Progress in the Development of Respiratory Syncytial Virus and Parainfluenza Virus Vaccines

Anna P. Durbin and Ruth A. Karron

Respiratory syncytial virus (RSV) and human parainfluenza viruses (hPIVs) are leading causes of viral lower respiratory tract illness in children and in high-risk adult populations. Despite decades of research, licensed vaccines for RSV and hPIVs do not exist. Recently, however, genetically engineered live attenuated RSV and hPIV candidate vaccines have been generated, several of which are already being evaluated in clinical trials. Recombinant technology allows candidate vaccines to be “fine-tuned” in response to clinical data, which should hasten the development of vaccines against these important respiratory pathogens.

RSV as important causes of viral LRTI in young children [16]. Like RSV, hPIV-3 can cause severe LRTI in immunocompromised patients [17, 18].

Licensed vaccines for RSV and hPIVs are not currently available. This review will describe both the obstacles to success and the recent progress made in the development of vaccines against these important respiratory pathogens.

EPIDEMIOLOGY

By the time they are 2 years of age, almost all children will have been infected with RSV, and ~50% will have been infected twice [19]. HPIV-3 also infects children early in life: ~60% and ~80% will have been infected before the ages of 2 and 4 years, respectively [20, 21]. Infection with hPIV-1 and hPIV-2 occurs when children are slightly older, but, by 5 years of age, most children have been infected with these viruses at least once [20, 21]. RSV epidemics occur during the winter and early spring in temperate climates and during the rainy season in some, but not all, tropical climates [22, 23]. Currently, in the United States, hPIV-1 epidemics occur in the fall of odd-numbered years, hPIV-2 epidemics occur biennially or annually in the fall, and hPIV-3 epidemics occur annually in the spring and summer [16].

IMMUNITY

Both RSV and hPIVs can reinfect individuals throughout life, although infection in healthy older children and young adults
is usually associated with URTI. Primary infection with RSV or hPIV-3 does not always elicit immune responses that will protect the lower respiratory tract, because RSV- and hPIV-3–associated LRTI can occur in young children experiencing second infections [12, 19]. Immunity to RSV and hPIVs is mediated via humoral and cellular effectors, including serum antibody (acquired as a result of infection or maternally derived in young infants), secretory antibody, and major histocompatibility complex I–restricted cytotoxic T lymphocytes [1, 24, 25]. The RSV F glycoprotein may also elicit innate immune responses via toll-like receptors and CD14 [26, 27]. In general, humoral immune responses consisting of secretory and serum antibodies protect against infection of the upper and lower respiratory tracts, respectively, whereas cell-mediated responses directed against internal proteins terminate infection. Recent studies involving mice using vectored vaccines against hPIV-1 and hPIV-3 suggest that brief cross-protection between these viruses may occur via cell-mediated immunity associated with internal proteins common to both viruses [28, 29]. However, significant cross-protection does not appear to occur in humans [30].

Because RSV and hPIVs cause reinfections, the goal for vaccines against these pathogens is not to prevent infection but, rather, to prevent virus-associated LRTI and complications, such as otitis media. The suboptimal responses of young infants to primary infection with either RSV or hPIV-3 has important implications for vaccine development, because it suggests that the administration of >1 dose of vaccine will likely be needed for such individuals.

**OBSTACLES TO VACCINE DEVELOPMENT**

Although the importance of RSV as a respiratory pathogen has been recognized for >40 years, a vaccine is not yet available because of several problems inherent in RSV vaccine development. The peak incidence of severe disease and mortality associated with pediatric RSV infection occurs in infants <3 months of age, who often have high titers of maternally derived antibody to RSV. These young infants may not respond adequately to vaccination, because of immunologic immaturity and/or suppression of the immune response by maternally-derived antibody [1]. An RSV vaccine will also need to protect against the antigenically divergent groups RSV A and RSV B. It is most important to note that the vaccine must not potentiate naturally occurring RSV disease, as was observed with the formalin-inactivated RSV vaccine (FI-RSV).

