Virology **297**, 153–160 (2002)
doi:10.1006/viro.2002.1416

w metadata, citation and similar papers at core.ac.uk
brought to you by CO
provided by Elsevier - Publisher Conne

Sendai Virus, a Murine Parainfluenza Virus Type 1, Replicates to a Level Similar to Human PIV1 in the Upper and Lower Respiratory Tract of African Green Monkeys and Chimpanzees

Mario H. Skiadopoulos,*[1] Sonja R. Surman,* Jeffrey M. Riggs,* William R. Elkins,† Marisa St. Claire,‡ Machiko Nishio,§ Dominique Garcin,§ Daniel Kolakofsky,§ Peter L. Collins,* and Brian R. Murphy*

*Respiratory Viruses Section and †Experimental Primate Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; ‡Bioqual, Inc, Rockville, Maryland 20850; and §Department of Genetics and Microbiology, University of Geneva School of Medicine, CMU, 9 Avenue de Champel, CH1211, Geneva, Switzerland

Received December 14, 2001; returned to author for revision January 31, 2002; accepted February 11, 2002

Human parainfluenza virus type 1 (HPIV1), a major cause of croup in infants and young children, accounts for 6% of hospitalizations for pediatric respiratory tract disease. The antigenically related Sendai virus, referred to here as murine PIV1 (MPIV1), is being considered for use as a live-attenuated vaccine to protect against HPIV1 (J. L. Hurwitz, K. F. Soike, M. Y., Sangster, A. Portner, R. E. Sealy, D. H. Dawson, and C. Coleclough, 1997, *Vaccine* 15(5), 533–540) and also as a recombinant vaccine vector expressing antigens to protect against viral disease in humans. However, in the 1950s MPIV1 was reported to have been isolated from humans, suggesting that zoonotic transmission might have occurred. It is therefore important to examine the ability of MPIV1 to replicate in nonhuman primates, i.e., surrogate hosts for humans. In the present study the level of replication of MPIV1 and HPIV1 was compared in African green monkeys and chimpanzees. Surprisingly, MPIV1 replicated as efficiently as HPIV1 in the upper and lower respiratory tract of African green monkeys at doses of 10^4 and 10^6 and replicated only slightly less efficiently at both sites in chimpanzees. African green monkeys immunized with MPIV1 were highly resistant to subsequent challenge with HPIV1 even though MPIV1 did not induce a detectable HPIV1-neutralizing antibody response. The high level of replication of MPIV1 observed in the upper and lower respiratory tract of these primates suggests that MPIV1 likely would require significant attenuation before it could be given to humans as a vaccine against HPIV1 or as a vaccine vector. Its ability to efficiently replicate in nonhuman primates suggests that MPIV1 lacks a significant host range restriction in primates and could theoretically cause zoonotic disease in humans. © 2002 Elsevier Science (USA)

INTRODUCTION

Paramyxoviridae is a large family of viruses that includes human parainfluenza virus types 1, 2, 3, 4A, and 4B (HPIV1, HPIV2, HPIV3, HPIV4A, and HPIV4B, respectively). Sendai virus (MPIV1), simian virus 5 (SV5), and bovine PIV3 (BPIV3) are the animal counterparts of HPIV1, HPIV2, and HPIV3, respectively. Human and animal PIV1s and PIV3s are classified together in the genus *Respirovirus*, and the PIV2s and PIV4s are classified in the genus *Rubulavirus*. The natural host of Sendai virus has not been clearly identified, but the virus is referred to here as murine PIV1 (MPIV1) because it infects mice efficiently, causes disease, and spreads readily to uninfected animals. SV5 was originally recovered from monkey tissue, but appears to be a canine virus. HPIV1, HPIV2, and HPIV3 cause severe lower respiratory tract disease that can lead to hospitalization of infants and young children (Chanock *et al.*, 2001). In a study of infants and children over a 20-year period, HPIV1, HPIV2, and HPIV3 were identified as etiologic agents responsible for 6.0, 3.2, and 11.5%, respectively, of hospitalizations for respiratory tract disease (Murphy *et al.,* 1988). Infection with parainfluenza viruses can also lead to otitis media in children (Heikkinen *et al.,* 1999). Thus, a vaccine is needed to protect against these viruses.

HPIV1 and MPIV1 are very similar in their nucleotide and amino acid sequences (Newman *et al.,* 2002). The major antigenic determinants, the F and HN glycoproteins, are 68 and 72% identical, respectively (Gorman *et al.,* 1990; Komada *et al.,* 1992; Lyn *et al.,* 1991). The antigenic relatedness that exists between MPIV1 and HPIV1 suggested that MPIV1 could be used as a Jennerian vaccine to protect against HPIV1 (Hurwitz *et al.,* 1997). In the Jennerian approach to vaccine development, immunization against a human virus is achieved using an antigenically related animal virus that is attenuated due to a natural host range restriction. There are a number of instances where an animal virus has been shown to be attenuated in humans and protective against a related human virus (Kapikian *et al.,* 1992; Karron *et al.,* 1995; Murphy and Chanock, 2001).

