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Review

Influenza and respiratory syncytial virus (RSV) vaccines for infants: Safety, immunogenicity, and efficacy

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ABSTRACT

Respiratory viral infections in infants and young children frequently cause illness that can easily progress to hospitalization and death. There are currently no licensed vaccines to prevent respiratory viral disease in children younger than 6 months, reflecting safety concerns and the difficulty in inducing effective immune responses in infants. This review discusses vaccines that have been developed, or are currently being developed, against influenza and respiratory syncytial virus, with a focus on studies performed to demonstrate their safety and efficacy, and the impact of immunologic immaturity and maternal antibodies on the infant response to vaccines.

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1. Introduction

Acute lower respiratory infections are the most common cause of infant mortality worldwide. Respiratory syncytial virus (RSV) is frequently the infecting agent responsible for infant hospitalization, although other viruses such as influenza, parainfluenzaviruses types 1, 2 and 3, human metapneumovirus, adenovirus, coronavirus, and rhinoviruses are also responsible for upper or lower respiratory tract illness and death [1]. In addition, new viruses such as human bocavirus and polyomaviruses are being recognized as causing respiratory disease in infants and young children [2]. While there are currently no vaccines to protect children against most of these pathogens, licensed influenza vaccines are available for children >6 months of age, and progress has been made in developing vaccines to protect the young against RSV. This review discusses factors that need to be considered for the further development of safe and effective vaccines against influenza and RSV for infants.

2. Incidence of influenza and respiratory syncytial virus infections in children, and vaccines currently available to protect against these respiratory viruses

The incidence of RSV and influenza infection in young children varies substantially from year to year, with mortality due to these infections occurring predominantly in the developing world [1]. Infants infected with respiratory viruses are more likely to have severe disease than older children, largely because virus replication is not controlled by immature immune system, and because destruction of lung epithelial cells and the inflammatory response are more likely to have a significant impact on the fine architecture and function of the infant lung. Seroprevalence studies indicate that most children have experienced at least one influenza infection by 6 years of age whilst virtually all have been infected with RSV prior to their second or third birthday [3,4]. While most respiratory virus infections are acute illnesses that resolve without complications, in 2008, an estimated 28,000–111,500 children younger than 5 years died worldwide as a result of influenza-associated acute lower respiratory

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Abbreviations: ALRI, acute lower respiratory infection; BCG, bacille Calmette-Guerin; FI, formalin-inactivated; F, (RSV) fusion protein; H and HA, hemagglutinin; HI, hemagglutination inhibition; N and NA, neuraminidase; LAIV, live attenuated influenza vaccine; TIV, trivalent inactivated influenza vaccine; RSV, respiratory syncytial virus; PRISM, post-licensure rapid immunization safety monitoring; VSDL, Vaccine Safety Datalink; VAERS, Vaccine Adverse Event Reporting System.

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virus infection (ALRI) [5]. Mortality is similarly high for RSV-associated ALRI with approximately 66,000–199,000 deaths worldwide estimated for the year 2005 for children in this age group [6]. Hospitalization rates for RSV or influenza virus infection are highest among infants less than one year of age; in the United States (US), RSV infection accounts for 75,000–125,000 hospitalizations per year among children <1 year of age (peak age between 2 and 5 months) with as many as 1.5 million clinic visits [3,7,8]. Hospitalization rates for infants in the US with influenza virus infection are estimated to be on the order of 180,000 per year for infants <6 months of age with clinic visits approximately 10-fold higher [9–12]. In addition to the burden of disease caused by infections during infancy, children also serve as important vectors, transmitting these viruses to adults and the elderly who may be vulnerable to infection due to absence of immunity to drifted influenza strains or due to waning of immunity to RSV, with susceptibility to associated complications further increased by underlying medical conditions. Vaccines targeted for the prevention of influenza and RSV disease in infants would benefit the public health by providing not only individual protection but by improving herd immunity.