In the early 1960s, FI-RSV (lot 100) was administered intramuscularly to infants and children aged 2 months to 7 years [31–34]. Lot 100 not only failed to protect against disease due to wild-type RSV, but it induced an exaggerated clinical response to wild-type RSV infection in infants who were naive to RSV before vaccination. Many vaccinees were hospitalized with LRTI; in one study, the hospitalization rate among vaccinees approached 80%, compared with 5% among control subjects [31]. Tragically, 2 infants who received lot 100 died at 14 and 16 months of age following wild-type RSV infection [31], RSV was readily isolated from the lower respiratory tracts of these infants.

The mechanisms responsible for the FI-RSV vaccine–associated disease enhancement are incompletely understood. However, data on recipients of lot 100 [34–36] and from studies in rodent models [37–40] have led to the hypothesis that children vaccinated with FI-RSV remained susceptible to infection with wild-type RSV because vaccination produced inadequate levels of serum-neutralizing antibodies and did not induce local immunity. Once infected with wild-type RSV, virus was not readily cleared because FI-RSV did not prime recipients for a CD8+ cytotoxic T cell response, and the viral infection produced a direct cytopathic effect in the lower respiratory tracts of these infants. In addition, immunization with FI-RSV primed recipients for a Th-2–like response, with increased local production of IL-4, IL-5, and IL-10, an influx of lymphocytes and eosinophils, the possible release of additional mediators, and resultant inflammation and bronchoconstriction [41–43]. Recently, we showed that immune complex deposition also appears to have contributed to the enhanced disease observed in these children [44].

The clinical experience with FI-RSV and the information gleaned from animal models about disease enhancement suggest key features of an RSV vaccine for seronegative infants. The vaccine should induce protective levels of neutralizing antibody, as well as CD8+ RSV-specific cytotoxic T cells, and a pattern of CD4 cell response similar to that evoked by wild-type RSV. Although a live attenuated vaccine is most likely to exhibit these characteristics [30, 43, 45], it is possible that novel immunization strategies that combine nonreplicating vaccines with cytokines or new adjuvants might achieve these goals [42, 46].

**VACCINES IN CLINICAL DEVELOPMENT**

**Live, attenuated vaccines.** Live, attenuated vaccines may offer several advantages over nonreplicating vaccines, especially for infants and young children. Intranasal immunization with a live, attenuated vaccine should induce both systemic and local immunity and may, therefore, protect against URTI, as well as LRTI. The immune response to a live vaccine should closely resemble the response to natural infection and, therefore, should not produce enhanced disease after exposure to wild-type virus [45]. Like other live, attenuated intranasal respiratory virus vaccines [47, 48], live intranasal RSV and hPIV-3 candidate vaccines replicate in young infants in the presence of
maternally acquired antibody [49–51], which will be critical for the success of these vaccines in young infants.

As described below, achieving an appropriate balance between attenuation and immunogenicity in these vaccines is not straightforward, because the levels of vaccine virus replication tend to correlate with both induction of immune responses and with clinical symptoms [1, 16, 30]. The level of reactogenicity that can be tolerated will depend on the age and physical condition of the target population. For example, vaccine-induced nasal congestion would likely be acceptable in toddlers and older persons but unacceptable in very young infants, who are obligate nose breathers ([50] and described below). hPIV-3 cp45 is an example of a live, attenuated vaccine that is sufficiently attenuated yet immunogenic in young infants [51].

Biologically derived live, attenuated RSV vaccines. Several strategies for the development of a live, attenuated vaccine were originally explored, including the creation of host-range mutants, cold-passaged (cp) mutants (serially passaged at low temperature), and temperature-sensitive (ts) mutants (unable to grow efficiently at high temperatures). On the basis of previous experience with live, attenuated influenza vaccines [52], growth of the cp and ts mutants in vivo was expected to be restricted, particularly in the lower respiratory tract (at core body temperature). The clinical evaluation of cp or ts mutants developed between 1968 and 1976 and has been extensively summarized elsewhere [43, 45, 53]. In brief, these vaccine candidates were either underattenuated (cp RSV and ts-1 RSV) or overattenuated (ts-2 RSV), and reversion to wild-type (ts') phenotype was observed in virus isolates obtained from children who received ts-1 RSV. Transmission of ts-1 RSV from children who were vaccinated to placebo recipients also occurred [54, 55]. It is important to note that enhanced disease was not observed when infants who received ts-1 RSV or cp RSV were naturally infected with wild-type RSV [54, 56]. Although these early attempts to develop a live, attenuated RSV vaccine were unsuccessful, they established the use of placebo-controlled, double-blind trials with postvaccination surveillance during RSV epidemics as the model for future evaluation of live, attenuated RSV vaccines in children. In addition, cp RSV is the progenitor of the cpts RSV vaccines recently evaluated in children (table 1, figure 1A, and below).