Pertinent to the parainfluenza viruses, BPIV3 is a promising Jennerian vaccine candidate against HPIV3.

[1] To whom correspondence and reprint requests should be addressed at NIH, Building 50, Room 6511, 50 South Drive, MSC 8007, Bethesda, MD 20892-8007. Fax: (301) 496-8312. E-mail: mskiadopoulos@niaid.nih.gov.

0042-6822/02 $35.00
© 2002 Elsevier Science (USA)
All rights reserved.

BPIV3, which is very similar to HPIV3 with regard to genome organization and shares extensive sequence and antigenic relatedness (Bailly *et al.,* 2000), is restricted in replication in the respiratory tract of rhesus monkeys and chimpanzees (Clements *et al.,* 1991; Coelingh *et al.,* 1988) as well as in seronegative human infants and children (Karron *et al.,* 1995). Despite its reduced level of replication in the respiratory tract of primates, BPIV3 is immunogenic and protective against challenge with HPIV3 and is currently being further evaluated in clinical trials for use as a vaccine to protect against HPIV3 (Karron *et al.,* 1995; Lee *et al.,* 2001).

With regard to PIV1, mice immunized with HPIV1 were partially protected against a lethal dose of MPIV1 (Sangster *et al.,* 1995) and, similarly, African green monkeys immunized with MPIV1 were protected against replication of HPIV1 following experimental challenge (Hurwitz *et al.,* 1997). However, in this previous study only the upper respiratory tract was sampled for MPIV1 replication, and the replication of HPIV1 was not examined in parallel to evaluate the expectation that MPIV1 was attenuated in the primate host. Since a comparison of the level of replication of MPIV1 and HPIV1 in the upper and lower respiratory tract of primates has not been undertaken and since MPIV1 is also being evaluated as a vaccine vector to protect against human viral pathogens (Matano *et al.,* 2001), it was essential to determine whether MPIV1 is satisfactorily attenuated in primates.

The issue of how well MPIV1 replicates in primates also is of interest for reasons of epidemiology and natural history. Specifically, MPIV1 was reported to have been isolated from infants in the early 1950s (Kuroya and Ishida, 1953). However, during the ensuing years there have been no further outbreaks of human disease. It has thus remained unclear whether MPIV1 indeed had caused disease and had been isolated from these young children, perhaps as a consequence of zoonotic spread from an animal reservoir, or whether the clinical samples were contaminated with MPIV1 in the laboratory.

To assess the ability of MPIV1 to replicate in nonhuman primates, which serve as surrogates for humans, the replication of MPIV1 was directly compared with that of HPIV1 in the upper and lower respiratory tract of seronegative African green monkeys and chimpanzees. Both of these experimental animals have been shown previously to support moderate levels of HPIV1 replication and are among the most permissive animal models for evaluating candidate HPIV vaccines (Durbin *et al.,* 2000). Unexpectedly, our findings indicate that MPIV1 is not significantly restricted in its replication in these primates compared to HPIV1. The implications of this observation for the use of MPIV1 as a Jennerian vaccine and as a vector and for its putative association with disease in humans are discussed.

RESULTS

HPIV1 and MPIV1 replicate to a similar level in the upper and lower respiratory tract of African green monkeys

Previously it was determined that seronegative African green monkeys (*Cercopithecus aethiops*) and chimpanzees (*Pan troglodytes*) are suitable primate models in which to evaluate the replication of HPIV1 (Durbin *et al.,* 2000), although it should be noted that these animals do not develop HPIV1 disease and are therefore thought to be less permissive than humans. To determine the relative level of replication, immunogenicity and protective efficacy MPIV1, derived from a cDNA of the Sendai virus Z strain, or HPIV1, a virulent wild-type (wt) virus (Murphy *et al.,* 1975), was administered to a group of four African green monkeys, which were confirmed to be seronegative for HPIV1. These animals were infected simultaneously by the intranasal (i.n.) and intratracheal (i.t.) routes with 10^4 $TCID_{50}$ of MPIV1 or HPIV1 in a 1 ml inoculum at each site. Two additional groups of four animals each were immunized in the same way with 10^6 $TCID_{50}$ of MPIV1 or HPIV1 per site. The quantity of virus in nasopharyngeal swab and tracheal lavage samples was determined by limiting dilution assay on LLC-MK2 monolayers incubated at 32°C and is expressed as $\log_{10}$ $TCID_{50}$/ml. The mean daily virus titer of nasal swab and tracheal lavage samples throughout the course of the study are presented in Fig. 1, and the mean peak virus titers are shown in Table 1.

