon sensing an infection, neutrophils must travel from the bloodstream to the site of inflammation (chemotaxis), leave the vasculature (diapedesis), find and engulf the invading pathogen (phagocytosis), and destroy it via bactericidal activity in the phagolysosomes. Deficiencies in each of these functions have been documented in preterm infants and likely contribute to infectious risk(14). To exit the bloodstream, neutrophils must roll along the vascular endothelium, an activity mediated by the selectins (L-selectin on neutrophils and P-selectin on the endothelium). Compared to adults, neonatal neutrophils express less than half the L-selectin on their cell surface than adult neutrophils(15) and preterm endothelium has decreased P-selectin expression(16). Basal expression of β2 integrins [Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18)] are required for arrest of rolling and adhesion to the endothelium but these are decreased on preterm neutrophils and not upregulated in response to a stimulus(17). Diapedesis through the endothelial lining requires the ability to deform/reform the actin cytoskeleton, which is impaired in neonatal neutrophils(18). Once at the site

of infection, neutrophils phagocytose microbial products that are coated with either complement or specific antibody; these complexes are recognized by complement receptors (e.g. Mac-1) and Fcγ receptors(19). The decreased phagocytic capability of preterm neutrophils(20) can be explained in part by low opsonizing activity, as adult neutrophilic phagocytosis is diminished in preterm serum. Once the neutrophil has ingested the pathogen, it is killed primarily via NADPH oxidase-dependent respiratory burst. Although term neonates have a largely intact respiratory burst, preterm neonates [especially those that are critically ill (21)] display decreased respiratory burst and killing upon exposure to GBS, Staphylococcus, and Pseudomonas(22). The expression of other bactericidal molecules in neutrophilic granules, such as lactoferrin, myeloperoxidase, and bactericidal/permeability increasing protein (BPI) is also decreased in preterm neutrophils(1). Finally, neutrophils also trap bacteria by extruding DNA, chromatin, and antibacterial proteins by forming neutrophil extracellular traps (NETs). However, NET formation is diminished by NET inhibitors present in cord blood of preterm and term neonates(23).

Monocytes, macrophages, and dendritic cells are antigen presenting cells that secrete inflammatory mediators (cytokines, complement, APPs), have phagocytic function, and present antigen to T and B cells, linking the innate and adaptive arms of the immune system. These cells have a similar expression pattern of TLRs as adults but a cytokine response that is skewed toward a Th17 profile (IL-6, IL-23) and away from a pro-inflammatory Th1 profile (low IFNγ, IL-12, and TNFα) in favor of Th2 cytokines (IL-5, IL-10, IL-13)(8). Preterm monocytes exhibit decreased chemotaxis and phagocytosis during infection(24), and have decreased upregulation of co-stimulatory molecules (MHCII, CD40, CD80, CD86) necessary for successful antigen presentation to and activation of T and B lymphocytes(1). Natural killer (NK) cells play a significant role in the host defense to a variety of infections, especially those caused by viruses. Fetal and neonatal NK cells are phenotypically and functionally immature with significant reductions in IFNγ and TNFα production as well as reduced cytotoxic function(25).

Adaptive immunity

In general, the adaptive immune system can be separated into cell-mediated responses [e.g. T helper cells (Th, $CD4^+$) and cytotoxic T cells (CTL, $CD8^+$)], humoral responses (e.g. immunoglobulins), and immunoregulatory functions [e.g. T regulatory cells (Tregs)]. Because immunological tolerance to the growing fetus is critical for a successful pregnancy, several immune mechanisms that likely contribute to neonatal infectious risk are in place to limit the maternal response to microbial invasion at the materno-fetal interphase.

