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Safety and Efficacy of Neonatal Vaccination

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Abstract

Newborns have an immature immune system that renders them at high risk for infection while simultaneously reducing responses to most vaccines, thereby posing challenges in protecting this vulnerable population. Nevertheless, certain vaccines, such as Bacillus Calmette Guérin (BCG) and Hepatitis B vaccine (HBV), do demonstrate safety and some efficacy at birth, providing proof of principle that certain antigen-adjuvant combinations are able to elicit protective neonatal responses. Moreover, birth is a major point of healthcare contact globally meaning that effective neonatal vaccines achieve high population penetration. Given the potentially significant benefit of vaccinating at birth, availability of a broader range of more effective neonatal vaccines is an unmet medical need and a public health priority. This review focuses on safety and efficacy of neonatal vaccination in humans as well as recent research employing novel approaches to enhance the efficacy of neonatal vaccination.

Keywords

adjuvant; immunization; neonate; newborn; Toll-like receptor

Introduction

Neonates and infants suffer a high frequency and severity of microbial infection resulting in millions of deaths worldwide [1]. The same immune deficiencies that render newborns susceptible to infection also reduce their memory responses to most antigens, thereby potentially frustrating efforts to protect this high-risk population. As birth is the most reliable point of healthcare contact worldwide [1] and effective vaccination at birth would provide early protection for newborns and infants, expanding and improving the available means of neonatal vaccination is a global health priority.

Newborns have impaired immune responses due to a range of deficiencies in both adaptive immunity [2] and innate immunity [3], as well as the potentially suppressive effects of maternally-derived antibodies (MatAb) [4,5]. Newborns exhibit increased activity of suppressive T regulatory cells [6,7] coupled with impairments in functional activity of antigen-presenting cells (APC) [8,9]. Thus study of neonatal vaccination is in part a quest for antigen (Ag)/adjuvant (Aj) combinations that will be efficacious at birth. In addition, neonates and infants have a limited Ab repertoire and may produce suboptimal Ab in response to some Ag [10,11].

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This review summarizes clinical data on the safety and efficacy of human neonatal vaccination, as well as translational studies aimed at developing novel approaches to effective neonatal vaccination. Throughout, our emphasis will be on safety and efficacy of approaches to neonatal vaccination, bearing in mind that basic aspects of neonatal immunity (with a specific focus on dendritic cell cells) are reviewed in an accompanying article by Willems et al [12].

Potential barriers to neonatal immunization

Safety concerns

Concerns that have been raised regarding vaccination of neonates and infants include: i) doubts about efficacy given the limited capacity of neonates to respond to many Ag; and ii) potential effects on immune system polarization, including potential for triggering autoimmunity *via* epitope mimicry or Aj effect [13,14]. From a theoretical perspective, these concerns are in part mitigated by: i) the documented ability of newborns to respond to several vaccines including Bacillus Calmette Guérin (BCG) and hepatitis B vaccine (as outlined below), which serves as proof of concept that neonatal vaccination can be safe and effective and; ii) the presence of extensive immunologic mechanisms for central and peripheral tolerance that eliminates self-reactive T and B cells in newborns, coupled with; iii) evidence that multiple pediatric vaccines, including BCG, are not linked to allergy or autoimmunity [15]. Nevertheless, despite these conceptual reassurances, novel vaccines, as any new drugs, do have the potential of inducing side-effects and must certainly undergo rigorous and on-going safety analysis, including that provided in the U.S. by the Vaccine Adverse Event Reporting System (VAERS), a program of the U.S. Food and Drug Administration (FDA) and the Centers for Disease Control (CDC). Indeed, safety concerns have prompted discontinuation and/or changes in some pediatric vaccines, with two examples discussed below.

In 1998, the measles-mumps-rubella (MMR) vaccine was the subject of controversy in the UK when Andrew Wakefield *et al.* [16] reported on twelve children who developed symptoms of autism spectrum disorder soon after they had received MMR. The interpretation section of this study was later retracted in 2004 by ten of Wakefield's coauthors, and subsequent large studies concluded that there was no evidence of a link between MMR and autism [17]. Early thiomersal exposure was also hypothesized to be associated with neuropsychological deficits in children, although this link was not supported in a study of 1047 children aged 7 to 10 years [18]. Nevertheless, in 1999, the American Academy of Pediatrics and Centers for Disease Control and Prevention requested removal of thiomersal from all pediatric vaccines, and this ethylmercury-containing preservative was no longer used in routine childhood vaccines in the U.S. as of 2001. Although the autism link has been refuted, the need for stringent safety monitoring in the development of all vaccines remains, particularly those that may be given to newborns.

The live attenuated rotavirus vaccine RotaShield (Wyeth-Ayerst) contained three rotavirus reassortants, with different genes encoding specific serotypes (VP4 or VP7) evoking virus-specific Ab, along with genes of Rhesus macaque-passaged rotavirus that attenuated virulence [19]. After approval, 76 cases of intussusception, in which one segment of the bowel enfolds within another segment, causing obstruction, were reported to the VAERS surveillance system. 70% of intussusception cases occurred after the first dose of vaccine. Due to this surveillance, the CDC recommended the suspension of the rotavirus vaccine until further studies could be performed. One study found one case in every 5000 to 9500 vaccinated infants, with the highest risk after the first dose. Due to the possible association with intussusception, Rotashield was withdrawn from the market in 1999.