2.1. *Influenza virus vaccines for infants*

Both RSV and influenza virus are enveloped, negative-sense, single-strand RNA viruses that belong to the Paramyxoviridae and Orthomyxoviridae families respectively. RSV is a single serotype but has two main antigenic subtypes, A and B. There are three types of influenza viruses that infect humans, A, B and C, type A viruses are further subdivided into subtypes based on the antigenic profile of the two envelope glycoproteins, hemagglutinin (HA) and neuraminidase (NA) and some subtypes may be further characterized into genotypes or clades based on sequence obtained for envelope genes. Two distinct lineages have also been identified for influenza B viruses based on the results of antigenic profiles obtain mainly from cross-hemagglutination testing. In the US, an inactivated, split trivalent influenza vaccine is licensed for children ≥ 6 months old, and a live, attenuated vaccine can be used in healthy children ≥ 2 years old. Trivalent inactivated influenza vaccines (TIV) contain a mixture of three viruses representative of circulating influenza A (subtypes H1N1 and H3N2) strains and a single B strain. Quadrivalent inactivated vaccines containing both influenza A subtypes as well as both B/Yamagata and B/Victoria lineages are undergoing clinical development. A quadrivalent formulation of the live attenuated influenza (LAIV) vaccine was recently approved in the US. Monovalent vaccines also exist for the prevention of infection due to pandemic (pdm) influenza A strains such as 2009 H1N1pdm and H5N1. Influenza immunization is recommended for all individuals older than 6 months in the US. To allow time for production of protective antibody levels, vaccination should occur before influenza begins to circulate in the community. For children aged 6 months through 8 years who are being vaccinated against influenza for the first time the recommended regimen includes 2 doses administered 4 weeks apart. Annual vaccination is recommended because vaccines are usually reformulated to include new virus strains that have small changes in the HA and NA surface glycoproteins allowing escape from pre-existing immunity (antigenic drift); the annual dose also provides a boost to pre-existing immunity for antigens and strains that have not changed – this is needed since antibody titers often decrease to levels below those associated with protection in the months between epidemics.

2.2. *RSV vaccines for infants*

In contrast, no vaccine is currently licensed for the prevention of RSV disease during childhood in spite of multiple attempts and persistent effort. The initial vaccine tested was an alum-precipitated, formalin-inactivated (FI), whole-virus vaccine developed in the 1960's [13]. Infants immunized with a 3-dose series administered between 2 and 7 months of age subsequently developed RSV-specific serum antibody responses that had poor in vitro neutralizing activity [14]. However, these responses were not protective. Following subsequent exposure to RSV there were similar rates of infection in vaccinees and controls, and ~65% of children given the formalin-inactivated vaccine were hospitalized for severe RSV lower respiratory tract infection as compared with 2.5% of controls who received a similarly prepared parainfluenza virus type 3 (PIV3) vaccine [15]. Two of the RSV-vaccinated children died. The unfortunate legacy of the formalin-inactivated RSV vaccine has haunted efforts to develop an RSV vaccine ever since and has led to much research to investigate and understand the causes of enhanced post-vaccination RSV disease in infants. No single unifying hypothesis entirely explains the disaster although several lines of investigation provide important clues to factors that may have contributed to enhanced disease. A description of these factors is beyond the scope of the present report however, the details have been reviewed recently [16–18], and include (i) skewing of the immune response to favor Th2-type cytokines and an allergic response upon exposure to virus, (ii) dysregulation of the cytokine response with heightened Th2- and Th1-type responses that upon exposure to RSV resulted in a "cytokine storm", (iii) failure to induce adequate neutralizing antibody responses due to modification of critical epitopes on the fusion (*F*) glycoprotein following formaldehyde inactivation, (iv) low antibody avidity following immunization with FI-RSV vaccine that increases tissue damage in the small airways due to deposition of complement on cells decorated with anti-RSV-IgG immune complexes, (v) modulation of type 1 interferon responses as a result of binding of the RSV fusion glycoprotein (RSV-F) to toll like receptor 4 (TLR4), and (vi) contributions of host factors that enhance susceptibility to severe disease, such as genetic polymorphisms in TLR4 that have been detected with higher frequency among infants with severe RSV infection and disease as compared with controls who had only mild disease.