Depending on the cytokine milieu, $CD4^+$ T cells can differentiate into Th1 (IL-12 and IFN-γ), Th2 (IL-4), Th17 (IL-6 + TGF-β), or Treg (TGF-β + retinoic acid + IL-2) cells. Th1 cells produce IL-2 and IFN-γ and are involved in cellular immunity, Th2 cells produce IL-4, IL-5 and IL-13 and are involved in humoral immunity, while Th17 cells produce the proinflammatory cytokine IL-17, and Tregs are immune suppressor cells. Consistent with their limited exposure to foreign antigens, most T cells in the healthy neonate are immature with limited capacity to produce cytokines. With increasing postnatal age, the proportion of

effector $CD4^+$ T cells rises and effector memory T cells generated during infancy are functionally similar to those of adults(26).

Effective T cell function depends on CD40 ligand (CD154) expression, which is reduced on preterm and term $CD4^+$ T cells, and important for the activation and differentiation of antigen-specific $CD4^+$ T cells including those with Th1 immune function(27). Naive $CD4^+$ T cells derived from cord blood have reduced activation and impaired early Th1 differentiation including IFN-γ production compared with peripheral blood naive $CD4^+$ T cells from healthy adults(28). IFN-γ production by stimulated naive cord blood $CD4^+$ T cells is reduced 5- to 10-fold relative to adult $CD4^+$ T cells, which may play a role in persistent viral infections acquired *in utero* like human cytomegalovirus (HCMV) or Zika virus(29). Similarly, $CD4^+$ T cell proliferation and cytokine production (IL-2 and IFN-γ) are delayed in neonatal herpes simplex virus (HSV) infection compared to adults with primary infection(30). Intrinsic limitations in $CD4^+$ T cell function may also explain less robust immune responses to inactivated and life vaccines during early infancy. In contrast, IFN-γ production by stimulated naive cord blood $CD8^+$ T cells can be considered similar in neonates and adults(29). In addition, following congenital HCMV infection differentiated $CD8^+$ T cells showed potent perforin-dependent cytolytic activity and produced antiviral cytokines(31). Of interest HIV-specific $CD8^+$ T-cell responses can be detected in the first days of life in most *in utero*-infected infants(32), which is encouraging for immunization efforts targeting in utero responses. Tregs are forkhead box protein 3 (FOXP3)-expressing $CD4^+$ T cells that are $CD25^{high}$ and $CD127^-$. They are abundant in the peripheral blood and tissues of the human fetus and preterm infant, suppress fetal anti-maternal immunity, and persist at least until early adulthood(33). Proportions of Tregs in cord blood of preterm infants may higher compared to healthy tem neonates.

B cells/Immunoglobulins

Upon stimulation, activated B lymphocytes differentiate into plasma cells, which produce large amounts of antibodies. During this process B cells are able to change from IgM to other antibody isotypes (IgG, IgA, IgE), a process known as class switching, without changing antigen specificity. The inability of the neonate to effectively class switch, or produce antibodies in response to polysaccharides (e.g. bacterial capsular polysaccharides such as for *H. influenzae* type b, limits resistance to bacterial pathogens to which the mother has made little or no IgG antibody. Because the transfer of antibodies is interrupted after premature birth as the majority of maternal IgG transfer occurs in the last trimester, IgG concentrations are significantly lower in preterm infants and reach a nadir already at 2-3 months of age. However, multiple clinical trials of prophylaxis or treatment with polyclonal IgG, IgM-enriched IgG, or monoclonal antibody preparations in over 10,000 babies have not demonstrated benefit(34). IgA, IgD, IgM and for most cases IgE do not cross the placenta in significant amounts. IgA-producing plasma cells are largely absent in the infant intestine until after 1 month of age(35). Balanced microbial colonization of the newborn intestine may be critical for normal intestinal immune development and IgA induction(36) and low IgA levels in the preterm intestine has been considered a risk factor for necrotizing enterocolitis. Infants mount relatively poor quality and low titer antibody responses to primary viral infections. However, a lack of somatic mutations rather than fetal bias of the B

cell repertoire may be the limiting determinant of good quality antibody responses to viruses in neonates(37). Immunization with protein antigens such as tetanus and diphtheria toxoids or Hepatitis B surface antigen induce lower antibody titers during the first weeks of life especially in very low birth weight infants but not subsequently. Thus, chronologic age and body weight are likely more important determinants of antibody responses to T-cell-dependent antigens than gestational age.