A series of live, attenuated, intranasally administered cpts RSV A candidate vaccines were derived from further attenuation of cp RSV through chemical mutagenesis (figure 1A). This process generated ts candidate vaccines that displayed a spectrum of attenuation in rodents and nonhuman primates [57–60]. Several of these were evaluated in phase I clinical trials (table 1) [50, 61]. The cpts248/955 and cpts530/1009 vaccines were evaluated sequentially in adults and RSV-seropositive and -seronegative children as young as 6 months of age. Although

Table 1. Respiratory syncytial virus (RSV) and human parainfluenza virus (hPIV) vaccines evaluated in clinical trials since 1990.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type</th>
<th>Manufacturer</th>
<th>Population evaluated</th>
<th>Vaccine status</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV subunit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FG</td>
<td>Recombinant FG fusion protein</td>
<td>SmithKline Beecham</td>
<td>Adults</td>
<td>Inactive</td>
</tr>
<tr>
<td>BBG2Na</td>
<td>RSV G peptide-BB&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pierre Fabre</td>
<td>Adults&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Inactive</td>
</tr>
<tr>
<td>PFP-1, -2, -3</td>
<td>Purified F protein</td>
<td>Wyeth</td>
<td>Adults, children&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Active</td>
</tr>
<tr>
<td>RSV A subunit</td>
<td>Copurified F, G, M proteins</td>
<td>Aventis Pasteur</td>
<td>Adults</td>
<td>Active</td>
</tr>
<tr>
<td>RSV live attenuated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ts-1 A, B, C</td>
<td>RSV A ts mutants</td>
<td>MRC</td>
<td>Adults</td>
<td>Inactive</td>
</tr>
<tr>
<td>cp or cpts&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Serially passaged derivatives of cp RSV A2</td>
<td>NIH or Wyeth/NIH</td>
<td>Adults, children, infants</td>
<td>Inactive</td>
</tr>
<tr>
<td>Recombinant RSV</td>
<td>Recombinant derivatives of cp RSV A2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Wyeth/NIH</td>
<td>Adults, children, infants</td>
<td>Active</td>
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<tr>
<td>hPIV-3 live attenuated</td>
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<tr>
<td>Bovine PIV-3</td>
<td>Host range mutant</td>
<td>NIH, MedImmune</td>
<td>Adults, children, infants</td>
<td>Inactive</td>
</tr>
<tr>
<td>Recombinant human/bovine PIV-3</td>
<td>hPIV-3 with bovine N gene&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NIH</td>
<td>Adults, children, infants</td>
<td>Active</td>
</tr>
<tr>
<td>cp45</td>
<td>Serially passaged derivative of JS wt</td>
<td>NIH, Wyeth</td>
<td>Adults, children, infants</td>
<td>Active</td>
</tr>
</tbody>
</table>

NOTE. cp, cold passaged; NIH, National Institutes of Health; PFP, purified F glycoprotein; ts, temperature sensitive.
<sup>a</sup> Includes healthy adults, older persons, pregnant women, and healthy and high-risk RSV-seropositive children.
<sup>b</sup> Healthy adults and older persons.
<sup>c</sup> BB is the albumin-binding domain of streptococcal protein G and functions as a carrier protein.
<sup>d</sup> Includes cp RSV, which was evaluated in adults, and cpts248/955, cpts530/1009, cpts530/1030, and cpts248/404, each of which was evaluated in children as young as 6 months who were naive to RSV. cpts248/404 was evaluated in infants as young as 1 month of age.
<sup>e</sup> Includes mutants with multiple ts mutations and/or deletions of nonessential genes.
<sup>f</sup> Other recombinant human/bovine PIV3 vaccines are in preclinical development.
both \textit{cpts}248/955 and \textit{cpts}530/1009 were attenuated in adults and seropositive children, neither was sufficiently attenuated in seronegative children to permit evaluation in very young infants [61].