Many lines of evidence demonstrate the important role of neutralizing antibody elicited in response to the two RSV envelope glycoproteins, including the fusion protein (RSV-F) and attachment protein (RSV-G), in providing long term protection against infection. In a randomized, double-blind, placebo-controlled clinical trial, monthly infusions of polyclonal human immunoglobulin (Respigam) containing high titers of RSV neutralizing antibodies (yielding post-infusion trough levels in excess of 1:300) decreased RSV hospitalization in high-risk infants by 60% when compared with infants given an IgG-free albumin control [19]. Likewise, monthly injections of an anti-RSV-F humanized monoclonal antibody (palivizumab) that has neutralizing and fusion-inhibiting activities, also significantly decreased the incidence of hospitalization due to RSV lower respiratory tract disease among high-risk infants [20]. These studies not only provide proof of efficacy for passively-administered neutralizing anti-RSV antibodies, but also provide support for maternal immunization programs that seek to elicit high titers of RSV neutralizing antibodies in the third trimester that will transfer across the placenta and provide newborns with protection for at least the first 3–6 months of life. These studies also suggest that RSV vaccines capable of eliciting neutralizing antibody responses, particularly those directed against RSV-F protein, have the potential to succeed. Although no RSV vaccine is currently licensed, a variety of experimental vaccines are being developed and many have been, or are undergoing, testing in the clinic. These include cold-adapted, live-attenuated viruses, recombinant-chimeric murine or bovine-human strains, vectored vaccines including replication incompetent recombinant adenovirus, alphavirus vaccines, various vector-expressed virus-like-particles containing RSV proteins, BCG recombinants expressing RSV-F, and subunit RSV-F vaccines (reviewed by Schmidt [18]).

3. Pre-clinical and clinical trial needs for respiratory virus vaccines

Licensure of vaccines for the prevention of influenza and RSV disease in infants is based on a demonstration of safety and efficacy in well-controlled clinical trials in the target population. Typically, this evaluation includes pre-clinical testing, and Phase 1, Phase 2, and Phase 3 clinical studies. Sometimes additional post-marketing studies, also known as Phase 4, are required subsequent to licensure. Pre-clinical studies may include proof-of-concept studies in animals to demonstrate immunogenicity and evidence of efficacy following a live virus challenge; for influenza and RSV vaccines these studies are typically performed in small animals such as mice, cotton rats, and/or ferrets (see Table 1 for summary). For RSV vaccines, animals should ideally be challenged with viruses that represent both subtype A and B viruses since it is expected that an effective vaccine will need to provide immunity against both subtypes. The need for an adjuvant to enhance or modify the immune response can also be justified using these pre-clinical tests. Repeat-dose toxicology studies in rats or rabbits may be needed as well as reproductive toxicology studies (if the vaccine is intended for use in a maternal immunization program). Pre-clinical testing of influenza and RSV vaccines in animal models also includes studies to demonstrate the attenuated phenotype and stability of live virus vaccines. For non-replicating RSV vaccines, additional testing in animals is required in order to provide evidence that the vaccine is unlikely to prime for enhanced susceptibility when vaccines are subsequently exposed to wild-type RSV infection post-vaccination. This safety study to evaluate vaccine priming for enhanced disease is necessary before testing may proceed in RSV-naïve children but is generally not needed prior to testing the candidate vaccine in RSV-seropositive individuals.