Inadequate immunogenicity of most vaccines at birth

Immunization in early life is a major public health imperative, but remains a challenging field. The neonatal immunological milieu, skewed towards Th2 immunity to prevent recognition of the developing fetus as an allograft by the maternal immune system [20], represents an important obstacle that vaccination during neonatal period must overcome. In addition to the challenge posed by immaturity of the neonatal leukocyte compartment, effective neonatal vaccines, must also overcome the potential inhibitory effect of MatAb [20]. It is believed that inhibition of adaptive immune responses by MatAb depends on the ratio between MatAb titers and vaccine antigen dose and is due to determinant-specific masking of B cell epitopes [21]. Infant APC uptake and T cell responses appear to be largely unaffected. For example, with respect to the *Haemophilus influenzae* type b (Hib)-conjugate vaccines, MatAb to the tetanus toxoid (TT) carrier protein inhibit infant responses to TT, but do not inhibit Ab responses to the Hib polysaccharide moiety [22]. Thus MatAb result in specific masking of TT but not of Hib antigenic determinants to infant B cells, preserving APC uptake of MatAb:Ag immune complexes, and allowing response to the Hib polysaccharide moiety. Overall, responses of human newborns to vaccines are not predictable from studies of older infants or adults. Nevertheless, several vaccines have been shown to elicit a clinically significant immunogenic response at birth, as reviewed below.

Of note, in assessing the potential efficacy of neonatal vaccines, although the prevention of infection is the ultimate goal and most important end-point, correlates of vaccine-induced immunity must be carefully considered, as recently reviewed by Plotkin [23]. Both quantitative and qualitative (*i.e.*, functional activity) of Ab can serve as “co-correlates” and surrogate markers for protection and are predominantly used in vaccine studies. Nevertheless, cell-mediated immunity is critical in protection against intracellular infections and, through the function of CD4⁺ cells, necessary to enhance B cell development, as illustrated below in the case of BCG.

Early studies with whole cell pertussis vaccine given alone or combined with diphtheria and tetanus vaccines within the first 24 hours of life demonstrated safety, without any signs of erythema, infiltration, fever, irritability, vomiting or anorexia [24]. However, pertussis immunization at birth resulted in serologically inadequate responses and blunting of booster responses to pertussis in 75% of study subjects until 5 months of age, suggestive of antigen-specific “immunologic paralysis” or tolerance induced by the immunization. This failure was believed to be independent of any effects of MatAb, as these were low or undetectable. In contrast, immunization at 3 weeks of age resulted in adequate serologic response [24].

Purified polysaccharide vaccine (PRP), the first vaccine licensed to prevent Hib disease, was neither immunogenic in neonates nor consistently immunogenic in children older than 18 months [25]. In contrast, the current Hib conjugate vaccine, diphtheria CRM 197 protein conjugate (HbOC) is given as a series of three injections starting at two months of age. Lieberman *et al.* [25] attempted to enhance antibody response to HbOC by administering the diphtheria-tetanus (DT) vaccine at birth, only to find that at 7 months, children exposed to DT at birth had a lower antibody response than those immunized beginning at 2 months of age. Impairment in neonatal Th1 helper T cell response compared to adults may contribute to reduced neonatal responses to some vaccines. For example, after oral polio vaccination (OPV), young infants produce a relatively weak IFN- γ and cell-mediated response compared to adults, although they produce high titers of neutralizing antibodies [26], thought to be essential for protective immunity against poliovirus [27].

In general, neonates mount impaired responses to T-independent polysaccharide antigens, and their antibody responses to T-dependent protein antigens are short-lived [5]. Accordingly, the 23-valent *Streptococcus pneumoniae* polysaccharide vaccine (PPV23) is

not immunogenic in children younger than 2 years [28]. Although the pneumococcal protein-polysaccharide conjugate vaccine is safe and effective when administered to infants as a four dose series (2, 4, 6, and ≥ 12 months), its efficacy at birth is unknown and currently under investigation [29].

Vaccines currently given at birth

Although newborns generally mount weaker responses than older persons to a wide range of vaccines, some vaccines do have a measure of efficacy when given at birth.

Bacillus Calmette-Guérin

The Bacillus Calmette-Guérin (BCG) vaccine is a live attenuated *Mycobacterium bovis* vaccine administered within the first few days of life in most countries to prevent childhood tuberculous meningitis and miliary disease. With more than 3 billion people having received it, it is the most widely used vaccine worldwide [30]. It is not generally recommended for use in the United States due to a relatively low prevalence of tuberculosis and the variable effectiveness of immunization against adult pulmonary tuberculosis.