The \textit{cpts}248/404 vaccine was, however, subsequently evaluated in children and infants as young as 1 month of age. The \textit{cpts}248/404 vaccine was highly attenuated in these infants but caused nasal congestion that, in some instances, interfered with feeding and sleeping [50]. This vaccine also induced serum neutralizing antibody and RSV IgG and IgA responses in children >6 months of age, whereas predominantly IgA responses were observed in infants ≤6 months of age [50]. Surveillance conducted in the RSV season after vaccination did not demonstrate disease enhancement but provided preliminary evidence of protection against symptomatic RSV infection [50].

Genetically engineered (cDNA-derived), live, attenuated RSV vaccines. The recovery of infectious virus from cDNA clones of RSV [62] has provided insight into the genetic basis of attenuation of biologically derived vaccines and has hastened the development of additional live, attenuated vaccine candidates by means of recombinant technology [1, 63]. Mutations present in \textit{cp} RSV and 6 of its \textit{ts} derivatives were inserted into wild-type RSV individually and in combination, and the majority of attenuating mutations were found to occur in the polymerase (\textit{L}) gene, with a notable exception being the 404 mutation in the \textit{M} gene start signal (figure 1A) [1, 63–65]. Using this information, attenuating mutations from biologically derived vaccines have been combined to produce further attenuated vaccine candidates [1, 30, 43, 63, 66]. Deletion of a nonessential gene (\textit{SH}, \textit{NS1}, \textit{NS2}, or \textit{M2-2} [67]), in combination with \textit{cp} and \textit{ts} mutations, might also produce a highly attenuated, genetically stable vaccine (figure 1A) [30, 68, 69].
Recombinant RSV vaccines containing cp, ts, and deletion mutations are currently being evaluated in clinical trials [70]. Because foreign genes can also be inserted into an rRSV genome [71], a cDNA-derived bivalent RSV vaccine might be developed that contains the G genes from RSV A and RSV B [71–73]. Recombinant technology also provides the opportunity to create chimeric viruses containing RSV F and G genes, with internal genes provided by related respiratory viruses such as PIV-3 (see below).

**Subunit RSV vaccines.** RSV F and G, the viral glycoproteins that induce neutralizing and protective antibodies [1], have been evaluated as potential candidate vaccines. Subunit vaccines are most likely to be useful for immunization of older persons and high-risk children and might also be used for maternal immunization. Vaccines which have recently been evaluated in clinical trials include purified F glycoprotein (PFP-1, PFP-2, and PFP-3) [74–81]; copurified F, G, and matrix (M) proteins [82]; and BBG2Na, a peptide from the G glycoprotein conjugated to the albumin-binding domain of streptococcal protein G (table 1) [83–86]. A chimeric RSV FG fusion protein vaccine was evaluated in phase I trials in adults but is not being investigated further.

RSV PFP-1, PFP-2, and PFP-3 vaccines have been evaluated in healthy adults, in children >12 months of age with and without chronic underlying pulmonary disease (i.e., chronic lung disease due to premature birth or cystic fibrosis [CF]), in institutionalized and ambulatory older persons, and in pregnant women [75–80, 87]. These vaccines contain the purified F glycoprotein (PFP) adsorbed to aluminum hydroxide (PFP-1 and PFP-2) or aluminum phosphate (PFP-3). The PFP vaccines have been well tolerated in these populations; acute reactions were minimal, and enhanced disease was not observed [75–81, 87]. In addition, ≥4-fold increases in RSV neutralizing antibody titers were observed in 50%–75% of vaccinees who received 50 μg of vaccine, depending on the preimmunization level of neutralizing antibodies. A meta-analysis of PFP-1 and PFP-2 studies involving adults and children concluded that these vaccines appeared to reduce the incidence of RSV infection, although the incidence of RSV LRTI was not significantly reduced. The heterogeneity of the populations studied raised doubts about the validity of this conclusion [88].

