Determinants of the Host Range Restriction of Replication of Bovine Parainfluenza Virus Type 3 in Rhesus Monkeys Are Polygenic

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Received 19 July 2002/Accepted 7 October 2002

The Kansas strain of bovine parainfluenza virus type 3 (BPIV3) is 100- to 1,000-fold restricted in replication in the respiratory tracts of nonhuman primates compared to human PIV3 (HPIV3), an important pathogen of infants and young children. BPIV3 is also restricted in replication in human infants and children, yet it is immunogenic and is currently being evaluated in clinical trials as a vaccine candidate to protect against illness caused by HPIV3. We have examined the genetic basis for the host range attenuation phenotype of BPIV3 by exchanging each open reading frame (ORF) of a recombinant wild-type HPIV3 with the analogous ORF from BPIV3, with the caveats that the multiple ORFs of the P gene were exchanged as a single unit and that the F and N genes were exchanged as a unit. Recombinant chimeric bovine-human PIV3s were recovered from cDNA, and the levels of viral replication in vitro and in the respiratory tract of rhesus monkeys were determined. Recombinant chimeric HPIV3s bearing the BPIV3 N or P ORF were highly attenuated in the upper and lower respiratory tracts of monkeys, whereas those bearing the BPIV3 M or L ORF or the F and HN genes were only moderately attenuated. This indicates that the genetic determinants of the host range restriction of replication of BPIV3 for primates are polygenic, with the major determinants being the N and P ORFs. Monkeys immunized with these bovine-human chimeric viruses, including the more highly attenuated ones, developed higher levels of HPIV3 hemagglutination-inhibiting serum antibodies than did monkeys immunized with BPIV3 and were protected from challenge with wild-type HPIV3. Furthermore, host range determinants could be combined with attenuating point mutations to achieve an increased level of attenuation. Thus, chimeric recombinant bovine-human PIV3 viruses that manifest different levels of attenuation in rhesus monkeys are available for evaluation as vaccine candidates to protect infants from the severe lower respiratory tract disease caused by HPIV3.

Human parainfluenza virus type 3 (HPIV3) and its animal counterpart, bovine PIV3 (BPIV3), are enveloped, nonsegmented negative-strand RNA viruses of the genus Respirovirus in the family Paramyxoviridae (4, 26). HPIV3 is a common cause of respiratory disease in infants (8, 28), and presently a licensed vaccine is not available. HPIV3 and BPIV3 share a moderate to high level of nucleotide and amino acid sequence identities (2) and are 25% antigenically related by cross-neutralization studies (6). The Kansas strain of BPIV3 is restricted in replication in the respiratory tracts of humans and nonhuman primates (6, 23) and is being evaluated as a candidate vaccine to prevent the severe lower respiratory tract disease caused by infection of infants and young children with HPIV3 (22–24, 27).

BPIV3 and HPIV3 have the same genome organization (4), encoding nine proteins from six contiguous genes. These nine proteins include the three nucleocapsid-associated proteins, the nucleoprotein (N), the phosphoprotein (P) and the large polymerase protein (L), which form the nucleocapsid complex and the associated polymerase; an internal matrix protein (M); and the fusion (F) and hemagglutinin-neuraminidase (HN) envelope glycoproteins. The P gene also encodes three additional polypeptides by use of an alternative reading frame for the accessory protein C or by pseudotemplated nucleotide insertion (RNA editing) for the generation of the accessory V and D polypeptides. By analogy to other parainfluenza viruses, one or more of the HPIV3 accessory proteins might be involved in regulation of viral RNA synthesis and function as antagonists of the host antiviral interferon response (15).