Irrespective of vaccine type, Phase 1 testing of vaccines targeted for use in an infant population generally proceeds using a dose-ranging step-down design characterized by evaluating candidates in small number ($N = 15–20$) of healthy adults followed by dose-ranging tests in small groups of progressively younger seropositive children prior to proceeding with dose-ranging studies in progressively younger sero-negative, immunologically-naïve infants. Serostatus of the youngest infants, for example, those less than 6 months of age, is typically complicated by the presence of maternal antibodies. Usually a range of doses are tested within each age cohort beginning with the lowest and proceeding incrementally with tests using higher doses of vaccine. Once a safe dose is identified in the target population, further Phase 1 testing in small numbers of children may be used to optimize the number of doses required to achieve maximum vaccine take.

At this point, clinical studies enter Phase 2 during which the vaccine and immunization schedule are tested in larger numbers of children ($N = 100–300$ subjects) in order to accrue additional safety data and evidence of effectiveness. For live virus vaccines, transmission studies may be conducted to assess the ability of the vaccine virus to spread and infect naïve contacts. The reliability of clinical tools that will be used to demonstrate efficacy in pivotal trials (including diaries, questionnaires, case definitions, scoring methods to assess disease severity and critical laboratory tests such as assays to assess immunogenicity or to identify infected individuals) are proven during Phase 2.

Table 1
Characteristics of pre-clinical and clinical studies performed to support licensure of pediatric influenza and RSV vaccines.

Pre-clinical animal studies	• Immunogenicity and efficacy in small animals models • Evaluate potential to prime for enhanced disease following live virus challenge • Repeat-dose toxicology studies • Reproductive toxicology studies[a]
Phase 1	• Small numbers of subjects (10–20 per group) • Dose-ranging, step-down studies • Primarily to evaluate safety • Evaluate immunogenicity and optimize number of doses to achieve seroconversion/seroresponse
Phase 2	• Larger numbers of subjects in the target population (100–300) • Evaluate safety and immunogenicity • For live virus vaccines, evaluate transmission to naïve contacts (family or day care studies) • Follow up during winter months to evaluate efficacy and/or possibility of enhanced disease • Assess laboratory and clinical tools that will be used in pivotal Phase 3 trials
Phase 3	• Number of subjects: 5000–10,000 (or more) • Evaluate safety • Evaluate clinical efficacy (some immunogenicity data may be collected)
Phase 4	• Post-licensure studies • Active and passive surveillance for rare adverse events (VAERS[b], PRISM[c]) • Confirm safety and effectiveness with distributed product

[a] Reproductive toxicology studies are needed when the vaccine is likely to be used in pregnant women.
[b] VAERS: vaccine adverse events reporting system.
[c] PRISM: post-licensure rapid immunization safety monitoring.

One retrospective look at data acquired over 20 years of clinical trials involving various RSV candidate vaccine strains showed that the rates of RSV-associated lower respiratory tract illness (LRI) were similarly low (∼3–4%) in both vaccinees and controls in the winter subsequent to vaccine administration; this included data for approximately 60 children given live-attenuated vaccines between 1 and 3 months of age, spanning the age group at highest risk for enhanced disease [21]. None of the children involved in these studies were hospitalized with RSV illness during the winter season following immunization. These findings suggest that live-attenuated viruses administered by the intranasal route are unlikely to prime for enhanced disease. Nevertheless, as a part of the safety assessment for these vaccines, RSV-naïve infants enrolled in Phase 1 and 2 vaccine trials are typically followed through one or two RSV seasons after vaccine administration to detect any signal that might indicate enhanced susceptibility to severe disease.