Most recently, a phase I study of PFP-2 was conducted with pregnant women, and a phase III study of PFP-3 was conducted with children with CF. Between weeks 30 and 34 of uncomplicated pregnancies, 35 women were randomized to receive either 50 μg of PFP-2 vaccine or saline placebo. PFP-2 was well tolerated and immunogenic in these women. All 35 infants were healthy at birth, and there was no difference in neonatal and perinatal outcomes between vaccine and placebo recipients. During RSV season, there were no increases in the rate or severity of respiratory illnesses among infants of vaccine recipients. The geometric mean titers of RSV F antibody were 4-fold higher in the children born of immunized mothers than in those born of placebo recipients at birth and at 2 and 6 months after delivery. Levels of RSV neutralizing antibody were not reported [81].

The efficacy trial of PFP-3 vaccine in children with CF was conducted on the basis of previous studies that demonstrated reductions in the number of LRTI episodes (although not in the number of episodes of RSV infection) in 34 children with CF who received PFP-2 vaccine or placebo [89]. In the phase III trial, 298 children 1–12 years of age with CF were vaccinated with 30 μg of PFP-3/aluminum phosphate vaccine or aluminum phosphate alone. The vaccine was safe, well tolerated, and immunogenic, with 67% and 55% of subjects showing a 4-fold increase in neutralizing antibody titer to RSV A and RSV B, respectively. However, there were no statistically significant differences in the frequency of LRTI episodes between vaccine and placebo recipients (V. LaPosta, personal communication).

A subunit vaccine consisting of copurified F, G, and M proteins from RSV A has been administered intramuscularly to healthy adults, with either alum or polydicarboxylatophenoxyphosphazene as an adjuvant (table 1) [82]. Both formulations of the vaccine were well tolerated and comparably immunogenic, with 2- and 4-fold increases in neutralizing antibody titers detected in 96%–100% and 76%–83% of vaccinees, respectively. In this previously primed population, neutralizing antibody responses to RSV A and RSV B were detected with comparable frequency. Studies of this vaccine in other populations are in progress.

BBG2Na is a prokaryotically expressed fusion protein that consists of the central conserved region of the G glycoprotein from the RSV A Long strain (residues 130–230) fused to the albumin binding domain of streptococcal protein G, which acts as a carrier protein [84, 86, 90, 91]. Residues 158–190 are conserved among RSV A isolates, and 163–174 are conserved among RSV A and RSV B isolates [92]. Despite induction of modest levels of RSV neutralizing antibody [85], BBG2Na protected rodents against challenge with RSV, and serum samples obtained from RSV-seropositive individuals were found to react with peptides derived from this region [93]. In phase I trials, 10-, 100-, or 300-μg doses of BBG2Na in alum were well tolerated in healthy young adults [86]. Four weeks after vaccination, the 100- and 300-μg doses of vaccine induced ≥2-fold increases in neutralizing antibody in 33%–71% of vaccinees [86]. In phase II studies of this vaccine, 2 healthy young adults developed type III hypersensitivity (purpura). An efficacy trial of BBG2Na in older persons was recently completed.

