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Vaccines against Respiratory Viral Pathogens for Use in Neonates: Opportunities and Challenges

Martha A. Alexander-Miller

The first six months of life reflect a time of high susceptibility to severe disease following respiratory virus infection. Although this could be improved significantly by immunization, current vaccines are not approved for use in these very young individuals. This is the result of the combined effects of poor immune responsiveness and safety concerns regarding the use of live attenuated vaccines or potent adjuvants in this population. Vaccines to effectively combat respiratory viral infection ideally would result in robust CD4+ and CD8+ T cell responses, as well as high-affinity Ab. Inclusion of TLR agonists or single-cycle viruses is an attractive approach for provision of signals that can act as potent stimulators of dendritic cell maturation, as well as direct activators of T and/or B cells. In this article, I discuss the challenges associated with generation of a robust immune response in neonates and the potential for adjuvants to overcome these obstacles. *The Journal of Immunology*, 2014, 193: 5363–5369.

Respiratory infections are one of the leading causes of morbidity and mortality throughout the world. Among the most prevalent are infections with respiratory syncytial virus (RSV), rhinovirus, and influenza virus (1). These infections are particularly problematic for infants, resulting in increased morbidity and mortality compared with older children and adults. There are an estimated 11.9 million episodes of severe acute lower respiratory tract infection (ALRI) in young children each year (2). Children under 1 y of age account for 6.4 million instances of severe ALRI and nearly 3 million cases that are grave enough to be considered very severe (2). Further, children <12 mo of age exhibit a 3-fold increase in the rate of fatality following infection compared with children 12–59 mo of age (2). Not surprisingly, the likelihood of severe disease decreases as age increases. For example, in the case of RSV infection, approximately half of children requiring hospitalization are ≤3 mo of age (3), and infants younger than 27 d have the highest incidence of ALRI-associated disease (2). Together, these findings demonstrate the extreme susceptibility of the newborn to disease caused by respiratory pathogens.

The increased disease severity associated with respiratory infection in infants is the result of the naive status of these individuals, as well as the reduced ability of the immune system to respond to infection. Defects in infant immunity span both innate and adaptive components, both of which are critical contributors to immune-mediated clearance of infection (4–6). Reported defects in the innate response include reduced migration, phagocytosis, and bactericidal activity (6, 7). Adaptive immune defects include decreased cytokine production and costimulatory molecule expression by APCs, reduced T cell sensitivity following ligand engagement, decreased T cell repertoire diversity, decreased T cell effector function, a bias toward Th2 development, and impaired B cell differentiation and survival (4–7) (Fig. 1).

Effective control of respiratory virus infection begins with a robust innate antiviral response that is dominated by the production of type I IFN. The production of this critical innate antiviral mediator is diminished in neonates as a result of decreased production on a per-cell basis, as well as a reduction in the number of plasmacytoid dendritic cells (DCs) (3, 8, 9), the cell type specialized for high-level type I IFN production. Beyond type I IFN, the innate response to virus infection that results in the production of cytokines and chemokines that promote inflammation and immune cell recruitment is decreased in infants (10).

Innate immune responses to virus infection are dependent on activation through TLRs, as well as cytoplasmic innate sensors (e.g., RIG-I and MDA-5). Both TLR- and RIG-I–mediated responses are impaired in neonates (3, 9, 11–13). The reduced activity of these innate sensors has implications for the generation of the adaptive immune response because they are important mediators of DC maturation that promotes competence for naive T cell activation. Specifically, DCs from neonates produce low amounts of IL-12 and are impaired in their ability to upregulate costimulatory molecules (e.g., CD80 and CD86) following exposure to virus-derived signals (9). These deficiencies are the first obstacle in the generation of an efficacious adaptive immune response in the neonate.

In addition to the impaired function of DCs, T lymphocytes from neonates exhibit inherent defects in their ability to undergo activation and differentiation (14–16). Reported defects include reduced levels of the signaling molecules lck and...
ZAP-70 (17), as well as a decrease in AP-1–mediated transcription (18). The combined deficiencies in DC maturation and T cell responsiveness are likely contributors to impaired T cell responses observed in vivo following infection or vaccination (19, 20).