Phase 3 clinical trials provide proof of vaccine efficacy and a thorough look at safety. For a new vaccine with no predecessor, proof of efficacy is obtained from large, multi-centered, randomized, placebo-controlled double-blinded clinical trials that show that the attack rate (i.e., culture-confirmed infection and/or a disease endpoint such as hospitalization) is significantly reduced among vaccinees as compared to the incidence or attack rate in the unvaccinated population. Clinical investigators have estimated that ∼500 children between birth and 12 months of age would be needed per arm to have 90% power to detect a vaccine with 80% efficacy in preventing culture-confirmed symptomatic respiratory tract infection due to RSV [22]. Alternatively, if a vaccine is already licensed, as is the case for seasonal inactivated influenza vaccine, a non-inferiority trial may be used to compare immunogenicity of the new vs. the licensed product using a surrogate serological marker of efficacy such as hemagglutination inhibiting (HI) antibody titer. In this scenario, the lower bounds of the confidence intervals for the seroresponse rates for each vaccine are compared and immunogenicity of the new vaccine is considered to be acceptable if the response does not differ from that seen following immunization with the licensed product by some small, clinically acceptable margin (FDA Guidance for Industry, *Clinical Data Needed to Support Licensure of Seasonal Inactivated Influenza Vaccines*, 2007, http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091990.pdf accessed on 11/16/2012). Geometric mean titers or concentrations of antibodies are also compared between groups to show that these values do not vary by more than 50%. In some cases, the Phase 3 trial demonstrates that the new vaccine can achieve a minimum response rate or a minimum protective titer among the majority of vaccinees. However since the immune correlates of protection in infants given influenza vaccine are controversial and may vary by vaccine type, clinical efficacy studies may still be required. Since many vaccines are administered to children, some Phase 3 studies are designed to assess safety and immunogenicity of the candidate vaccine when given concomitantly with other routinely recommended vaccines using a non-inferiority design. Phase 3 studies also extend the safety database for the target population by testing the vaccine in large numbers of subjects (e.g., 5000 or more). When evaluating a live virus vaccine, the majority of the population used for the safety database needs to be virus-naïve in order to reliably assess the reactogenicity of the vaccine strain. Most pre-licensure vaccine studies are conducted in healthy populations; use of the vaccine in special populations such as those with asthma, HIV infection, or those using biological response modifiers, may require separate study depending on vaccine type in order to obtain approval for use in individuals with these conditions.

After licensure, post-marketing Phase 4 studies are used to obtain a more complete assessment of effectiveness (decreased illness) when the vaccine is used in the general population and, sometimes, to obtain additional safety data for rare adverse events once the vaccine is given to larger numbers, as would be the case if recommended for universal use in the US with a birth cohort of ~4 million children per year. In the US, the FDA monitors the safety of new vaccines in children using the post-licensure rapid immunization safety monitoring (PRISM) program while the CDC performs rapid cycle analysis to detect safety signals in real-time through centers participating in the Vaccine Safety Datalink [23]. These programs supplement passive reports accrued into the Vaccine Adverse Events Reporting System (VAERS) which is also monitored continuously for potential signals of serious adverse events.

4. Safety and efficacy of influenza and RSV vaccines in infants

As outlined above, the path to licensure for any vaccine designed for newborns and very young infants is carefully scrutinized to assure that those exposed to these vaccines are likely to derive benefit with minimal risk. Toward that end, vaccines for the prevention of influenza and respiratory syncytial virus disease are assessed for local and systemic toxicity. In general, live-attenuated respiratory virus vaccines (typically given intranasally) should not cause lower respiratory tract illness (bronchiolitis, pneumonia, or wheezing), a significant increase in fever or upper respiratory infection (URI) symptoms such as cough, nasal congestion, or acute otitis media. This is particularly important for infants <1 month of age since they are obligate nose breathers who may not tolerate nasal stuffiness. While small amounts of rhinorrhea or nasal congestion might be acceptable in older children, levels that interfere with nursing, feeding, or sleeping in infants are not likely to be acceptable. The most common adverse events seen after administration of live attenuated influenza vaccine (LAIV) were runny nose and nasal congestion and the incidence of serious adverse reactions was very low. Episodes of wheezing were increased in LAIV-vaccinated children less than 2 years of age and among those of any age with a history of recurrent episodes of wheezing or with asthma. As a result, the lower age limit for the live attenuated influenza virus vaccine was set at 24 months of age and this vaccine is not recommended for use in those with asthma or predisposed to wheezing.