MPIV1 replicated to a similar level as HPIV1 in both the upper and the lower respiratory tract of monkeys infected with either 10^4 $TCID_{50}$ or 10^6 $TCID_{50}$. Specifically, the two viruses were very similar with regard to the duration and pattern of shedding at the two sites (Fig. 1, Table 1) and also were very similar at each site on the basis of the mean peak titer of the individual animals in each group (Table 1). Thus, over a 100-fold range of virus titer administered to the African green monkeys, MPIV1 was not attenuated for replication compared to HPIV1. Illness was not observed in monkeys that were infected with either MPIV1 or HPIV1.

Infection of African green monkeys with MPIV1 protected the monkeys from challenge with HPIV1

In the experiment described above, serum samples were taken from the monkeys on day 0 immediately prior to infection and on day 28. The African green monkeys infected with HPIV1 or MPIV1 developed a high titer of homologous serum hemagglutination inhibition (HAI) antibodies (Table 1) and virus-neutralizing antibodies (data not shown). However, the serum antibodies raised against each virus did not cross-react efficiently with the other (Table 1 and data not shown). In contrast, hamsters infected with Sendai virus induced neutralizing antibody to HPIV1, but not vice versa (Cook and Chanock, 1963).

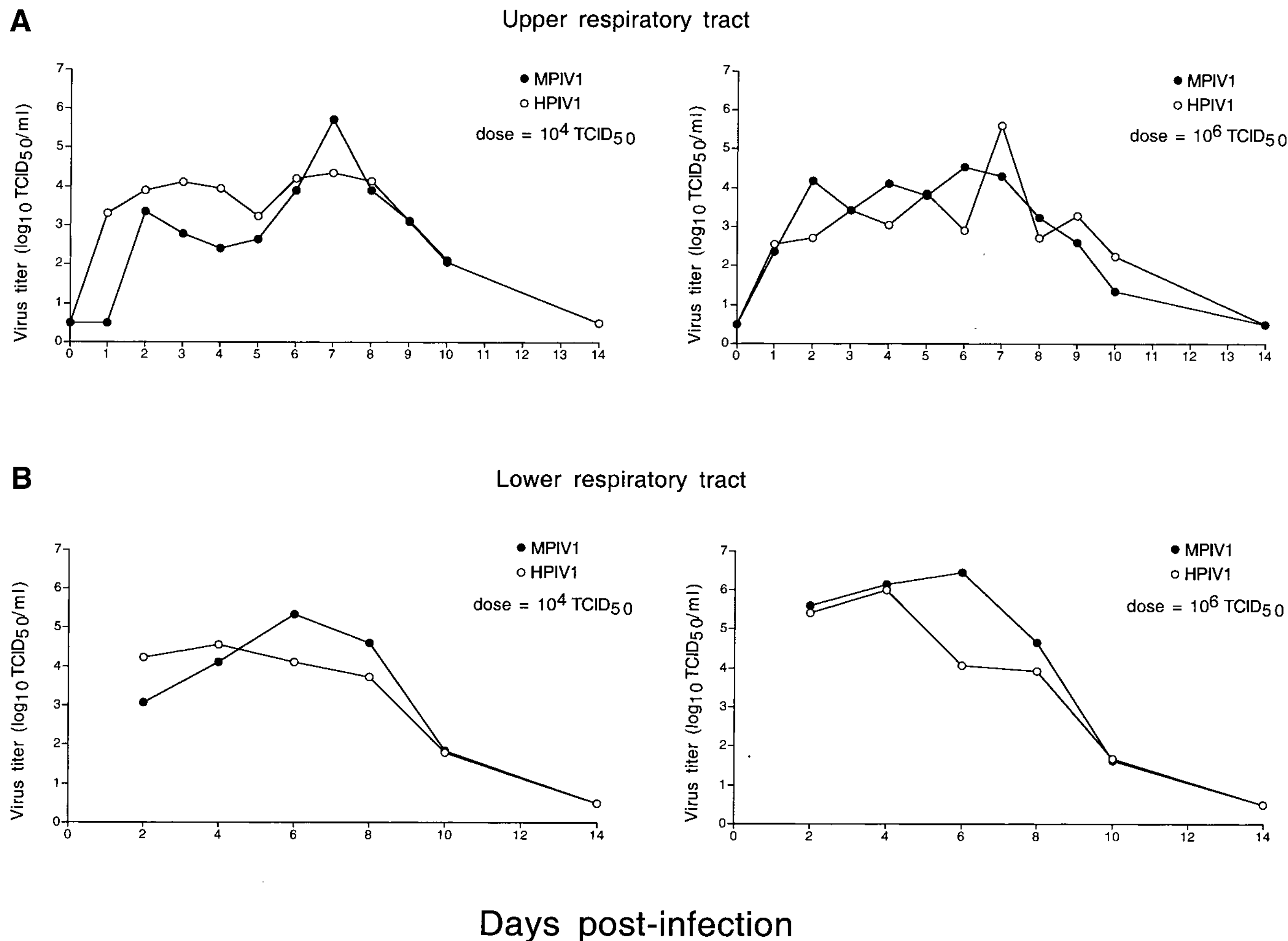

FIG. 1. The level of replication of MPIV1 and HPIV1 in the upper and lower respiratory tract of African green monkeys. Mean daily virus titers in nasopharyngeal swab (A) or tracheal lavage (B) specimens obtained on the indicated day postinoculation from animals infected i.n. and i.t. with 10^4 (left) or 10^6 (right) $TCID_{50}$ of the indicated virus: ●, MPIV1 n = 4; or ○, HPIV1 n = 4. Limit of detection = 1.0 log_{10} $TCID_{50}$/ml.

On day 28 postinfection, the animals described above were challenged i.n. and i.t. with 10^6 $TCID_{50}$ of wt HPIV1 per site, and the titer of virus in the nasopharyngeal swab and tracheal lavage samples was determined as described above. Shedding of the HPIV1 challenge virus was not detected in the upper or lower respiratory tract of animals that were previously infected with HPIV1, whereas a very low level of HPIV1 shedding was detected at both sites in animals that had been infected with MPIV1 (Table 1).

MPIV1 replicates similar to HPIV1 in the upper and lower respiratory tract of chimpanzees