While the cis-acting transcription and replication control regions are highly conserved between the two viruses, the amino acid sequence identities between homologous proteins of the two viruses range from approximately 59% for the P protein to 90% for the M protein (2). Thus, the host range determinants of the attenuation phenotype of BPIV3 for primates are likely encoded by one or more of the BPIV3 open reading frames (ORFs) rather than the highly conserved cis-acting regulatory sequences. Previously, the N ORF of BPIV3 was shown to confer a high level of attenuation when substituted for the analogous ORF in HPIV3 (1), and the BPIV3 F and HN genes were shown to confer a moderate level of attenuation when substituted for their F and HN counterparts in HPIV3 (34). In the present study, we have extended these observations to show that the P and, to a lesser extent, the M and L ORFs can also confer attenuation when substituted for the homologous HPIV3 ORF in the HPIV3 genome, demon-
described previously (12, 20, 35); titers are expressed as 50% tissue culture
plaque forming units (TCID50/ml) at 32°C and at graded temperatures from 35 to 40°C were
determined by titration on LLC-MK2 monolayer cultures as described above.

Recovery of rHPIV3s with BPIV3 ORF substitutions. The Kansas strain of HPIV3 was
restricted in replication in the respiratory tracts of humans and other primates. To identify the
host range attenuation phenotype of BPIV3, as described previously (11, 39). For some groups, the
data presented includes data collected from two previous studies (1, 34). Groups of
animals in the present and previous studies were infected with identical virus
preparations, and the samples were collected and quantitated by using similar
procedures.

RESULTS
Recovery of rHPIV3s with BPIV3 ORF substitutions. The Kansas strain of HPIV3 was
restricted in replication in the respiratory tracts of humans and other primates. To identify the
host range attenuation phenotype of BPIV3 in primates, the antigenomic cDNA of HPIV3 was
modified to contain the N, P, M, or L ORF of BPIV3 in place of the analogous HPIV3 ORF (Fig. 1). In addition, the F and
HN genes were transferred together as a pair to replace their
HPIV3 counterparts, as described previously (34).
Each of the chimeric bovine-human HPIV3s containing the N, P, M, F, and HN, or L ORFs of BPIV3 in place of the analogous HPIV3 ORF (Fig. 1). In addition, the F and
HN genes were transferred together as a pair to replace their
HPIV3 counterparts, as described previously (34).

To confirm the identity and structure of each chimeric recombinant, vRNA was
isolated from each biologically cloned virus as described previously (36) and was used as the template for reverse transcription-PCR (RT-PCR) to amplify the
region of interest. Thus, an RT-PCR fragment containing the substituted ORF flanked by the backbone HPIV3 sequence was generated, and the amplified product was analyzed by restriction endonuclease digestion and DNA sequencing
to confirm its structure (36).

Recovery of rHPIV3s in vitro. Multicycle growth kinetics of wild-type and chimeric rHPIV3s were determined in LLC-MK2 cells. Each virus tested was inoculated in triplicate into LLC-MK2 monolayers in six-well plates at a multi-
plicity of infection (MOI) of 0.01, and the cultures were incubated at 32°C. Medium (0.5 ml) from each well was harvested and replaced with 0.5 ml of fresh medium at 0 h and at 24-h intervals thereafter for 5 days postinfection. For determination of temperature sensitivity of replication in vitro, the virus titers (TCID50/ml) at 32°C and at graded temperatures from 35 to 40°C were deter-
mained by titration on LLC-MK2 monolayer cultures as described above.

Primate studies. Rhesus monkeys (Macaca mulatta) that were negative for
serum hemagglutination-inhibiting (HAI) antibody to HPIV3 were inoculated
simultaneously by the intranasal (i.n.) and intratracheal (i.t.) routes with 1 ml per
site of L15 medium containing 105 TCID50/ml of virus, as described previously (1, 34). Nasopharyngeal (NP) swab samples were collected on days 1 through 10 postinfection, and tracheal lavage (TL) samples were collected on days 2, 4, 6, 8, and 10 postinfection. Virus present in the NP and TL specimens was quantified by
plaque assay as described previously.