In general, the BCG vaccine exhibits an excellent safety profile. The main adverse events to vaccination are local reactions, including scarring (up to 92% of healthy neonates), pustule formation and drainage [31]. These usually respond to conservative management. Axillary and cervical lymphadenopathy are the most common regional adverse effects, and may persist for a few months, occasionally resulting in surgical drainage [32]. Disseminated BCG infection is a rare complication, occurring in less than one per million individuals. It has been reported in children with congenital immune disorders, such as severe combined immunodeficiency, chronic granulomatous disease, and the acquired immunodeficiency syndrome. About half of the cases of disseminated BCG infection in children are linked to rare immunodeficiencies of the IFN- γ and IL-12 pathways [33], including a report of fatal BCG infection in an infant with IFN- γ -receptor deficiency [34]. A relatively high incidence of osteitis, osteomyelitis and disseminated BCG infection was noted upon use of the Danish 1331 BCG vaccine strain manufactured by Statens Serum Institut, involving several hundreds of children in Finland between 2000 and 2006. Retrospective analysis suggested that the increased reporting rate, although within the expected frequency of adverse reactions expected for the product, might be due to a combination of factors including heightened awareness surrounding use of the newly available BCG Vaccine SSI following publicity associated with the withdrawal of the previously used product, the relatively higher potency/reactogenicity of the Danish 1331 strain, and administration errors (incorrect dose or route of administration) [35,36]. The low and declining rate of tuberculosis in Finland, prompted a change in vaccination policy in Finland from universal to risk-group targeting [37].

Studies of the efficacy of BCG vaccine have provided widely-varying results. Efficacy has ranged from 0 to 80% in case control studies using different BCG strains [32]. This variability has been attributed to disparate exposure to environmental mycobacteria among study populations, strain variation in BCG preparations, genetic or nutritional differences, and other environmental factors such as sunlight exposure and poor cold-chain maintenance [38]. In a meta-analysis by Rodrigues *et al.*, a 75 to 86% protective effect was noted against miliary and meningial tuberculosis [39]. In measuring vaccine efficacy with relative risk or odds ratio for tuberculosis in vaccinated *versus* unvaccinated infants, the protective effect was 0.74 when estimated from four randomized controlled trials, and 0.52 when estimated from nine case-control studies [40]. In a meta-analysis of the effect of BCG vaccination on childhood tuberculosis meningitis and miliary tuberculosis worldwide, Bourdin Trunz *et al.* estimated that the 100.5 million BCG vaccine doses given to neonates in 2002 prevented

~30,000 cases of tuberculous meningitis and ~11,500 cases of miliary disease during the first five years of life [41]. The greatest beneficial BCG immunization was noted in regions where both the risk of tuberculosis and rates of vaccine coverage were highest, including Southeast Asia, sub-Saharan Africa, and the western Pacific. Of note, the efficacy of neonatal BCG administration has been linked to its ability to effectively induce a Th1-polarized neonatal immune response [42]. Of note, BCG also effects the immune response to unrelated Ag in early life, boosting both Th1- and Th2-type responses to other Ag (*e.g.* HBV and oral polio vaccines), probably through its influence on DC maturation [43]. At the current cost of US\$2–3 per dose, the global cost of BCG vaccination is approximately US \$206 per year of healthy life gained.

Research directed at developing even more effective vaccines against tuberculosis continues, using two vaccination strategies. One strategy involves BCG priming at birth and introduces a booster dose to prolong immunity and protect the adult population. Heterologous boosting is also an option, employing one of the novel, more potent tuberculosis vaccines to replace BCG [44]. Novel TB vaccines include live recombinant BCG vaccines, such as rBCG30 that expresses high amounts of *M. tuberculosis* major secretory protein [45], modified vaccinia Ankara virus vaccine expressing protective AG 85A (MVA-85A), as well as adjuvant subunit vaccines, such as H1/IC31 given by parenteral delivery, and H1/LTK63 for mucosal delivery [46]. The DNA vaccine containing the hsp65 protein (see below) is a promising candidate both as a replacement for BCG and as a booster dose.

Hepatitis B vaccine

With over 2 billion individuals having serological evidence of hepatitis B (HBV) infection worldwide and suboptimal treatment provided by current antiviral therapy, primary prevention through immunization remains the most effective way of controlling the spread of HBV [47].

Safe and effective vaccines against HBV infection have been available since 1982. Three classes of vaccine are available, produced in plasma, yeast, or mammalian cells. The vaccine prepared by concentrating and purifying plasma from hepatitis B surface antigen (HBsAg) carriers to produce subviral particles, although highly efficient and safe, is no longer used in most developed countries because of concerns for potential transmission of blood-borne infections. Yeast-derived recombinant HBV vaccines are produced by cloning the HBV S gene in yeast cells, and contain thiomersal as a preservative. Mammalian cell-derived recombinant vaccine, in addition to the S antigen, contain either antigens from the pre-S2 region or both the pre-S1 and pre-S2 regions that assemble into a virus-like particle and produce an enhanced immunologic response [48]. In 1991, the United States Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) recommended HBV vaccination for all infants, regardless of the HBsAg status of the mother [49]. HBV vaccine is usually given as three intramuscular doses over a 6-month period, with the first dose given at birth. This vaccination schedule decreased the burden of HBV disease in the U.S., a protective effect also noted in many other countries. These guidelines were updated in 2005 to recommend implementation of universal vaccination of neonates before discharge from the hospital [50].

