Impaired innate immune responses in human neonates

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L. MARÓDI

Department of Infectious and Pediatric Immunology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary

A B S T R A C T

Human newborns are at greater risk of infection than children and adults because of the immaturity of their immune system. Mononuclear phagocytes from the cord generate blunted responses to an array of toll-like receptor (TLR) ligands and to physiologic stimuli of the inflammatory response. Neonatal deficiency of innate cellular immunity includes a decreased production of interferons, interleukin (IL)-12/IL-23, and IL-18, and other proinflammatory cytokines, an impaired type-1 response of macrophages to IFN-γ, the most potent macrophage-activating agent *in vivo*, and to lipopolysaccharide, the primary constituent of the outer membrane of Gram-negative bacteria. This review will describe recent advances in understanding innate cellular immunity in human neonates. As we learn more about neonatal innate immunity, new therapeutic avenues may come into sight. Drug development efforts could be directed toward augmenting innate cellular immune responses to prevent and treat neonatal infections more accurately.

Correspondence: Laszlo Marodi, Department of Infectious and Pediatric Immunology, Medical and Health Science Center, University of Debrecen, Nagyerdei Krt. 98, H-4012 Debrecen, Hungary.
Phone: 36.52.416841.
Fax: 36.52.430323.
E-mail: lmarodi@jaguar.dote.hu

Human neonates are more vulnerable to infectious agents than older children and adults, and are especially susceptible to infections with intracellular pathogens. Innate immunity against these pathogens represents the critical first-line barrier of host defenses. The past decade has brought grate strides in our understanding of innate immune mechanisms in humans. An increasing body of evidence suggests that neonatal innate responses may not be fully developed, allowing early dissemination of infections. This review describes recent advances and current understanding of innate cellular immune responses in human newborns.[1,2] A better understanding of molecular mechanisms that underlie neonatal immune functions may improve our ability to prevent and treat neonatal infections.

Major cytokine profiles of the fetus and neonatal infant

Nature endowed us with fundamental protective mechanisms against the constant danger of Th1-inductive placental and fetal damage. Biasing fetal immunity toward Th2 polarization appears to be an evolutionary adaptation orchestrated via production of cytokines and other regulatory molecules. The predominant production of Th2 cytokines in fetal and neonatal life appears to play a key role in damping the newborn's innate immune responses. Production of Th1 cytokines is reduced during fetal life and after birth. Deficiency of interferon (IFN)-γ production by neonatal T cells in response to stimulation by bacterial components has been well-documented.[3,4] A decreased production of IL-12 by cord mononuclear cells may be linked to IFN-γ deficiency in newborns.[5] Neonatal macrophages exposed to lipopolysaccharide (LPS) are also defective in the production of other proinflammatory cytokines, such as IL-1β, and tumor necrosis factor (TNF)-α.[6,7] Production of IL-18 (IFN-γ-inducing factor), by neonatal lymphocytes may also be decreased compared to adult cells.[8,9]

Responses of neonatal monocytes and macrophages to Toll-like receptor (TLR)-activating stimuli

Production of TNF-α and IL-6 in response to TLR-activating stimuli has recently been studied *in vitro*.[10,11] TNF-α release by cord and adult blood cells stimulated with an array of TLR ligands (triacylated bacterial lipopeptides, ligands for TLR1 and 2; mycoplasma-associated lipopeptide, ligand for TLR2 and 6; lipopolysaccharide (LPS), ligand for TLR4; imiquimod, ligand for TLR7) was compared. The results showed that ligand-induced TNF-α release was markedly decreased in cord blood compared to that in

adult blood despite similar basal mRNA expression of TLR1-10 and CD14 in monocytes.[10] Differences in ligand-induced TNF-α release correlated to both TNF-α mRNA synthesis and intracellular TNF-α production in isolated cord and adult monocytes exposed to LPS. Importantly, basal monocyte surface expression of TLR2, TLR4, and CD14 were all comparable in newborns and adults. Further, cord and adult blood responses to multiple TLR-activating microbial stimuli (poly dI:dC, ligand for TLR3; LPS, ligand for TLR4; flagellin, ligand for TLR5; CpG DNA, ligand for TLR9) were studied in parallel, with respect to production of the pro-inflammatory cytokine TNF-α and IL-6, a multifunctional cytokine. The results showed that cytokine release in cord blood was characterized by a higher IL-6/TNF-α ratio than in adult peripheral blood.[11] A high ration of stimulus-induced IL-6/TNF-α production may exert a modulatory effect on neonatal innate immunity.