FIG. 1. Schematic diagram (not to scale) of the genome structures of recombinant HPIV3s with BPIV3 ORF substitutions and of their parental HPIV3 wild-type and BPIV3 Kansas viruses. BPIV3 se-
quences are indicated by the shaded areas (E), and HPIV3 sequences are indicated by the clear areas (C). The percent amino acid identity for the D and V proteins is provided for the portion of the D and V
protein sequence that is present downstream of the P gene editing site and is represented in both viruses (2). \( V \), relative position of the
T1711I point mutation in rHPIV3-L-T1711I.
The ability to recover each of the chimeric recombinant bovine-human viruses indicates a substantial degree of compatibility of the substituted BPIV3 proteins with the HPIV3 proteins as well as with the cis-acting sequences of the HPIV3 genome.

Replication of chimeric rHPIV3s in vitro. For each chimeric virus, the possibility existed that the substituted BPIV3 protein might not be fully compatible with the encoded proteins and cis-acting sequences of the HPIV3 backbone. If such a partial incompatibility exists, it should be manifested by a decrease in the replication of the chimeric virus in a cell line such as LLC-MK2, which is permissive for both parent viruses.

Therefore, the kinetics of replication of each chimeric rHPIV3 in vitro was compared to that of their wild-type rHPIV3 and BPIV3 parent viruses by infecting LLC-MK2 cells at an MOI of 0.01 and measuring virus yield at 24-h intervals. Except for rHPIV3-LB(T1711I), all of the chimeric rHPIV3s bearing BPIV3 ORF substitutions grew at a rate similar to that of their parent viruses (Fig. 2), and all of the chimeric viruses grew to over 10⁷ TCID₅₀/ml by day 5 postinfection. This confirmed that each of the substituted wild-type BPIV3 proteins exhibited a high degree of compatibility with the proteins and cis-acting signals of the HPIV3 backbone. In contrast, the restricted replication of rHPIV3-LB(T1711I) in vitro indicates that one or both of the amino acid substitutions in its BPIV3 polymerase protein are attenuating in vitro.

Replication of chimeric viruses at permissive and restrictive temperatures. It was possible that one or more of the substituted BPIV3 proteins might have a marginal incompatibility with a protein in the HPIV3 backbone that was not detectable at 32°C but that might become evident at elevated temperatures. It also was of interest to further characterize the restricted replication exhibited by the mutant rHPIV3-LB(T1711I) virus (Fig. 2). To determine if the ORF substitutions in each rHPIV3 altered the ability of these viruses to grow at elevated temperatures, the level of temperature sensitivity of replication of each chimeric rHPIV3 was compared to that of the parent viruses and to that of rHPIV3cp45, which is a well-characterized ts and attenuated candidate HPIV3 vaccine that was previously shown to be appropriately attenuated and immunogenic in humans and nonhuman primates (19, 20, 24). The wild-type and chimeric rHPIV3s were evaluated for their abilities to grow on LLC-MK2 cells at the permissive temperature of 32°C and at a range of higher temperatures (Table 1). Surprisingly, both rHPIV3-Lₐ and rHPIV3-Lₐ(T1711I) were highly ts, with rHPIV3-Lₐ(T1711I) being more ts than either rHPIV3-Lₐ or rHPIV3cp45. As noted above, the BPIV3 L ORF present in the rHPIV3-Lₐ(T1711I) virus contained two amino acid coding changes relative to wild-type BPIV3, and it was of interest to determine which one, or both, was responsible for the increased ts phenotype of rHPIV3-Lₐ(T1711I) compared to rHPIV3-Lₐ. An alternative viral clone of rHPIV3-Lₐ(T1711I) was identified that contained the T1711I substitution but not the A425V mutation. This virus had the same shutoff temperature as rHPIV3-Lₐ(T1711I) (data not shown), indicating that the

![Graph](image1.png)

**FIG. 2.** Multistep growth curves of chimeric rHPIV3s. LLC-MK2 monolayers were infected in triplicate with the indicated PIV3 at an MOI of 0.01 and incubated at 32°C. Aliquots of the medium supernatants were harvested at 24-h intervals, and the virus titer was quantified on LLC-MK2 monolayers at 32°C.