Adverse events to HBV vaccine are mild and most commonly include pain at the injection site (3–29%), mild fever > 37.7°C (1–6%), malaise, headache, joint pain and myalgia. These effects were reported no more frequently among children receiving both HBV vaccine and diphtheria/tetanus/whole cell pertussis (DTP) vaccine than among children receiving the DTP vaccine alone. More serious adverse reactions have been described in the literature [49], but the strength of these associations remains unclear. The estimated incidence of anaphylaxis following HBV vaccination among children and adolescents is one case per 1.1 million

vaccine doses [50]. Although a retrospective case-control study suggested an association with multiple sclerosis in adults, and routine school-based vaccination was suspended in France in 1998, multiple sclerosis was not reported after immunization with HBV vaccine among children [50]. Likewise, a possible association with Guillain-Barré syndrome that was proposed in adult recipients of the plasma-derived HBV vaccine was not confirmed [50].

Efficacy of the HBV vaccine is measured by its ability to induce hepatitis B surface antibody (Hbs Ab) at a titer of >10 IU/L. In healthy infants, one dose provides ~30–50% protection, two doses 50–75% protection, and three doses $>90\%$ protection against HBV infection, thereby eliminating the need for booster doses [48]. A remarkable degree of protection had been demonstrated in the 1980's, although this effect was not as extensive as that obtained when the vaccine was used in conjunction with passive immunization with multiple injections of hepatitis B immune globulin. Immunization was estimated to reduce the carrier state of infants born to HBsAg-positive carrier mothers by ~90% [51,52]. Chang *et al.* have shown that universal vaccination in Taiwan was associated with $>50\%$ decline in the incidence of hepatocellular carcinoma in children [53].

Oral polio vaccine

Halsey *et al.* studied the efficacy of trivalent oral polio vaccine (TOPV) and DTP administered to human neonates [54]. The authors noted that although MatAb may modify or block the serum immune response during the first few weeks of life, the first or priming dose of DTP could be given effectively by 4 weeks of age. TOPV administered to infants during the first week of life resulted in intestinal infections and local immune responses in 50–100% of infants and induction of serum Ab in 30–70% of infants. By 4–8 weeks of age, TOPV administration induced serum Ab response matching that induced in older infants. Although the WHO Program on Immunization recommended initiating DTP and TOPV schedules at 6 weeks of age, the authors suggested considering administration of the first dose of TOPV at birth (or as close to birth as possible), for countries where poliomyelitis has not yet been controlled.

Pertussis vaccine

The severity of pertussis among young infants and the immunogenicity in newborn mice of acellular pertussis (aP), as opposed to the tolerogenicity of whole cell pertussis vaccine in human newborns [24], has prompted investigation of aP in human newborns. Knuf and co-workers [55] compared aluminium-adsorbed acellular pertussis vaccine (aP; containing pertussis toxoid, filamentous hemagglutinin, and pertactin) or HBV given intramuscularly at 2–5 days of age followed by DTaP-HBV-IPV/Hib at 2, 4, and 6 months. They [55] demonstrated that neonatal aP vaccination was safe (no significant differences in reactogenicity between groups), induced higher Ab responses to Pertussis Ag by 3 months (*i.e.*, did not induce immunologic tolerance), and resulted in earlier Ab responses to DTaP, but did dampen Ab response to Hib and HBV vaccines. The authors speculate that the dampening of responses to Hib and HBV was due to strong secondary T lymphocyte-specific pertussis responses after the first dose of DTaP-IPV/HBV/Hib potentially interfering with CD4⁺ T cell help, a phenomenon known as “bystander interference”. Given that the risk of death due to pertussis infection is diminished by the first infant dose of aP given at the currently standard time-point of 2 months of age [56], the authors speculate that a birth dose would further reduce the risks of pertussis-related deaths during the current early window of vulnerability.

Vaccines given in infancy

Vaccines given early in life, during infancy but after the neonatal phase, include Rotavirus, diphtheria-tetanus-acellular pertusis (DTaP; at 2, 4, 6, and 15–18 months), Hib (2, 4, 6, 12–15 months), pneumococcal conjugate vaccine (PCV; 2, 4, 6, 12–15 months), inactivated poliovirus vaccine (IPV; 2, 4, 6–18 months), influenza (yearly from 6 months to 18 years), MMR vaccine (12 months), Varicella (12 months), and Hepatitis A (12–18 months) [57]. Although a complete discussion of the safety and efficacy of all infant vaccines is beyond the scope of this review, rotavirus vaccine will be discussed as illustrative of safety and efficacy studies in vaccinating the very young.