**Biologically derived, live, attenuated PIV-3 vaccines.** Two biologically derived PIV-3 vaccine candidates have been evaluated in clinical trials (table 1). HPIV-3 cp45 (cp45) was derived from the JS wild-type strain of hPIV-3 by 45 passages in primary
African green monkey kidney cells at low temperatures [94]. The cp45 virus contains 20 point mutations (5 of which are silent) that differentiate it from the JS wild-type strain. Three of these mutations occur in the L (polymerase) protein of cp45 and contribute substantially to the attenuation and temperature-sensitive phenotypes of this candidate vaccine (figure 1B) [95, 96]. The second candidate, bovine parainfluenza virus type 3 (bPIV-3), is a host range variant that is closely related to hPIV-3 [97]. Comparison of bPIV-3 and hPIV-3 sequences demonstrated that the hemagglutinin-neuraminidase (HN) and fusion (F) proteins, the protective antigens of PIV-3, share amino acid identities of 74.6% and 78.5%, respectively [98]. The viruses are 25% related antigenically by cross-neutralization assays. Both cp45 and bPIV-3 have been evaluated in phase I and phase II trials in adults, hPIV-3-seropositive children, hPIV-3-seronegative children, and infants as young as 1 month (cp45) or 2 months (bPIV-3) of age [46, 51, 99–101]. Both candidates were overattenuated in adults and in seropositive children but were highly infectious in seronegative children and infants [46, 51, 99–101]. There were no significant differences in the incidence of respiratory or febrile illnesses among seronegative vaccinees and placebo recipients. Otitis media occurred more frequently among seronegative children vaccinated with hPIV-3 cp45 in phase I trials than among placebo recipients, but this was not observed in infants vaccinated with hPIV-3 cp45 [51], nor in phase II trials of hPIV-3 cp45 in seronegative children (R.B. Belshe et al., unpublished data).

Although both vaccines elicited hemagglutination-inhibiting antibody responses against hPIV-3 in most vaccinated seronegative children, the magnitude of the response to hPIV-3 was lower in children who received bPIV-3, which is consistent with the limited antigenic relatedness of the bovine and human PIV-3 HN [97]. For this reason, recombinant bovine/human PIV-3 candidate vaccines containing the hPIV-3 HN and F genes and 1 or more bPIV-3 internal genes are being developed (see below). Infants receiving a live, attenuated PIV-3 vaccine will likely require 2 or more doses of vaccine to achieve the level of immunity conferred by natural infection and, at least for cp45, the optimal interval between doses is likely to be <3 months [51].

Sendai virus, a murine PIV-1 virus, has been evaluated as an hPIV-1 vaccine candidate. African Green monkeys inoculated with 10<sup>3</sup>, 10<sup>4</sup>, or 10<sup>6</sup> egg infectious dose 50 units of Sendai virus were protected against challenge with hPIV-1 [102]. However, more recent studies have shown that Sendai virus replicates as well or better than hPIV-1 in the upper and lower respiratory tracts of African Green monkeys and chimpanzees, which suggests that Sendai virus may not be sufficiently attenuated for use as an hPIV-1 vaccine [103].

**Genetically engineered, (cDNA-derived), live, attenuated PIV vaccines.** As with RSV, infectious rhPIV-3 (wild type and cp45) and bPIV-3 have been recovered from cDNA using reverse genetics [96, 104, 105]. Recombinant DNA technology has allowed rapid expansion of the number of vaccine candidates for hPIV-3 by systematically introducing point mutations into the genome of hPIV-3 (figure 1B) and by creating chimeric human-bovine PIV-3 viruses [106–109]. The most promising recombinant hPIV-3 vaccine candidates are human-bovine chimeric viruses. In rhPIV-3–N<sub>h</sub>, the nucleocapsid (N) protein of hPIV-3 has been replaced with that of bPIV-3. In nonhuman primates, replication of this virus was as restricted in the upper and lower respiratory tracts as the parent bPIV-3 strain was, whereas protection against challenge was comparable to that induced by vaccination with hPIV-3 [107]. rhPIV-3–N<sub>b</sub> is currently being evaluated in phase I clinical trials (table 1). Two groups have reported development of chimeric bovine-human PIV-3 viruses that contain the internal genes of bPIV-3 and the HN and F genes of hPIV-3 [105, 109]. Preclinical studies have shown that these recombinant bovine-human PIV-3 viruses are attenuated but protect against challenge with wild-type hPIV-3.

Recombinant DNA technology has also hastened the development of vaccines for hPIV-1 and hPIV-2. Recombinant, chimeric parainfluenza viruses containing the internal genes of hPIV-3 (either wild type or cp45) and the HN and F genes of hPIV-1 (rPIV3–1) or hPIV-2 (rPIV3–2) [29, 110–112] have been evaluated in rodents and nonhuman primates. Although rPIV3–1 infected hPIV-3–immune hamsters, its replication, immunogenicity, and protective efficacy against hPIV-1 challenge was somewhat diminished, possibly mediated by resistance conferred by T cell immunity to shared internal proteins [30]. Because children would likely be vaccinated against hPIV-3 before hPIV-1 or hPIV-2, rPIV-1 might not be an optimal hPIV-1 vaccine candidate. In contrast to the experience with rPIV3–1, rPIV3–2 was overattenuated and did not protect against PIV-2 challenge in hamsters. Most recently, rhPIV1 rhPIV-2 viruses (rPIV-1 and rPIV-2) have been recovered from cDNA [113, 114]. These recombinant viruses may prove to be instrumental in the development of hPIV-1 and hPIV-2 vaccines.