![Table](image2.png)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mean titer (log₁₀ TCID₅₀/ml) at 32°C</th>
<th>Mean log₁₀ reduction in virus titer at the indicated temperature (°C)</th>
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<td>—</td>
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<td>0.0</td>
</tr>
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<td>1.6</td>
</tr>
<tr>
<td>rHPIV3 cp45</td>
<td>7.8</td>
<td>0.9</td>
</tr>
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*Data are the means of results of two or more titrations. Values in bold type are at or above the shutoff temperature, which is defined as a 100-fold or greater reduction in titer compared to the titer at 32°C after adjusting for the reduction in replication of the rHPIV3 wild-type control virus. rHPIV3cp45 is included as a well-characterized ts control virus.

—, Data not gathered.
T1711I mutation alone is responsible for the increased level of temperature sensitivity of rHPIV3-LB T1711I. 

**Introduction of a BPIV3 ORF into wild-type HPIV3 can attenuate rHPIV3 for rhesus monkeys.** To determine the effect of each ORF or gene substitution on the ability of the chimeric rHPIV3 to replicate in the upper and lower respiratory tracts of rhesus monkeys, each chimeric virus was administered i.n. and i.t. to rhesus monkeys at a dose of 10⁵ TCID₅₀ per site. The levels of replication of the chimeric viruses was compared to those of the wild-type rHPIV3 and BPIV3 parental viruses. The kinetics of replication for each virus was determined in the upper and lower respiratory tracts of rhesus monkeys over a 10-day period (Fig. 3). This was expressed as the mean peak titer, which gives an indication of the peak titer of virus shedding, and as the mean of the sum of the daily titers, which gives a comparison of the virus load over the 10-day period (Table 2). The mean sums of the titers are generally higher in the NP specimens than in the TL specimens, since the NP specimens were collected daily whereas the TL specimens were collected only every other day. 

Each BPIV3 ORF conferred restriction of replication in the upper or lower respiratory tracts of rhesus monkeys when substituted for the analogous ORF in HPIV3 (Fig. 3), demonstrating that the host range attenuation phenotype of BPIV3 is polygenic. By comparison of the mean peak titers of virus replication in the upper respiratory tract (Table 2), the chimeric rHPIV3s fell into three groups: (i) viruses bearing the BPIV3 M or L ORF or the F and HN genes, whose replication was restricted approximately 16- to 32-fold, (ii) viruses bearing the BPIV3 N or Lₜ₁₇₁₁₁ ORF, which exhibited 40- to 100-fold restrictions of replication, and (iii) the chimeric HPIV3 with the BPIV3 P ORF substitution, which exhibited a 1,000-fold restriction in replication, suggesting that the BPIV3 P ORF is the major contributor to the attenuation phenotype. The level of replication of rHPIV3-Pₐ was even lower than that of its BPIV3 parent virus in the upper respiratory tract, suggesting that some of its restricted replication in vivo may also be due to a gene incompatibility effect that was not evident in vitro. The pattern was somewhat different in the lower respiratory tract, where the level of attenuation was generally lower. Virus bearing the BPIV3 L gene or the F and HN gene pair was attenuated sixfold or less, while viruses bearing the N, M, or Lₜ₁₇₁₁₁ gene were attenuated 26- to 32-fold, and virus bearing the BPIV3 P gene was attenuated 100-fold, relative to wild-type rHPIV3 (Table 2). Because rHPIV3-Lₜ₁₇₁₁₁ was attenuated for replication in vitro and was highly ts, the level of attenuation of this virus observed in vivo likely is due to a combination of restricted replication specified by host range sequences and that specified by its high level of temperature sensitivity (the body temperature of rhesus monkeys is about 39°C). Interestingly, rHPIV3-Mₐ, rHPIV3-Fₐ HNₐ, and rHPIV3-Lₜ�ₐ, which were attenuated for replication in the upper respiratory tract, were not significantly restricted in the lower respiratory tract.
Chimeric rHPIV3s containing BPIV3 ORF substitutions are immunogenic and protect rhesus monkeys from challenge with wild-type HPIV3. Protection against repeated infection with HPIV3 is associated with high levels of antibodies against the major antigenic determinants, the F and HN glycoproteins (9, 29). Although the BPIV3 and HPIV3 F and HN proteins are 75 to 79% identical in amino acid sequence (2), they are only 25% antigenically related (6). To evaluate the immunogenicity of the rHPIV3s, serum samples were collected prior to infection and on day 28 or 31 following infection with the chimeric rHPIV3s or their parent viruses, and the levels of serum HAI antibodies to HPIV3 were determined. Each of the chimeric recombinants bearing the HPIV3 F and HN glycoproteins induced a high level of HAI antibodies to HPIV3, whereas rHPIV3-FGHNB and the BPIV3 parent virus bearing the BPIV3 glycoproteins induced 8- to 16-fold less HAI antibody reactive with the human virus. rHPIV3-LH T711I, rHPIV3-NB, and rHPIV3-PB replicated approximately 2- to 10-fold less efficiently in the respiratory tracts of rhesus monkeys compared to BPIV3, yet they induced approximately 4- to 32-fold more HPIV3 HAI antibodies, likely because they bear the homologous HPIV3 glycoproteins.