Rotavirus vaccine is the most recent addition to the panel of immunizations in early life and has been recently reviewed by the World Health Organization (WHO) Weekly Epidemiological Record [58] as well as by Dennehy [19]. Protection against rotavirus infection is of major clinical interest, as it is the leading cause of severe diarrhea in children less than 5 years globally, with over 25 million outpatient visits and over 2 million hospitalizations yearly. Licensed in 2006, two live attenuated oral rotavirus vaccines, monovalent human rotavirus vaccine Rotarix® and the pentavalent bovine-human vaccine RotaTeq, replaced their counterpart RotaShield® which was withdrawn from the market in 1999 because of a possible association with intussusception. The two new vaccines have a similar safety and efficacy profile but a different immunization schedule: Rotarix is administered in a 2-dose schedule between 6 and 12 weeks (at least 4 weeks apart) and RotaTeq as 3 doses at 2, 4 and 6 months (first dose between 6–12 weeks and subsequent doses at 4–10 weeks intervals, with the first dose given no later than 12 weeks and the third dose given before the age of 32 weeks). The first dose of these vaccines should not be given to infants older than 12 weeks, as the safety has not been established, and this confers a potentially higher risk of intussusception. According to the Global Advisory Committee on Vaccine Safety and their data on post-licensure surveillance until June 2007, the use of these vaccines was not associated with an increased risk of intussusception or other serious adverse events [58]. Rare complications included mild and transient symptoms from the respiratory or gastrointestinal tract. The vaccines are contraindicated in infants with a history of intussusception or anatomical malformations possibly predisposing for intussusception. Of note, neither of these vaccines contains thiomersal.

Both vaccines provide 74–85% protection against rotavirus diarrhea of any severity and ~90–100% protection against severe rotavirus disease that extends to the second year of follow-up. Both vaccine dose and host factors (*e.g.* MatAb, interfering bacterial and viral agents, and malnutrition) are believed to determine extent of the immune response. Although optimal surrogate markers for vaccine efficacy have yet to be clearly defined, intestinal virus-specific IgA has correlated with protection and serum IgA responses to the VP4 and VP7 surface structural proteins have been used as end points, though cell-mediated immunity is believed to contribute to anti-rotaviral defense as well [59]. As clinical efficacy has thus far been demonstrated mainly in the U.S., Europe and Latin America, the WHO has not yet recommended global inclusion of rotavirus vaccines into national immunization programs until its potential is confirmed in all regions of the world.

Clinical studies of novel early life vaccines

Malaria is a leading global health problem against which no effective vaccine has yet been introduced in clinical practice. The RTS,S/AS02D candidate malaria vaccine was found to be safe, well tolerated, and immunogenic in infants up to 18 weeks old living in a highly endemic area of Mozambique [60]. It is a hybrid recombinant protein consisting of tandem repeats from a *Plasmodium falciparum* protein and the S antigen of HBV, formulated with the adjuvant system AS02 (a mixture of the Toll-like receptor (TLR) agonist

monophosphoryl lipid A (the active moiety of lipopolysaccharide/endotoxin) and the detergent saponin QS21) [60,61]. Candidate HIV vaccines capable of generating robust immunologic responses in breastfeeding infants are also being developed [62]. Other novel early life vaccines current being studied include vaccines against *Salmonella typhi*, RSV, influenza, and parainfluenza. Additional studies are assessing co-administration at birth of hepatitis B vaccine in combination with hepatitis A or BCG, that may modify responses to other vaccines [43].

Need for novel approaches to enhance neonatal vaccination

The ability of certain vaccines such as BCG and HBV vaccine to exhibit some efficacy at birth provides proof of concept that despite generally impaired APC function and Th1 responses, neonatal vaccination is possible. The medical advantages inherent to neonatal vaccines effective at birth include: i) early protection that would close the window of vulnerability inherent to vaccination schedules that start later in life (*e.g.* 2 months), ii) the practicality of birth being a global point of contact with healthcare systems, and ii) potential advantages of novel vaccines that may require fewer doses to achieve efficacy. In this context, we review recent approaches to the development of neonatal animal models and recent *in vitro* work with human neonatal cells.

Animal models of neonatal vaccination

Applicability of neonatal animal vaccination models to humans

In assessing the relevance of animal studies to humans, it is important to recognize that mammalian species vary in the type of placentation and relative placental and colostral transfer of immunoglobulins (Ig) to the fetus/newborn [63]. For example, pigs, horses and ruminants have either epitheliochorial or syndesmochorial placentation and no placental Ig transfer, relying very heavily on colostral transfer [64]. In contrast, rodents and primates have haemendothelial and haemochorial placentation, respectively and both rely heavily on placental transfer with lesser colostral transfer. In general, species that allow early (placental) transfer of maternal Ig (*e.g.* mice and humans), demonstrate a slower rate of immune maturation. Of interest, B cell and antibody repertoire-development in rabbits requires gut-associated lymphoid tissues [65].

Another aspect to consider in interpreting animal models is the relatively high divergence of the innate immune system. For example, the innate immune system of mice is particularly divergent from that of humans [66]. Thus, although murine models are absolutely critical for immunologic research and provide powerful insights, results in mice do not always translate directly to humans.