A few small studies have assessed the safety of trivalent inactivated influenza virus vaccine (TIV) vaccine in infants <6 months of age [11,24]. Englund et al., enrolled substantial numbers of infants 6–12 weeks of age ($N = 1374$ with 913 given TIV and 459 given placebo) in a study to assess safety and immunogenicity of TIV in this age group; this trial did not detect any significant differences in rates of fever or serious adverse events between groups. Severe adverse events such as febrile seizures and narcolepsy that have been associated with some influenza vaccines administered to young children, highlight the need for careful studies to identify the root cause of these events so that the safety of these, as well as future formulations, can be ensured [25–27].

Since a number of pathogens can result in similar respiratory symptoms, efficacy of a respiratory virus vaccine is tested using a combination of clinical and virologic end-points. The primary efficacy end-points of pediatric phase 3 studies for inactivated and live influenza vaccines were disease signs (fever, in addition to upper and lower respiratory tract symptoms) accompanied by confirmation of influenza virus infection using nasal washes or throat swabs to identify the pathogen associated with respiratory illness [28]. Infection by RSV or an influenza virus is usually confirmed by culture of these samples in susceptible cells, but amplification of viral genes by PCR that can provide additional information about the infecting pathogen, has become a suitable alternate test method. Measures of vaccine immunogenicity have not replaced all clinical studies to establish efficacy because the immune mechanisms that contribute to protection are not completely understood, with different arms of the immune system playing a role in preventing infection and expediting clearance of infected cells. In addition, a specific immune endpoint used as a surrogate marker of protection in adults, cannot always be applied to young children. For example, a hemagglutination inhibition (HI) titer of 1:40 following inactivated influenza vaccination is often used as a correlate of protection against influenza disease in adults [29,30]. However, recent clinical studies of an adjuvanted inactivated influenza vaccine show that a higher HI titer (1:110 for a similar (50%) rate of protection) may be required to protect children younger than 3 years of age [31]. Even higher titers are required to achieve a greater degree of protection [31,32].

Another difficulty in inferring potential efficacy of a vaccine from serologic responses is that different protective immune mechanisms are induced by different vaccine types; while anti-HA antibodies contribute to efficacy of inactivated influenza vaccines, protection induced by live, attenuated influenza vaccines in young children is associated with the induction of IFN-γ-producing CD8+ T cells [33], and may also rely on NA-inhibiting antibodies and virus-specific mucosal IgA as demonstrated for adults [34]. As shown for live, attenuated influenza vaccines, it is likely that RSV vaccines that similarly induce cellular (CD8+ T cells) as well as mucosal sIgA and serum IgG neutralizing antibody responses will provide the greatest chance of protection against infection [35,36].

5. Impact of immunologic immaturity and maternal antibodies on infant responses to vaccines

It is difficult to induce either B or T cell responses in infants due to an immature immune system and an absence of pre-existing memory B and T cells (reviewed in Ref. [37]). Factors that contribute to an ineffective immune response in infants include defects in both innate [38] and adaptive [39] mechanisms, in addition to the presence of maternal antibodies [40]. Adaptive immune responses are controlled by regulatory T cells (Treg) that are increased in newborns [41,42]. These cells are clearly important *in utero* to protect against generating responses to maternal antigens, however, Tregs suppress cell-mediated responses in the first few weeks of life, possibly by decreasing the stimulatory capacity of antigen-presenting cells (APC) [43–45], resulting in reduced numbers of activated CD4+ and CD8+ T cells. Not only does this result in fewer antigen-specific T cells, but this environment also produces a qualitative difference in the neonatal milieu that preferentially supports development of CD4+ Th2 cells while simultaneously eliminating CD4+ Th1 cells by inducing apoptosis [46].