To evaluate the protective efficacy of the chimeric rHPIV3s, monkeys were challenged i.n. and i.t. with 10^3 TCID_{50} of wild-type HPIV3 (Table 2). NP and TL samples were collected at 2-day intervals for 10 days postchallenge, and the virus present in the samples was quantified on LLC-MK2 cells. Each of the chimeric recombinants tested afforded a high level of protection against HPIV3 replication, including the highly attenuated rHPIV3-PB and rHPIV3-LH T711I. Analysis of serum samples collected following the challenge showed that each of the groups developed a similar, high titer of HAI antibodies to HPIV3, indicating that all the monkeys had indeed been infected.

**DISCUSSION**

BPIV3 is highly restricted in replication in rhesus monkeys and in humans, providing an attenuation phenotype that is being used to develop live vaccines against HPIV3. The present study sought to determine the genetic basis of this attenuation by exchanging each of the HPIV3 ORFs with its corresponding BPIV3 counterpart and examining the effect of this exchange on the replication of each resulting chimeric virus in vitro and in rhesus monkeys. The findings indicated that each of the ORF substitutions attenuated rHPIV3 for rhesus monkeys and that there was a gradient of attenuation specified by the different ORFs. In general, chimeras containing a gene(s) in which the encoded protein is highly conserved between bovine and human PIV3 replicated to higher levels in vivo, whereas chimeras involving a gene(s) in which the encoded protein was less well conserved between bovine and human PIV3 replicated to lower titer.

The chimeric rHPIV3 bearing the BPIV3 P substitution, rHPIV3-PB, was highly attenuated in rhesus monkeys and, indeed, was somewhat more attenuated than the BPIV3 parent virus itself in the upper respiratory tract. Since rHPIV3-PB replicated in vitro as efficiently as its BPIV3 parent and was not
ts, it is reasonable to suggest that its restriction of replication in vivo primarily reflects host range differences rather than an incompatibility of bovine and human PIV3 proteins. Thus, it is clear that one or more of the proteins encoded by this gene complex, namely, the P protein itself and the C, D, or V accessory proteins encoded by alternative ORFs, is a major contributor to the overall attenuation of BPIV3 for rhesus monkeys. The P proteins of BPIV3 and HPIV3 share approximately 59% amino acid identity and have the highest number of amino acid differences (247 amino acids) of all of the PIV3 polypeptides. The C, D, and V accessory proteins share approximately 72, 30 and 56% amino acid identity, respectively, which corresponds to 56, 92, and 35 amino acid differences, respectively (2).