Finally, the timing of vaccine administration is also an important and at times controversial aspect of neonatal animal vaccination models. In particular, multiple studies have focused on mice that are 1 week of age to model neonatal responses [2]. However, given the importance of developing vaccines active on the first day of life, and growing evidence that of distinct perinatal physiology at birth, including high levels of immunosuppressive adenosine at birth [67], it will be important to also study vaccination of animals in the first day of life.

Safety and efficacy of neonatal vaccination in animal models

Multiple studies have documented that certain vaccines are apparently safe and effective when administered *in utero* or to newborn animals. Although serious side-effects due to vaccination of neonatal animals are generally rare, passive surveillance in the U.K. of dog vaccinations has demonstrated a relatively high prevalence of vaccine-associated adverse

effects in very young animals [68]. The most common adverse event appears to be facial edema and pruritis, believed due to immediate (type I) hypersensitivity reaction triggered by degranulation of mast cells sensitized by maternal IgE. These potential adverse effects may be secondary to high bovine serum albumin content in canine vaccines and are most prevalent in small breed dogs, suggesting that dose reduction may be in order. Alum- or lipid-adjuvanted vaccines induce greater tissue inflammation than non-adjuvanted vaccines after subcutaneous administration in 14–16 week old kittens [69].

Examples of efficacy of neonatal vaccination in animal models include avian studies of the live herpes virus of turkeys (HVT) vaccine, aimed at preventing the α -herpesvirus neoplastic Marek's disease of chickens, demonstrate protection even when administered in ovo or at day 1 [70]. Beagle puppies have been vaccinated sub-cutaneously with modified live canine parvovirus at 1 day of age [63]. Both kinetics and magnitude of Ab response were similar to those of older puppies. In this model, vaccination after colostral ingestion or of puppies of convalescent dams with high anti-canine parvovirus titers was unsuccessful, illustrating the potential inhibitory role of maternal antibody. However, under certain circumstances the hurdle of maternal Ab can be overcome. Puppies born to dams boosted during pregnancy with killed adjuvanted rabies vaccine and whom received colostral immunity, nevertheless mounted protective Ab responses after immunization with RABISIN vaccine comprised of rabies virus glycoproteins and aluminum hydroxide adjuvant [63]. The authors speculate that either greater antigenic content and/or vector properties may allow more efficient Ag presentation. Thus under certain conditions murine and human neonates can mount effective *adaptive immune* responses. Although these studies do not define the mechanisms by which the vaccine studied overcame impairments in neonatal immunity, they do illustrate the possibility of effective vaccination at birth.

Novel approaches to enhancing efficacy of neonatal vaccines

Multiple novel approaches are being explored in an effort to overcome deficiencies in neonatal immune responses and thereby allow effective neonatal vaccination [5]. We provide examples of such approaches below, selecting recent examples from the published literature.

Intracytoplasmic delivery of antigens

Several murine studies suggest that a key requirement for induction of effective neonatal adaptive response is entrance of Ag into the cytoplasm of APC. Chen and co-workers studied adult and neonatal (1 week old) BALB/c mice immunized i.p. with inactivated split-product influenza vaccine followed by a booster dose after 3 weeks or with intramuscular injection and *in vivo* electroporation of plasmid DNA [71]. Vaccination of neonates with hemagglutinin or neuraminidase DNA protected mice against influenza infection in the presence of MatAb. The authors concluded that in order to overcome potential inhibition of adaptive immune responses by MatAb, mothers and their offspring should be immunized with different influenza vaccines targeting distinct Ag (*e.g.* inactivated vaccine *versus* DNA vaccine; or use of DNA vaccines targeting different influenza products). If the same Ag is to be used, a study by Pertmer suggests that maternal antibodies do not blunt DNA vaccine-based responses to intracellularly expressed Ag [72].

Study of neonatal C57BL/6 and BALB/c mice immunized (i.p. and s.c.) within 24 hours of birth with disabled infectious single cycle HSV-1 variant reveals that a single round of viral replication dramatically enhances protective responses [73]. CD4⁺ and CD8⁺ T cells from neonatally vaccinated mice transferred to naïve recipients conferred protection against lethal viral challenge. UV-inactivated viral particles at up to 10⁴-fold higher doses were not able to

achieve this response, suggesting that cytoplasmic delivery of Ag can enhance neonatal immune responses.

Kollman and co-workers have demonstrated a novel approach to neonatal vaccination, employing an attenuated strain of the intracellular pathogenic bacterium *Listeria monocytogenes* to deliver Ag to the cytoplasm of APC [74]. Importantly, this approach appeared to be safe in neonatal mice in that they survived high-dose infection with the Δ actA strain of *L. monocytogenes* without any sign of disease nor any recoverable bacteria in spleen or liver 7 days post-vaccination. Neonatal mice vaccinated a single time with attenuated *L. monocytogenes* strain Δ actA mounted strong CD8⁺ and CD4⁺ T cell responses and were protected against subsequent challenge with wild type *L. monocytogenes*. Moreover, Δ actA served as an effective vehicle for delivery of heterologous Ag resulting in a strong CD8 and CD4 Th1-type memory response, suggesting that this strain may serve as an effective vaccine vehicle for neonatal immunization. Of note, recombinant attenuated strains of *L. monocytogenes* induce specific immunity even in the presence of preexisting immunity, potentially overcoming the hurdle of preexisting maternal immunity that might interfere with neonatal vaccine responses [75].