Ab responses are also significantly decreased in neonates (4, 21). Ab responses in young infants are largely IgM, with IgG production generally weak for the first year of life (22). Although increased relative to IgG, IgM responses are also impaired, as exemplified by RSV infection of human infants in whom both IgM and IgG responses are poor (23). Similar findings were reported in murine models (24). Isotype analysis showed a skewing toward IgG1, indicating a Th2-biased response (24). Important contributors to the poor Ab response in infants are impaired accessory cells [i.e., T follicular helper cells (25) and follicular DCs (26)], as well as inherent defects in B cell survival and differentiation (27). A potential contributor to the latter is the reduced expression of BCMA and BAFF-R on neonate B cells (28). Both of these receptors bind to BAFF, with BCMA having an additional ligand APRIL (29). Engagement of BAFFR or BCMA on B cells promotes survival through upregulation of antiapoptotic bcl-2 family members, together with downregulation of the proapoptotic factors bim and bad (30). The survival of plasmablasts and differentiation into long-lived Ab-secreting cells in the neonate are likely hampered by decreased levels of APRIL and BCMA (31). In the context of influenza virus infection, the absence of BAFF and APRIL was shown to result in an overall reduction in antiviral IgG (32). Whether there are defects in the initial activation of neonatal B cells is a matter of debate, although there are reports from studies of cord blood cells that suggest competence in this arena (33).

**Infant immune response to vaccination against respiratory viruses**

Although data from the analysis of respiratory virus vaccine responses in very young infants are limited, what are available support the inadequacy of current vaccine approaches for use in this population. These studies predominantly analyzed the Ab response, undoubtedly for practical reasons associated with sampling in this population. Delivery of the trivalent inactivated influenza vaccine in infants between 3 and 5 mo of age results in poor generation of Ab (34, 35). An initial dose of vaccine was not capable of inducing seroconversion for most strains (as defined by a fold-fold increase in Ab) (34). This low responsiveness was not the result of maternal Ab, because all individuals had prevaccination titers <1:8. A second dose resulted in a seroconversion rate of 27–32% for H1N1 strains and 17–93% across H3N2 strains. Not surprisingly, a correlation was observed between age and the rate of conversion, with older infants converting at a higher rate than younger infants (34). In a second study, conversion was assessed following completion of two doses of vaccine, with a reported conversion rate of 42–43% for H1N1 and 39–67% for H3N2 strains (35). For comparison, published studies assessing responses in older children (11–16 y) reported that >90% of individuals experienced a 4-fold increase in titer after a single vaccination (36).

Vaccine responsiveness in young infants also has been evaluated for measles and mumps. This vaccine is routinely given at 12 mo of age. Administration at an earlier time (i.e., 6 or 9 mo of age) resulted in a significantly reduced Ab response (37). A parallel impairment in the T cell response also was observed, with virus-specific cells exhibiting a reduction in the amount of IFN-γ produced in response to stimulation (37). Importantly, as with influenza virus, the reduced responsiveness in these individuals could not be accounted for by the presence of maternal Ab. The reduction in responsiveness in 6- and 9-mo-old infants suggests that the immune system continues to be impaired to some extent throughout the first 9 mo of life. It is difficult to state definitively when the immune system of the infant reaches full maturity. In this regard, four doses of the oral polio vaccine given to infants resulted in reduced IFN-γ–producing T cell responses compared with adults receiving a single dose (38), whereas infants administered bacillus Calmette–Guérin (BCG) vaccine had responses similar to adults (39). These findings suggest that the time at which responses in children can approximate adults varies with the nature/strength of the challenge (27). Although there is often significant impairment in immunity in young infants, the ability to obtain some degree of responsiveness following vaccination and instances in which responsiveness is relatively robust offer hope that the provision of additional stimulatory agents in the context of vaccination may be able to boost the response to levels that are protective in these individuals.
Desirable attributes of immune responses elicited by respiratory virus-specific vaccines and lung-specific challenges

The goal of vaccination is the generation of long-lived protective immunity. In the case of respiratory virus infection, this ideally includes the generation of high-affinity neutralizing Ab, central memory T cells, and lung-resident memory cells. This is a tall order in the context of adult vaccination and is even more challenging in the neonate.

We have an increasing appreciation for the importance of lung-resident memory T cells in the control of virus infection (40–42). The presence of these cells is the result of the combined effects of production in the local lymph node and in BAL. Although the benefit of vaccination strategies that could induce local immune responses in BAL is clear, approaches that could achieve this goal are less so. Lung-targeted delivery of a vaccine is certainly a difficult undertaking in a neonate. A further point of caution is that, although induction of BAL to facilitate immune responses is potentially advantageous, it is unclear how/whether the presence at very early ages of the strong inflammatory signals necessary for the induction of BAL (43) would impact establishment of regulatory processes in the lung. For example, an overly robust inflammatory challenge was shown to induce long-term changes in airway macrophages (i.e., these cells exhibit decreased responsiveness to TLR agonists, reduced phagocytosis, and increased production of IL-10) (44). These changes can be conferred to newly recruited macrophages, thereby maintaining altered function for extended times (44). This is clearly an area in which additional studies are needed to assess the potential for direct targeting of the lung for vaccination in this population.