One function of the accessory proteins of parainfluenza viruses is to counteract the antiviral effects of the host’s interferon system (14, 16, 17). The loss of this anti-interferon genes in negative stranded viruses by functional deletion is often associated with attenuation of replication in vivo (14, 15, 18). The accessory proteins of BPIV3 and HPIV3 have not yet been directly demonstrated to have anti-interferon activities, but the deletion of these genes is attenuating (13), making it likely that one or more of these PIV3 accessory proteins functions as an antagonist of the interferon system. Furthermore, it is possible that one or more of the BPIV3 genes in the P/C/D/V complex has evolved to inhibit the bovine interferon response in a host-specific fashion such that this anti-interferon protein might not function efficiently in primates. Thus, part of the host range attenuation phenotype of the BPIV3 P gene complex might be a consequence of the host specificity of one or more encoded host defense antagonists.

The rHPIV3-Nb, rHPIV3-Mb, rHPIV3-FbHNb, and rHPIV3-Lb chimeric viruses were each attenuated in the upper or lower respiratory tract, suggesting that the N and L genes, the F and HN pair of genes, and to a lesser extent the M gene each possess host range determinants of attenuation. The observations that the rHPIV3-Nb, rHPIV3-Mb, rHPIV3-FbHNb, and rHPIV3-Lb chimeric viruses are not significantly restricted in replication in vitro suggest that each of the substituted wild-type BPIV3 proteins functioned efficiently in the context of the HPIV3 proteins and cis-acting signals and that the attenuation observed in vivo for each substitution likely is not due to an incompatibility between the BPIV3 and HPIV3 components, but rather represents an authentic host range restriction. This is also suggested by the finding that some of these viruses were restricted for replication in the upper but not the lower respiratory tract. We do note that there was evidence of a possible small effect of incompatibility between the imported BPIV3 L ORF and the HPIV3 genetic background, since the rHPIV3-Lb chimera was found to be ts at elevated temperatures in vitro. This likely reflects a reduction in the efficiency of interaction between the BPIV3 L protein and the HPIV3 components at increased, nonphysiologic temperature. Whether this contributes to the attenuation of this chimeric virus in vivo is unknown. The finding that rHPIV3-Lb replicates to the same level as wild-type HPIV3 in the warmer lower respiratory tract but is restricted for replication in the cooler upper respiratory tract suggests that temperature sensitivity or viral protein incompatibility does not play a major role in its restriction of replication in primates. The lack of a significant level of attenuation conferred in the lower respiratory tract by the BPIV3 M, L, and F and HN ORFs indicates that the host range restriction specified by these genes may be tissue specific. Clearly, the host range restriction of replication of BPIV3 is polygenic, with the N, P, M, and L genes and the F and HN pair of genes each possessing host range determinants of attenuation. Since the host range restriction of replication of BPIV3 is specified by at least five genes, the attenuation phenotype of this virus should be highly stable phenotypically following replication in humans, and preliminary observations indicate that this is indeed the case (23).

Although the genetic stability of BPIV3 is a desirable characteristic for a live vaccine against HPIV3, the antigenic difference between BPIV3 and HPIV3 would reduce its protective efficacy against HPIV3. This was illustrated in the present study by the lower level of HPIV3-reactive HAI antibodies induced by rHPIV3-FbHNb versus wild-type rHPIV3. Thus, neither BPIV3 nor rHPIV3-FbHNb would be an optimal HPIV3 vaccine candidate, since they bear the major protective antigens of BPIV3 rather than HPIV3. In contrast, each of the other chimeric viruses described here bears the HPIV3 F and HN antigenic determinants combined with host range restriction determinants of BPIV3. Although several of these chimeric HPIV3s (rHPIV3-Nb, rHPIV3-Pb, and rHPIV3-Lb) were moderately to highly attenuated in vivo, they induced a higher level of serum HAI antibodies than BPIV3 and thus may prove to be useful as candidate vaccines for HPIV3. A chimeric virus, rBPIV3-FbHNb, in which BPIV3 was used as the backbone and its F and HN genes were replaced by those of HPIV3, has also been generated and is being evaluated as a vaccine candidate (21, 34). This vaccine candidate should also be phenotypically stable, since it possesses four of the BPIV3 genes that individually specify restricted replication in primates.