Innate responses of neonatal monocytes and macrophages to pro-inflammatory cytokines

Human neonates are not able to mount an efficient mononuclear phagocyte response to a large number of pathogens and it was proposed that the blunted immune response mediated by these cells could result from both effector and regulatory defects.[12-16] The responsiveness of human neonatal monocytes and macrophages to individual cytokines differs significantly from that of adult cells and the defect in neonatal macrophage activation involves pathways downstream from ligand-binding events, and includes signal transduction pathways.[15] In the innate macrophage response to pathogens, Th1 type cytokines play a key role in initiating early resistance, and induction of cell-mediated immunity.[17-19] IL-12 and IL-23 produced particularly by mononuclear phagocytes and dentritic cells upon direct infection or stimulation with microbial products induce the production of IFN-γ by NK cells. IFN-γ triggers the IFN-γ receptor (IFN-γR) on macrophages leading to a complex series of molecular changes at the plasma membrane, in the cytoplasm, and at the nucleus which results in macrophage activation.[19-21] IL-12 selectively promotes the differentiation of naive CD4+ T cells into effector Th1 CD4+ cells, which produce the same effector cytokines, i.e. IFN-γ. Therefore, the secretion of IL-12/IL-23 and a microenvironment dominated by these cytokines and IFN-γ initiate innate resistance to the pathogen and regulate the polarization of T cells to a Th1 response.

Monocyte-derived macrophages from human neonates are hyporesponsive to activation by IFN-γ, a finding that cannot be attributed to lower expression of IFN-γR or decreased affinity of these receptors to their natural ligand on neonatal cells.[13,14] This phenomenon was linked to a marked deficiency in phosphorylation of the IFN-γR-associated kinase-signal transducer and activator of transcription (STAT)-1.[22] STAT-1 is a convergent point for immunologic stimuli in a macrophage proinflammatory response and the strength of signal through the IFN-γR may influence immune responsiveness. These findings suggest the possibility that there are important differences in the way newborns and adults use STAT-1 to modify immune response to pathogens. The impaired production of the most critical Th1-type cytokines, together with the impaired Th1-type response of neonatal macrophages are likely to associate with the high susceptibility of newborns to infectious diseases in which type-1 differentiation is needed to combat the pathogen.

Impaired TLR-induced signaling in neonatal mononuclear phagocytes

Differential responses of cord and adult blood cells to innate immune receptors including TLRs, CD14, and IFN-γR may be due to intrinsic cellular factors or plasma components.[10,11,13,14,23] TNF-α release by washed neonatal blood cells may be enhanced by addition of adult plasma.[10] Yan *et al.*[23] have recently provided evidence that TLR4-mediated, nuclear factor-κB (NF-κB)-dependent transcriptional activation of neonatal monocytes and macrophages was depressed. Similary to others, they found that cord mononuclear cells secreted significantly lower amount of TNF-α compared with adult cells upon stimulation with LPS. Remarkably, newborns and adults had similar numbers of TLR4-positive and CD14-positive cells, and the effect of LPS stimulation on newborn or adult cells was similar for ERK and p38 phosphorylation. However, a markedly decreased expression of MyD88, the TLR-4 adapter protein, was found suggesting an impaired TLR4-mediated signaling in newborn monocytes.

The hyporesponsiveness of neonatal mononuclear cells to LPS, the most potent pathogen-derived activator, and to IFN-γ, the most potent macrophage-activating agent *in vivo* raises the possibility of an overall neonatal defect of stimulus-response coupling in mononuclear phagocytic cells. Such a signaling defect may result in suppression of inflammation and, as a consequence, an impaired anti-microbial response of monocytes and macrophages. The suppression of inflammation is obviously advantageous for survival of the fetus but makes the newborn susceptible to infections.

Conclusions

Neonatal monocytes and macrophages are qualitatively different from adult cells in that they are defective in secretion of a variety of Th1-type cytokines. Innate immune cells, such as monocytes and macro-

phages appear to be dormant in early life. The hyporesponsiveness of neonatal mononuclear phagocytes to both physiologic (IFN-γ) and pathogen-derived (LPS) activation suggests an overall neonatal defect of receptor-mediated signaling and the stimulus-response coupling. The mechanisms of recovery from the intrauterine depression of these cells after birth should be targeted by future research, and may provide the basis for specific immunotherapy of neonatal infections.

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