Therapeutic opportunities

Maternal and neonatal interventions including attention to handwashing, targeting early extubation and enteral feeding, as well as prompt removal of central lines have dramatically reduced infectious burden in neonates over the last few decades. The development and discovery of novel effective therapeutic approaches that will further reduce infection among preterm infants is likely to result from: 1) better understanding of human *neonatal-specific* immune function and ontogeny (cannot assume neonatal immunity is the same as adults), 2) maternal and neonatal vaccination strategies, and 3) neonatal immune priming approaches (maximizing human milk feeding, non-specific effects of early-life vaccination, healthy microbiome).

Conclusion

Many factors contribute to the increased susceptibility of preterm infants to develop and succumb to infection. Innate immunity comprised of barriers, inflammatory response elements, and cells attempt to eradicate the infection or hold it in check until an antigen-specific adaptive immune response can be generated. While adaptive immunity in human newborns is significantly more developed at birth compared to most animal species, significant factors exist that impair a robust immune response to pathogens or vaccine antigens. A more complete understanding of the unique immune capabilities of neonates is a prerequisite to the development of effective interventions.

Acknowledgments

Funding source: Dr. Wynn receives support from the National Institutes of Health (NIH)/National institutes of General Medical Science (K08GM106143) and the NIH/Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) (R01HD089939).

References

1. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immunol. 2007; 7:379–90. Online. [PubMed: 17457344]
2. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003; 3:710–20. published Online First: 2003/09/02. DOI: 10.1038/nri1180 [PubMed: 12949495]
3. Bae YM, Bae CW, Oh MH, Lee SH, Woo KM, Jung KB. Effect of exogenous surfactant therapy on levels of pulmonary surfactant proteins A and D in preterm infants with respiratory distress syndrome. J Perinat Med. 2009; 37:561–4. published Online. DOI: 10.1515/JPM.2009.100 [PubMed: 19492923]
4. Claud EC, Lu L, Anton PM, Savidge T, Walker WA, Cherayil BJ. Developmentally regulated IkappaB expression in intestinal epithelium and susceptibility to flagellin-induced inflammation. Proc Natl Acad Sci U S A. 2004; 101:7404–8. published Online First: 2004/05/05. DOI: 10.1073/pnas.0401710101 [PubMed: 15123821]