In addition to quantitative and qualitative deficiencies in antigen-specific T cell responses, B cell responses are reduced in infants due to a limited B cell repertoire and the lack of previous exposure to foreign antigens. Consequently, high avidity antibodies are usually not stimulated by an initial exposure to vaccine antigens or pathogens in the young. To generate an effective response, the infant must also overcome the presence of maternal antibodies that mask neutralizing antibody epitopes [47]. Epitope blocking in infants may be attributed to either pathogen-specific IgG transferred *in utero* [48], or maternal IgA obtained from breast-milk [49]. Data suggest that the balance between the quantity of maternal antibody and targeted antigen is predictive of successful response to inactivated vaccines, with interference by maternal antibodies resulting in suboptimal responses to influenza vaccines administered parenterally in animals [50,51] and humans [47]. In theory, mucosal vaccines have the potential to overcome this obstacle since vaccine immunogenicity at the mucosal surface is less likely to be hindered by passively acquired serum antibodies.

To ensure uniform and adequate protection of newborns against respiratory viruses, including infections due to influenza or respiratory syncytial virus, maternal immunization has been proposed. A randomized, controlled study showed immunization during the 3rd trimester of pregnancy with trivalent inactivated influenza vaccine reduced influenza illness by 63% in infants born to vaccinated mothers, and significantly reduced the overall incidence of febrile respiratory illness in both newborns and mothers [52,53], supporting the use of this strategy to protect infants from disease until they can be successfully vaccinated. This approach may be very important in the face of an influenza pandemic, when there is a shift in HA and NA antigens and maternal antibodies specific for seasonal influenza strains are likely to be ineffective in protecting either mother or her child. Antibodies detected against the H1N1 2009 pandemic virus in vaccinated mothers and their offspring demonstrate that transplacental transfer of antibodies is efficient, and can achieve protective levels that persist for at least 10 weeks in the majority of infants [54,55].

Respiratory illnesses in children younger than 6 months are predominantly due to RSV, reflecting the need for very high titers of transplacentally-transferred neutralizing antibodies [56,57]. RSV disease was reduced when levels of maternal neutralizing antibodies $>$1:300 were present [4,58,59], or when high-risk infants receiving monthly infusions of RSV-specific hyper-immunoglobulin maintained levels of serum neutralizing antibodies in excess of $\sim$1:300 [60]. Maternal immunization to prevent RSV infection in infancy is therefore a reasonable approach to protect young infants against this pathogen. One such study explored this possibility using an investigational purified RSV-F vaccine but titers were not boosted sufficiently above baseline to improve protection in infants born to vaccinated mothers [61]. If robust antibody responses were transferred to the newborn using this approach, it is likely that immunity would be provided to infants during the first few months of life. However, there are pitfalls associated with passive immunization; maternal antibodies still present at the time of infant vaccination may reduce immunogenicity of vaccines [50], or result in a less effective response due to induction of non-neutralizing antibodies. Studies in infants suggest this is the case for measles [62], and therefore careful consideration is given in recommending the appropriate age for measles vaccination.

Animal studies suggest that immunization in the presence of maternal antibodies can have a detrimental outcome on vaccine efficacy by preventing vaccine take and may even be harmful. For instance, piglets vaccinated against influenza in the presence of homologous maternally derived antibodies exhibited exacerbated disease and prolonged clinical signs when subsequently challenged with live virus [63–65]. However, this enhanced disease is avoided when the weanlings are immunized with a live-attenuated vaccine [66], suggesting that early intranasal vaccination of infants with live, attenuated RSV or influenza virus vaccine is likely to be safe and immunogenic, even when maternal antibodies are present.