In standard vaccine-delivery approaches, resulting memory T cells must be recruited to the lung airway following reactivation in the draining lymph node, an event that is a critical component of effective clearance and protection (45). In this regard, analysis of influenza infection of mouse neonates showed that T cells were impaired in their ability to migrate from the interstitium to the airways (46). Thus, efficient trafficking of effector T cells may pose an added obstacle to efficacious responses following infection.

An additional challenge in the lung is the apparent ability of the lung environment to negatively regulate effector cell function. The loss of function in effector cells was reported to occur in the context of a number of infectious processes, including RSV (47, 48), parainfluenza virus 5 (49), and murine pneumovirus (50). Our work in this area suggests that this is an intrinsic property of the lung (51, 52). Functional inactivation is observed even in the face of the inflammatory environment present following infection. That said, negative regulation is less apparent at early times postinfection when viral load and inflammation are high, suggesting that the presence of an inflammatory environment may dampen the inhibitory effect (47, 49). The extent to which this effect occurs in the lungs of neonates has not been explored. However, it seems likely it will be in play or even enhanced given the propensity for negative regulation of the immune response in this population.

Approaches to overcome the reduced responsiveness to vaccination in neonates

Virus infection often results in long-lasting, protective immunity. Arguably, the closest that we have come to achieving this goal in the context of vaccination against viral pathogens is through the use of live attenuated constructs. This approach has resulted in the eradication of smallpox and large reductions in infections with viral pathogens previously associated with childhood disease (e.g., measles, mumps, rubella, and chicken pox). The success of live attenuated vaccines is likely a consequence of elicitation of both robust Ab and CD8+ T cell responses (53–55), a goal that has not yet been realized with inactivated/subunit vaccines. However, although the ability to achieve both humoral and cell-mediated immune responses is highly desirable, the use of live attenuated constructs in neonates is undesirable because of safety concerns, including the potential for undiagnosed immune deficiencies. Consequently, alternative approaches that can elicit both arms of the immune response, combined with a superior safety profile, are sorely needed.

Generation of potent cell-mediated and humoral immune responses requires the participation of multiple cell types: at a minimum, DCs, CD4+ T cells, CD8+ T cells, and B cells. Optimal activation of these populations can be facilitated by mediators that act directly on individual cells (direct), as well as those that modulate function in accessory cells that subsequently provide activating signals to T cells in the form of cell surface molecules or cytokines (indirect). For example, vaccines that target DC maturation will promote T cell activation. However, T and B cells also can receive direct signals via stimulatory cell surface receptors. Targeting T and B cells by the combination of direct and indirect activation signals in the context of vaccination may aid in overcoming defects associated with neonatal immune responsiveness.

Vaccine responsiveness can be significantly improved by inclusion of adjuvants. Approved adjuvants in the United States and/or Europe include aluminum salts, oil-in-water emulsions (MF59, AS03, an AF03), virosomes, and AS04 (monophosphoryl lipid A preparation with aluminum salt) (56). Excellent reviews on the actions of adjuvants were recently published (56, 57). An area of intense focus in adjuvant development is the use of TLR agonists. TLRs are sensors of pathogen associated molecular patterns that survey the environment through residence at both the cell membrane and the endosome. Ten TLRs have been characterized in humans (58). Cell surface TLRs, including TLR1/2 and TLR2/6 heterodimers, together with TLR4, TLR5, and TLR10 homodimers, recognize a variety of pathogen associated molecular patterns associated with bacterial or viral infection. Endosomal TLRs (TLR3, 7, 8, 9) sense pathogen-derived nucleic acids and are key players in the context of virus infection (59). Respiratory viruses contain ligands for multiple TLRs, a portion of which are shared across many viruses and, thus, are attractive for vaccine development. For example, influenza virus and RSV, pathogens of major clinical importance in neonates, activate TLR3 and TLR7 (60, 61). Ligands have been identified for each TLR, with the exception of TLR10. Not surprisingly, work is underway to exploit these ligands in the context of vaccination.

Individual TLRs can be widely distributed on immune cells. For example, TLR7 is expressed by human T cells (62), B cells (63), and plasmacytoid DCs (64), and TLR8 is expressed on monocytes/macrophages and myeloid DCs (65, 66). As such, a TLR7/8 agonist would provide both direct and indirect activation signals for the elicitation of T and B cells following
There is evidence that TLR2 agonists also may be beneficial in neonates. Inclusion of the TLR2 agonist Pam3Cys increased activation of T cells in this population (73). Further, select TLR ligands can induce maturation of APCs, approaching the level observed in adults (12, 76). For example, the TLR8 agonist 3M-002 induces potent upregulation of CD40, CD80, CD83, and CD86, as well as production of the Th1-polarizing cytokine IL-12p70, in cells from neonates (12). A contributor to the effectiveness of TLR8 agonists in the context of neonate-derived cells appears to be the resistance of this pathway to inhibition by adenosine (12), a known inhibitory immune modulator in the blood of newborns (77).

