Table 2 Obstacles to RSV and PIV Vaccine Development

1. Immunity and immunopathology
   - Complete protection against infection and disease is difficult to achieve
   - Live attenuated vaccines are less immunogenic than wild-type virus
   - Multiple doses of vaccine are needed to provide durable mucosal immunity
   - Formalin-inactivated RSV vaccine induced enhanced disease
2. Young age of vaccinees
   - Decreased immune response in first 6 months of life
   - Maternal antibodies to RSV and PIV can decrease immune response to vaccine
   - Difficult to achieve a balance between attenuation and immunogenicity for live attenuated virus vaccines
   - Clinical trials for vaccines for use in infancy proceed slowly
   - Age-related temporal association with sudden infant death syndrome (SIDS) and reactive airway disease (RAD)
   - Interference with routine vaccines needs to be excluded
3. Virus specific factors
   - Highly infectious viruses able to infect partially immune subjects
   - Limited or no cross-protection (RSV A vs. RSV B)—five vaccines needed (RSV A, RSV B, PIV1, PIV2, PIV3)
   - Lack of highly permissive animal models
   - Poor growth and limited physical stability (RSV only)

Abbreviations: RSV, respiratory syncytial virus; PIV, parainfluenza virus.

require multiple doses of vaccine to achieve durable immunity. Second, most of the severe RSV and PIV3 disease occurs in young infants aged one to six months (18), the target population for a RSV and PIV3 vaccine, but infants infected with wt virus in the first six months of life generate less antibody to the viral surface glycoproteins than older infants (19,20). In addition, younger infants have a less diverse B cell repertoire, their antibody affinity maturation is less efficient, and T-cell help is limited (19,21–24). Thus, the immature immune response of the young infant is a significant obstacle for a vaccine designed to protect that age group. Third, maternal antibodies present in the young infant can modify the response to immunization. These transplacentally acquired RSV and PIV neutralizing serum antibodies reduce the immunogenicity of parenterally administered subunit vaccine, vectored vaccine, and wt virus infection through a poorly defined immunological mechanism termed antibody-mediated immune suppression (25–27). Fourth, for RSV, two antigenic subgroups (designated subgroup A and B) cocirculate and are only 25% related antigenically (18). A second infection within the same season is often caused by virus belonging to the heterologous subgroup, indicating that antigenic diversity is responsible in part for the high frequency of second infections with RSV, and more importantly, for LRT disease upon second infection (18). Therefore, a total of five separate vaccines will be needed to protect against RSV and PIV disease, two for RSV (RSV subgroup A and RSV B) and three for PIV (PIV1, PIV2, and PIV3). Fifth, during RSV vaccine trials in the 1960s, immunization of infants and young children with an inactivated RSV vaccine unexpectedly potentiated RSV disease following subsequent natural wt RSV infection (28,29). This observation, in the context of comparable observations for inactivated measles virus vaccine, mandates that vaccine development for RSV proceeds with great caution in the pediatric population.

In addition, infants will be vaccinated at an age when sudden infant death syndrome (SIDS) occurs, and this will raise a concern for the use of any topical RSV or PIV vaccine even though these viruses are not thought to be causal agents. This is not unlike the concern regarding the occurrence of intussusception in recipients of a live rotavirus vaccine. This and other reasons given in Table 2 make the development of a live virus vaccine for use in early infancy especially challenging.

In summary, successful vaccines against RSV and PIV must (*i*) be immunogenic in young infants, even in the presence of maternally-acquired serum antibodies; (*ii*) protect against LRTI following first infection with wt virus; (*iii*) induce resistance to both subgroup A and B strains of RSV; and (*iv*) not induce a immune response that can lead to enhanced RSV or PIV disease during subsequent natural infection. Despite these obstacles, it should be possible to successfully immunize the target pediatric population against RSV and PIV.

VACCINES FOR RSV AND PIV

Nonreplicating Virus Vaccines for RSV

Nonreplicating virus vaccines include inactivated whole virus vaccines, virus-like particles, subunit vaccines such as purified glycoproteins, and DNA or RNA vaccines that express one or more protective antigens of RSV or PIV. Inactivated whole RSV vaccines for seronegative infants are not currently being evaluated in clinical trials, but instructive information was derived from their previous use. These vaccines have been extensively reviewed elsewhere and will not be discussed in detail here (30). Formalin-inactivated RSV (FI-RSV) was evaluated in the 1960s and resulted in disease potentiation (increased frequency and severity of bronchiolitis and pneumonia) in vaccinees following infection with wt RSV (28,29) (Table 3). At least two factors are thought to have contributed to disease potentiation in FI-RSV vaccinees. First, FI-RSV failed to induce a significant level of resistance to RSV replication because (*i*) the antibodies induced by FI-RSV had greatly diminished neutralizing activity and (*ii*) FI-RSV failed to induce a protective $CD8^+$ T-cell response. Second, FI-RSV induced a nonprotective but disease enhancing Th2 type $CD4^+$ T-cell response (40) and was also associated with immune complex deposition in the airways (41). Thus, RSV replicated in FI-RSV vaccinees without significant immunological restriction, but the T cell–mediated inflammatory cell response was accelerated and augmented, and this translated into an increase in the frequency and severity of bronchiolitis and pneumonia. Enhanced disease has not been observed with natural RSV or PIV infection, reinfection, or with live attenuated RSV or PIV vaccines, and was not seen in seropositive subjects immunized with FI-RSV or RSV subunit vaccine (18). Thus, disease potentiation is not associated with replicating RSV vaccines, probably because they induce highly functional antibodies, $CD8^+$ T cells, and a more Th1-biased response.

A number of subunit RSV vaccines have been evaluated in preclinical trials, and several of them have progressed into clinical trials (Table 3). Most of these candidate vaccines consist of either one or both of the viral surface glycoproteins that mediate membrane fusion (F) or virus attachment (G), or parts thereof. Some of these candidate vaccines contained adjuvants such as aluminum hydroxide or aluminum phosphate, CpG nucleotides, monophosphoryl lipid A (MPL), saponins, or oil-in-water emulsions, while others were conjugated to bacterial toxins or formulated as immunostimulating complexes (31,34). The safety of many of these adjuvants for infants remains to be determined. Two observations will make