6. Designing vaccines against respiratory viruses for infants

Rational designs of influenza and RSV vaccines that are safe and immunogenic in very young infants have to overcome the hurdles of immune immaturity and maternal antibodies. Live, attenuated vaccines administered via the mucosal route offer one approach to surmount interference by maternal antibodies and to avoid potential disease exacerbation induced by inactivated vaccines administered by the parenteral route. In addition, live virus stimulates a more balanced helper T cell response that is (at least in theory) less likely to produce a predominant Th2-type cytokine environment that is often associated with exacerbation of disease. Mucosal vaccination against a respiratory virus has additional advantages, particularly for very young children in that local immune responses are induced that include secretion of virus-specific IgA antibodies that may be necessary for protection [67]. Consequently, vaccine efficacy may be excellent, even when virus-specific IgG responses in serum are not robust, as demonstrated for live, attenuated influenza vaccine in very young children [68,69].

Since RSV infections can have a severe outcome in newborns, there is a need to develop effective vaccines that protect infants from birth to 6 months. Clinical trials of live RSV vaccines therefore aim to identify a vaccine virus that is suitably attenuated and yet still immunogenic in this age group [70]. The development of vaccine candidates has greatly improved due to the use of sequence analysis to identify attenuating mutations, genetic engineering to construct new viruses, and the use of non-human primates as a preclinical animal model to rule out virus constructs that are insufficiently or overly attenuated [71–73]. Clinical trials of a vaccine candidate shown to be safe and immunogenic in 1–2 month old infants identified reversions of attenuating sequences in the viruses shed from vaccinees that were no longer temperature sensitive [74]. Recent studies suggest that reversion can be decreased by stabilizing codons associated with attenuating mutations [75], providing some confidence that a further modified virus may prove to be a suitable vaccine seed.

The immunogenicity and consequent effectiveness of influenza and RSV vaccines that are under development could potentially be improved by the inclusion of adjuvants. Since reinfection with RSV is common throughout childhood, there is an expectation that a successful vaccine may need to elicit immune responses that exceed those seen following natural infection. Depending on the adjuvant, increased immunogenicity may be a result of improved antigen uptake by antigen-presenting cells, activation of innate responses that support induction of a Th1-type response, or by creating an environment in germinal centers that allows greater proliferation of antigen-specific B and T cells, with large numbers of cells driven to become memory cells [76]. An example of an adjuvant that has been proposed to improve neonatal responses is IL-12; when co-administered with an inactivated influenza vaccine, IL-12 supported development of a Th1-type response in newborn mice and increased protection when the mice were challenged with virus as adults [77]. The adjuvant activity of several alternative formulations has been demonstrated in pediatric clinical trials. These include squalene-based adjuvants MF-59 [78,79] and AS-03 [80]. As discussed earlier, the benefit of each added vaccine component and its safety needs to be demonstrated through clinical testing. Immunization of young children with a monovalent inactivated H1N1 vaccine containing a squalene-based adjuvant AS-03 with α-tocopherol (vitamin E) was discontinued due to an observed increase in the incidence of narcolepsy in Scandinavia [81]. Interestingly, narcolepsy was also described following infection with the 2009 H1N1pdm virus [82]. Therefore, it is not clear whether this unusual adverse event was due to adjuvant components or whether a similar increase would have occurred in this population if exposed to unadjuvanted vaccine. Findings like this point to the importance of surveillance programs to identify and evaluate adverse events in real-time in order to respond to signals quickly and make or modify recommendations for vaccine use.

7. Conclusion

There is a critical need for vaccines against respiratory viruses, including RSV and influenza, in infants <6 months of age. Immature immune systems and the presence of maternal antibodies may prevent induction of robust neutralizing antibody responses to vaccines in infants. While live, attenuated virus vaccines administered intranasally provide the potential means to overcome some of these obstacles it is extremely difficult to identify suitably attenuated virus strains that retain immunogenicity. Non-replicating vaccines run the risk of priming for enhanced wild-type disease and need to be evaluated carefully before testing in the youngest of infants who are naturally predisposed to Th2-type responses. Further evaluation of programmatic efforts that consider maternal immunization together with pediatric vaccination is needed, as this approach may provide neonates and infants with sufficient immunity until they can be safely and successfully immunized.

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