An alternative strategy for increasing the efficacy of these immune modulators is the delivery of multiple TLR agonists. Simultaneous engagement of several TLRs has been shown to change DC maturation qualitatively and quantitatively (78, 79). T cells derived from human cord blood stimulated concomitantly with TLR2 and TLR5 agonists underwent greater proliferation and cytokine production compared with cells stimulated with either agonist alone (73). Although unknown, studies of tuberculosis vaccination may suggest that this approach is useful in the context of newborn vaccination. The tuberculosis vaccine (BCG), which is routinely delivered within 48 h of birth, is one of a limited number of vaccines that has shown success in very young infants. BCG contains ligands for five distinct TLRs (TLR1, TLR2, TLR4, TLR6, and TLR9) (80). It is tempting to speculate that the engagement of multiple TLRs may be a contributing factor to the ability of this vaccine to induce immune responses in these very young infants.

Although TLR agonists hold great promise as adjuvants, other approaches are under active investigation. Given the remarkable success of live attenuated viruses as vaccines, much effort has focused on the production of viral constructs that can achieve similar levels of immune stimulation while obviating the safety concerns associated with replicating virus. One promising area is the generation of single-cycle virus constructs. These constructs enter cells and produce significant amounts of viral RNA, as well as protein (81). This results in effective Ag presentation, as well as DC maturation and inflammatory cytokine production (82). Although not yet tested, it seems likely that a contributor to the efficacy of single-cycle vaccines is the induction of innate immune responses similar to those resulting from virus infection (i.e., activation of endosomal TLRs [TLR3, TLR7, and TLR8] and RLR [e.g., RIG-I or MDA-5]). However, because these constructs are incapable of making infectious virus and, as such, do not result in spread of virus beyond the initially infected cell, they have significantly reduced safety concerns.

Vaccines generated using single-cycle virus constructs showed usefulness in mice and nonhuman primates, eliciting both cell-mediated and Ab responses (82–86). In addition, they show promise in the context of the neonate. Infant mice vaccinated with a single-cycle HSV-1 variant within 24 h of birth had dramatically improved CD4+ and CD8+ T cell responses and were protected from virus challenge (87). In addition, infant rhesus macaques vaccinated with chimeric Venezuelan equine encephalitis/Sindbis virus replicon particles generated neutralizing Ab and were protected from disease following virus exposure, even at 1 y postvaccination (88). Protection from virus challenge at this time is consistent with the generation of long-lasting memory responses in infants, a goal that has proven challenging in the setting of the neonate. Despite their success in experimental models, from a practical standpoint
Experimental challenges to forward movement in the development of vaccines that are effective in neonates

One of the significant challenges associated with a more complete understanding of the defects in the neonatal immune response and the development of vaccines is the limitations associated with current experimental models. Neonatal mice, although extremely tractable, have a highly abbreviated period of infancy, making assessment of responses following prime-boost strategies difficult. A limitation to their use in the development of TLR agonists as adjuvants is the differential distribution and function of these molecules in mice versus humans. For example, in contrast to humans, murine T cells do not respond to TLR5 agonists (89). Further, mice do not have a functional TLR10 (90), and they express TLRs for which functional human equivalents appear to be lacking. The distribution of TLRs among DC subsets and B cells also differs between mice and humans (91, 92).

With regard to T cell differentiation, there is evidence that mice may overestimate the Th2 bias of the neonate. Specifically, the strong Th2 bias apparent in neonatal mice following vaccination with acellular pertussis is not replicated in human infants where a more balanced Th1/Th2 response is observed (93).

Human studies are also challenging as a result of the understandable difficulty in obtaining cells from neonates and restriction to in vitro analyses. Cells derived from cord blood have been used as a surrogate, but responses in these cells may not reflect the newborn past the first few days of birth, because their function is likely modulated by transient changes associated with delivery (e.g., the increase in adenosine) (10). New models (e.g., nonhuman primates) could markedly benefit this area of research because innate sensor distribution and function more closely resemble those of humans. In addition, the more extended period of infancy allows assessment of prime-boost regimens.

Conclusions

Although there is still much to learn with regard to the infant immune system, there is evidence that, under the right circumstances, the neonate can make a strong and effective response to challenge. At its core, the goal of a vaccine is to reproduce the quantity and quality of the immune response that is generated following pathogen clearance. As our understanding of the receptors and pathways involved in pathogen recognition and immune activation continues to expand, we will undoubtedly gain an appreciation for new targets and strategies that can be exploited to generate vaccines capable of providing protection in the highly vulnerable neonate population.

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Disclosures

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References