Recombinant DNA technology has also been used to create novel chimeric PIV-3 candidate vaccines for RSV and measles virus [115–118]. The recombinant chimeric bovine PIV-3 expressing the F and N proteins of human PIV-3 (rPIV3–F[H]HN[H]) was used as the backbone into which the F and G ORF of RSV A or RSV B were inserted [117, 118], either singly (F or G alone rB/HPIV3–G<sub>a</sub>, rB/HPIV3–F<sub>a</sub>, rB/HPIV3–G<sub>b</sub>, rB/HPIV3–F<sub>b</sub>) or in pairs (F and G, rB/HPIV3–G<sub>a</sub>+G<sub>b</sub>, rB/HPIV3–F<sub>a</sub>+F<sub>b</sub>). In rhesus monkeys, these viruses are attenuated and highly immunogenic, inducing RSV neutralizing antibody titers comparable to those induced by wild-type RSV. Intranasal immunization of young infants with a multivalent
vaccine comprised of rB/HPIV3-GA+F and rB/HPIV3-GB+F might provide protection against disease caused by RSV A, RSV B, and hPIV-3 in this vulnerable population. A similar strategy is being used to develop vaccines for other Paramyxoviruses, such as human metapneumovirus [119].

CONCLUSIONS

Significant progress has been made in the development of vaccines against RSV and hPIVs. At the time of this writing, 2 types of RSV vaccines were being evaluated in clinical trials: subunit vaccines for vaccination of older persons, RSV-seropositive children at high risk for severe RSV disease, and pregnant women; and live, attenuated recombinant vaccines, which would be used primarily for immunization of young infants and, possibly, older persons. The hPIV-3 candidate vaccine cp45 was shown to be safe and immunogenic in young children in phase I and II trials and should be evaluated in phase III efficacy studies. Bovine/human PIV chimeras may also prove to be attractive hPIV candidate vaccines, although clinical evaluation of these is just beginning. The availability of recombinant technology should allow further refinement of existing live, attenuated rRSVs and rhPIVs to produce engineered vaccines that are satisfactorily attenuated, immunogenic, and phenotypically stable.

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References

29. Tao T, Skiadopoulos MH, Durbin AP, Davoodi F, Collins PL, Murphy BR. A live attenuated chimeric recombinant parainfluenza virus (PIV) encoding the internal proteins of PIV type 3 and the surface glyco-
47. Crowe JE Jr, Bui PT, Siber GR, Elkins WR, Chanock RM, Murphy BR. Cold-passaged, temperature-sensitive mutants of human respiratory syncytial virus (RSV) are highly attenuated, immunogenic, and protective in seronegative chimpanzees, even when RSV antibodies are infused shortly before immunization. Vaccine 1995; 13:847–55.
54. Juhasz K, Whitehead SS, Boulanger CA, Firestone CY, Collins PL, Murphy BR. The two amino acid substitutions in the L protein of cp530/1009, a live-attenuated respiratory syncytial virus candidate...
vaccine, are independent temperature-sensitive and attenuation mutations. Vaccine 1999; 17:1416–24.
adapted human, and wild-type human parainfluenza type 3 viruses in adult volunteers and in chimpanzees. J Clin Microbiol 1991; 29: 1175–82.


109. Schmidt AC, McAuliffe JM, Murphy BR, Collins PL. Recombinant bovine/human parainfluenza virus type 3 (B/HPIV3) expressing the respiratory syncytial virus G and F proteins can be used to achieve simultaneous mucosal immunization against RSV and PIV3. J Virol 2001; 75:4594–603.