DNA vaccines

Pelizon and co-workers vaccinated 5 day old neonatal BALB/c mice by the intramuscular route with a cytomegalovirus intron-based plasmid containing an inserted fragment encoding the *Mycobacterium leprae* heat shock protein 65 (hsp65) [76]. pVAXhsp65 was transcribed at 2 to 7 days in the muscle tissue of newborn mice. 15 days after the last of a 3 series dose (5, 12 and 19 days of age), an increased ConA-induced splenic production of Th2-polarizing cytokines (IL-4 and IL-5) and inconsistent increases in anti-hsp65 IgG1 and IgG2a serum levels were noted. pVAXhsp65 appeared to be safe, in that Southern blot analysis did not reveal an evidence of integration in a range of organs, including spleen, liver, thymus, and regional lymph nodes. Moreover, similarly to BCG, pVAXhsp65 when given as a single dose, was able to prime 5 day old mice for a mixed Th1 and Th2 immune response to pVAXhsp65 boosting later during adulthood.

DNA-based vaccines have also shown promise in the effort to protect newborns against malaria. Neonatal BALB/c mice (7d) were immunized with a *Plasmodium yoelii* circumsporozoite protein (PyCSP) DNA vaccine mixed with a plasmid expressing murine granulocyte macrophage-colony stimulating factor then boosted at 28 d with pox virus expressing PyCSP [77]. Immunized neonates, including those born to immune mothers, were noted to mount CD8⁺ T cell-mediated protection similarly to adults.

A measles virus (MV) DNA vaccine consisting of measles H, F, and N genes was administered *via* the intradermal route with an IL-2 adjuvant to neonatal Rhesus macaques (4–5 d) that had received passive immunization with measles immunoglobulin (to mimic the presence of MatAb) [78]. All macaques were boosted with the same regimen at 2 months after vaccination. Although it did not enhance MV-induced Ab responses, MV DNA vaccine did prime MV-specific T cell responses as measured by MV-induced IFN- γ production by PBMC. Moreover, MV vaccine protected infant Rhesus macaques from subsequent MV challenge-induced rash and immunosuppression. Overall, DNA-based immunization represents a viable option in developing novel neonatal vaccines.

Intranasal administration of live attenuated vaccines

Mucosally delivered live attenuated *Salmonella enterica* vector vaccines have been studied as a platform to deliver the model antigen tetanus toxin fragment C in neonatal mice immunized by the intranasal route at days 7 and 22 of life [79]. *Salmonella* live vectors

colonized and persisted primarily in nasal tissue and induced high (adult level) titers of Frag C-specific antibodies, mucosal and systemic IgA- and IgG-secreting cells, T cell proliferative responses, and IFN- γ secretion. One week after the boost, a long-term mixed Th1- and Th2-type response to Frag C was established. Such effects were evident even in the presence of high levels of maternal antibodies.

Mielcarek and co-workers have developed a live attenuated strain of *Bordetella pertussis*, the causative agent of whooping cough [80]. Attenuated by deletion of genes encoding tracheal cytotoxin, pertussis toxin, and dermo-necrotic toxin, the strain BPZE1 was given to infant (3 week old) and adult Balb/C mice as a single intranasal dose. This attenuated intranasal vaccine induced stronger neonatal anti-Bordetella IgG responses than the acellular pertussis vaccine, demonstrating sterilizing immunity to subsequent intranasal challenge with *B. pertussis* and *B. parapertussis*. BPZE1 induced a reduced Th2-polarized response as measured by anti-filamentous hemagglutinin IgG1/IgG21 ratio. The authors speculate that BPZE1 could also represent a platform for delivery of heterologous antigens. This study focused on a 3 week old infant mouse model, and no data were provided about potential efficacy of this attenuated strain in newborn mice. However, a different study of intranasal administration of live *B. bronchiseptica* to 2 day-old neonatal piglets demonstrated efficacy against subsequent atrophic rhinitis challenge [81], suggesting that live attenuated Bordetella strains may induce effective immunity in newborns upon intranasal administration.

Recent studies have explored intranasal administration of *E. coli*-expressed rotavirus VP6 protein and the adjuvant *E. coli* labile toxin (LT-R192G) to neonatal (7 days old) and adult mice, and protection against fecal rotavirus shedding following challenge with the murine rotavirus strain EDIM [82]. In contrast to adult mice that developed both CD8⁺ T cell responses (rotavirus-inducible, Th1-cytokine producing spleenocytes) and Ab within 10 days, neonatal mice did not show protection until 28 days, at which point they possessed memory rotavirus-specific T cells, but did not produce anti-rotavirus Ab. These studies highlight the potential of intranasal immunization of newborns with live vaccines.