5. Collado MC, Cernada M, Neu J, Perez-Martinez G, Gormaz M, Vento M. Factors influencing gastrointestinal tract and microbiota immune interaction in preterm infants. Pediatr Res. 2015; 77:726–31. published Online. DOI: 10.1038/pr.2015.54 [PubMed: 25760550]
6. Cotten CM, Taylor S, Stoll B, et al. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. Pediatrics. 2009; 123:58–66. published Online First: 2009/01/02. DOI: 10.1542/peds.2007-3423 [PubMed: 19117861]
7. Patel AL, Johnson TJ, Engstrom JL, et al. Impact of early human milk on sepsis and health-care costs in very low birth weight infants. Journal of perinatology : official journal of the California Perinatal Association. 2013; 33:514–9. published Online First: 2013/02/02. DOI: 10.1038/jp.2013.2 [PubMed: 23370606]
8. Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O. Protecting the Newborn and Young Infant from Infectious Diseases: Lessons from Immune Ontogeny. Immunity. 2017; 46:350–63. published Online. DOI: 10.1016/j.immuni.2017.03.009 [PubMed: 28329702]
9. McGreal EP, Hearne K, Spiller OB. Off to a slow start: under-development of the complement system in term newborns is more substantial following premature birth. Immunobiology. 2012; 217:176–86. published Online. DOI: 10.1016/j.imbio.2011.07.027 [PubMed: 21868122]
10. Wolach B, Carmi D, Gilboa S, et al. Some aspects of the humoral immunity and the phagocytic function in newborn infants. Isr J Med Sci. 1994; 30:331–5. Online. [PubMed: 8034475]
11. Dzwonek AB, Neth OW, Thiebaut R, et al. The role of mannose-binding lectin in susceptibility to infection in preterm neonates. Pediatric research. 2008; 63:680–5. published Online First: 2008/03/05. DOI: 10.1203/PDR.0b013e31816fdbff [PubMed: 18317236]
12. Schmutz N, Henry E, Jopling J, Christensen RD. Expected ranges for blood neutrophil concentrations of neonates: the Manroe and Mouzinho charts revisited. J Perinatol. 2008; 28:275–81. published Online First: 2008/01/18. DOI: 10.1038/sj.jp.7211916 [PubMed: 18200025]
13. Pammi M, Brocklehurst P. Granulocyte transfusions for neonates with confirmed or suspected sepsis and neutropenia. Cochrane Database Syst Rev. 2011; CD003956, published Online. doi: 10.1002/14651858.CD003956.pub2
14. Raymond SL, Mathias BJ, Murphy TJ, et al. Neutrophil chemotaxis and transcriptomics in term and preterm neonates. Transl Res. 2017; published Online. doi: 10.1016/j.trsl.2017.08.003
15. Anderson DC, Abbassi O, Kishimoto TK, Koenig JM, McIntire LV, Smith CW. Diminished lectin-, epidermal growth factor-, complement binding domain-cell adhesion molecule-1 on neonatal neutrophils underlies their impaired CD18-independent adhesion to endothelial cells in vitro. J Immunol. 1991; 146:3372–9. Online. [PubMed: 1709192]
16. Lorant DE, Li W, Tabatabaei N, Garver MK, Albertine KH. P-selectin expression by endothelial cells is decreased in neonatal rats and human premature infants. Blood. 1999; 94:600–9. Online. [PubMed: 10397727]
17. McEvoy LT, Zakem-Cloud H, Tosi MF. Total cell content of CR3 (CD11b/CD18) and LFA-1 (CD11a/CD18) in neonatal neutrophils: relationship to gestational age. Blood. 1996; 87:3929–33. Online First: 1996/05/01. [PubMed: 8611722]
18. Linderkamp O, Ruef P, Brenner B, Gulbins E, Lang F. Passive deformability of mature, immature, and active neutrophils in healthy and septicemic neonates. Pediatr Res. 1998; 44:946–50. Online First: 1998/12/16. [PubMed: 9853933]
19. Wynn JL, Seed PC, Cotten CM. Does IVIg administration yield improved immune function in very premature neonates? J Perinatol. 2010; published Online First: 2010/01/08. doi: 10.1038/jp.2009.197
20. Tissieres P, Ochoda A, Dunn-Siegrist I, et al. Innate immune deficiency of extremely premature neonates can be reversed by interferon-gamma. PLoS One. 2012; 7:e32863. published Online. doi: 10.1371/journal.pone.0032863 [PubMed: 22427899]
21. Strunk T, Temming P, Gembruch U, Reiss I, Bucsky P, Schultz C. Differential maturation of the innate immune response in human fetuses. Pediatr Res. 2004; 56:219–26. published Online. DOI: 10.1203/01.PDR.0000132664.66975.79 [PubMed: 15181184]

22. Kallman J, Schollin J, Schalen C, Erlandsson A, Kihlstrom E. Impaired phagocytosis and opsonisation towards group B streptococci in preterm neonates. Arch Dis Child Fetal Neonatal Ed. 1998; 78:F46–50. Online First: 1998/04/16. [PubMed: 9536841]

23. Yost CC, Schwertz H, Cody MJ, et al. Neonatal NET-inhibitory factor and related peptides inhibit neutrophil extracellular trap formation. J Clin Invest. 2016; 126:3783–98. published Online. DOI: 10.1172/JCI83873 [PubMed: 27599294]