The high level of attenuation of rHPIV3-Lb T1711I in rhesus monkeys reflects the additivity of the restriction of replication specified by host range sequences of its L gene and one or both of the T1711I and A425V point mutations. The T1711I mutation was shown to confer the ts phenotype in vitro and thus likely also is responsible for the attenuation phenotype in vivo, although a contribution by the A425V mutation cannot be excluded at this time. Although the individual contributions of these two mutations to attenuation in vivo remain to be defined, the increased attenuation of rHPIV3-Lb T1711I versus rHPIV3-Lb illustrates that ts and host range determinants of attenuation can be combined to fine-tune the level of attenuation of a candidate vaccine virus.

Several other animal or avian viruses, such as the bovine and rhesus rotaviruses, avian influenza A viruses, bovine respiratory syncytial virus, and vaccinia virus (whose natural host is unknown), have a host range restriction of replication in humans or in nonhuman primates and are considered “Jennerian” vaccine candidates able to protect humans against the respective human pathogens (30). However, not all animal counterparts of a human viral pathogen exhibit a host range attenuation phenotype. For example, Sendai virus is a murine PIV1 that is approximately as similar in amino acid and nucleotide sequence to human PIV1 (HPIV1) as are the bovine and human PIV3s (33). However, Sendai virus replicates to the same level as wild-type PIV1 in primates (37), indicating that
it lacks the host range determinants of attenuation for primates manifested by BPIV3. Thus, a large number of amino acid differences between analogous viral polypeptides does not automatically confer a host range attenuation phenotype.

In the examples where host range attenuation in humans does occur, its genetic basis usually is not well understood and previously had been systematically evaluated only in a single case. An avian influenza A virus was evaluated as a live attenuated vaccine candidate for humans, since it was highly attenuated in nonhuman primates (32). Influenza A reassortant viruses bearing the hemagglutinin and neuraminidase genes of an H3N2 wild-type human virus and the set of six remaining RNA segments from the avian influenza A virus were found to be highly attenuated yet immunogenic in humans (31). Single-gene reassortant analysis showed that the avian influenza A virus NS, M, PB2, and PB1 gene segments each attenuated the wild-type H3N2 influenza A virus for humans (5). Interestingly, the NS1 protein encoded by the NS gene segment of the influenza A virus has been shown to be an interferon antagonist, and viruses lacking it are attenuated in vivo (38), raising the possibility that BPIV3 and the avian influenza A virus are each partially attenuated by functional loss of an interferon antagonist.

The attachment or fusion proteins can also play an important role in host range attenuation of respiratory viruses for primates. For example, receptor specificity has been shown to be a determinant for host range restriction of avian versus human influenza A viruses (7, 40). The host range attenuation phenotype of bovine respiratory syncytial virus, a virus that is highly restricted in replication in our closest animal relative, the chimpanzee, is partially mediated by its attachment G and/or fusion F protein (3). Therefore, it was not surprising to find that the BPIV3 attachment or fusion protein, i.e., the HN or F protein, could contribute to the host range attenuation phenotype of this virus for humans (34).

Thus, the genetic determinants of attenuation of two Jennen Vaccine candidates, BPIV3 in this study and a previously studied avian influenza A virus (5), have been defined for nonhuman primates or humans. In both cases, multiple genes contribute to the host range restriction of replication. This suggests that there may be multiple mechanisms by which this restriction of replication is achieved. This information provides a partial explanation for the safety and phenotypic stability of such viruses for use as vaccines, and sets groundwork for future studies that can specifically address the mechanism by which each of the BPIV3 proteins acts to restrict replication in the primate host.

ACKNOWLEDGMENTS

We thank Paul Duprex for a helpful PCR suggestion and Tammy Tobery, Ernest Williams, and Fatimah Davoodi for technical assistance.

REFERENCES