Novel adjuvants

Impaired responses of neonatal APC to many stimuli is a key hurdle to overcome in developing effective neonatal vaccines [83]. One approach to overcoming deficits in neonatal APC is to exogenously administer co-stimulatory signals whose endogenous production is deficient, such as the neonatal impairment of IL-12 production [84]. Co-administration of IL-12 and influenza subunit vaccine within 24 hours of birth elevated splenic expression of IFN- γ , IL-10, and IL-15 mRNA and the protective efficacy of antiviral vaccination [85]. In addition, IL-12 co-administration also increased IFN- γ -, IL-2-, and IL-4-secreting cells, and IgG2a Ab levels and enhanced survival in a B-cell dependent fashion after adult lethal challenge with infectious influenza virus.

Discovery of novel innate immune pathways and agonists that engage them has opened the door to assessment of novel vaccine Aj. Activation of TLR, transmembrane proteins that mediate recognition of microbial products, activates APC, including enhancement of DC maturation. Therefore, TLR agonists represent potential vaccine Aj, several of which (*e.g.* lipid A that signals through TLR4) are currently in clinical use [86,87]. The synthetic dsRNA polyriboinosinic:polyribocytidylic acid is a TLR3 agonist that induces type I/II IFN production and enhances primary anti-tetanus toxoid (TT) immune response of neonatal mice, increasing production of anti-TT IgG1, IgG2a and IgG2b isotypes [88]. Enhancement of the secondary anti-TT IgG response was noted when polyriboinosinic:polyribocytidylic acid was combined with retinoic acid/Vitamin A, a combined immunological/nutritional intervention that represented an effective vaccine Aj in neonatal mice. CpG oligonucleotides that activate TLR9 have also been shown to enhance neonatal Th1 responses in neonatal

murine models [89], although they appear to induce relatively weak responses in human newborn cord blood plasmacytoid DC tested *in vitro* [90].

With respect to *in vitro* studies of human neonatal APC, TLR8 agonists, including certain synthetic imidazoquinolines and single stranded viral RNA, are particularly effective at activating human neonatal APC *in vitro*, correlating with strong activation of the p38 MAP kinase and NF- κ B signaling pathways [91,92]. TLR8 (and TLR7/8) agonists were remarkably more effective in inducing production of TNF and IL-12 p40/70 as well as enhancing up-regulation of the co-stimulatory molecule CD40. In addition to their ability to effectively activate APC, TLR8 agonists may also contribute to enhancing neonatal adaptive immune responses by their ability to reverse the inhibitory effects of T regulatory cells that suppress adaptive immune responses [93], and that are particularly potent and abundant at birth [6,7]. Importantly, TLR7/8 agonist R-848 is an effective vaccine Aj when covalently linked to HIV Gag protein in a Rhesus macaque model *in vivo* [94], suggesting that these promising Aj merit further study, including assessment in neonatal animal models wherein transient and selective local amplification of APC and Th1-function including reversal of Treg function might safely and effectively enhance local neonatal adaptive immune responses to vaccines without effecting overall central and peripheral tolerance [13], nor the systemic skewing of responses against Th1 [2,3]. There are thus theoretical grounds, which coupled with emerging evidence of the apparent safety of this approach in adult non-human primates (Rhesus macaques) *in vivo* [94,95] and the efficacy of these agonists towards human neonatal APC *in vitro* [91] suggest that such an approach might be both safe and efficacious [92]. Nevertheless, as with all novel drug development, all novel neonatal vaccines will need to undergo rigorous safety evaluation, to ensure that doses and routes of administration avoid any harmful side-effects, including potentially over-exuberant inflammatory responses/reactogenicity [96] or risk of autoimmunity [14].

Concluding remarks

Worldwide, infectious diseases cause death of > 2 million newborns and infants less than 6 months of age. Significant reduction of this burden will require development of early life vaccination, including vaccines effective when given at birth, the most reliable point of global healthcare contact. Based on animal and human studies, neonatal vaccination is feasible, but requires strong immune signals such as those provided by *in vivo* replication of attenuated agents, and perhaps by certain adjuvants. Advances in manipulating attenuated microbial strains and recent characterization of innate immune recognition pathways provide opportunities for developing novel delivery systems and/or adjuvants to meet this crucial challenge. Safety considerations will be paramount, but the large burden of early life infections coupled with the practicality of immunizing at birth provide strong motivation to pursue effective neonatal vaccines.

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Abbreviations

Ab	antibody
Ag	antigen
Aj	adjuvant
aP	acellular pertussis

DTP	diphtheria/ tetanus/whole cell pertussis vaccine
DTaP	diphtheria-tetanus-acellular pertusis
HBV	hepatitis B vaccine
Hib	<i>Haemophilus influenzae</i> type b
IPV	inactivated poliovirus vaccine
MatAb	maternally-driven antibody
MMR	measles-mumps-rubella
OPV	oral polio vaccine
TOPV	trivalent OPV
TT	tetanus toxoid

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