24. Marodi L, Goda K, Palicz A, Szabo G. Cytokine receptor signalling in neonatal macrophages: defective STAT-1 phosphorylation in response to stimulation with IFN-gamma. Clin Exp Immunol. 2001; 126:456–60. Online. [PubMed: 11737062]

25. Li J, Li H, Mao H, et al. Impaired NK cell antiviral cytokine response against influenza virus in small-for-gestational-age neonates. Cell Mol Immunol. 2013; 10:437–43. published Online. DOI: 10.1038/cmi.2013.31 [PubMed: 23872919]

26. Tu W, Chen S, Sharp M, et al. Persistent and selective deficiency of CD4+ T cell immunity to cytomegalovirus in immunocompetent young children. J Immunol. 2004; 172:3260–7. Online. [PubMed: 14978134]

27. Stuber E, Strober W, Neurath M. Blocking the CD40L-CD40 interaction in vivo specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion. J Exp Med. 1996; 183:693–8. Online. [PubMed: 8627184]

28. Chen L, Cohen AC, Lewis DB. Impaired allogeneic activation and T-helper 1 differentiation of human cord blood naive CD4 T cells. Biol Blood Marrow Transplant. 2006; 12:160–71. published Online. DOI: 10.1016/j.bbmt.2005.10.027 [PubMed: 16443514]

29. Cimini E, Castilletti C, Sacchi A, et al. Human Zika infection induces a reduction of IFN-gamma producing CD4 T-cells and a parallel expansion of effector Vdelta2 T-cells. Sci Rep. 2017; 7:6313. published Online. doi: 10.1038/s41598-017-06536-x [PubMed: 28740159]

30. Burchett SK, Corey L, Mohan KM, Westall J, Ashley R, Wilson CB. Diminished interferon-gamma and lymphocyte proliferation in neonatal and postpartum primary herpes simplex virus infection. J Infect Dis. 1992; 165:813–8. Online. [PubMed: 1314868]

31. Marchant A, Appay V, Van Der Sande M, et al. Mature CD8(+) T lymphocyte response to viral infection during fetal life. J Clin Invest. 2003; 111:1747–55. Online. [PubMed: 12782677]

32. Thobakgale CF, Ramduth D, Reddy S, et al. Human immunodeficiency virus-specific CD8+ T-cell activity is detectable from birth in the majority of in utero-infected infants. J Virol. 2007; 81:12775–84. published Online. DOI: 10.1128/JVI.00624-07 [PubMed: 17881456]

33. Mold JE, Michaelsson J, Burt TD, et al. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. Science. 2008; 322:1562–5. published Online. DOI: 10.1126/science.1164511 [PubMed: 19056990]

34. Ohlsson A, Lacy JB. Intravenous immunoglobulin for suspected or proven infection in neonates. Cochrane Database Syst Rev. 2015; CD001239, published Online. doi: 10.1002/14651858.CD001239.pub5

35. Gustafson CE, Higbee D, Yeckes AR, et al. Limited expression of APRIL and its receptors prior to intestinal IgA plasma cell development during human infancy. Mucosal Immunol. 2014; 7:467–77. published Online. DOI: 10.1038/mi.2013.64 [PubMed: 24045575]

36. Mirpuri J, Raetz M, Sturge CR, et al. Proteobacteria-specific IgA regulates maturation of the intestinal microbiota. Gut microbes. 2014; 5:28–39. published Online. DOI: 10.4161/gmic.26489 [PubMed: 24637807]

37. Weitkamp JH, Lafleur BJ, Greenberg HB, Crowe JE Jr. Natural evolution of a human virus-specific antibody gene repertoire by somatic hypermutation requires both hotspot-directed and randomly-directed processes. Hum Immunol. 2005; 66:666–76. published Online. DOI: 10.1016/j.humimm.2005.02.008 [PubMed: 15993712]