To determine the relative level of replication and immunogenicity of MPIV1 and HPIV1 in chimpanzees, animals seronegative for HPIV1 were inoculated i.n. and i.t. in groups of 2 with 10^5 $TCID_{50}$ of MPIV1 or HPIV1. Nasopharyngeal swab and tracheal lavage samples were collected as described for the African green monkeys, and virus present in the samples was titered on LLC-MK2 monolayers. As shown in Fig. 2, the duration and pattern of shedding of MPIV1 and HPIV1 in the upper and lower respiratory tract were similar, although on most days the mean titer of MPIV1 was somewhat less than that of HPIV1. However, the mean peak virus titer of the individual animals in each group was similar (Table 2). The small difference in virus shedding between MPIV1 and HPIV1 in the upper and lower respiratory tract of the chimpanzee (Fig. 2) is reminiscent of the difference observed between the RSV antigenic subgroups A and B in the same experimental animal (Whitehead *et al.,* 1999) and represents only a marginal difference in replication rather than a significant level of attenuation. Chimpanzees immunized with HPIV1 or MPIV1 developed a high titer of homologous serum HAI antibodies (Table 2). With the exception of one animal immunized with HPIV1, which developed HAI antibodies to both HPIV1 (1:45) and MPIV1 (1:11) (Table 2), the HAI responses were virus-specific. Illness was not observed in chimpanzees that were infected with either MPIV1 or HPIV1.

TABLE 1

Replication of MPIV1 and HPIV1 in the Upper and Lower Respiratory Tract of African Green Monkeys

Immunizing virus[a] (dose; $TCID_{50}$)	Number of animals	Response to immunization: Mean peak virus titer[b,c] (log_{10} $TCID_{50}$/ml ± S.E.) NP swab[d]	Tracheal lavage[e]	Serum HAI antibody titer (mean recip. log_2 ± SE) against MPIV1 on day 28[f]	Serum HAI antibody titer (mean recip. log_2 SE) against HPIV1 on day 28[f]	Response to challenge[g]: Mean peak HPIV1 challenge virus titer[b] (log_{10} $TCID_{50}$/ml ± S.E.) NP swab[h]	Tracheal lavage[h]
MPIV1 (10^4)	4	5.7 ± 0.2	5.4 ± 0.5	9.0 ± 0.7	≤1.0 ± 0.0	1.6 ± 0.7	1.9 ± 0.7
HPIV1 (10^4)	4	4.9 ± 0.4	4.7 ± 0.3	≤1.0 ± 0.0	7.5 ± 0.3	≤0.5 ± 0.0	≤0.5 ± 0.0
MPIV1 (10^6)	4	4.9 ± 0.3	6.9 ± 0.2	9.8 ± 0.7	≤1.0 ± 0.0	1.4 ± 0.4	1.6 ± 0.8
HPIV1 (10^6)	4	5.6 ± 0.3	6.0 ± 0.5	≤1.0 ± 0.0	8.3 ± 0.3	≤0.5 ± 0.0	≤0.5 ± 0.0

[a] Monkeys were inoculated intranasally and intratracheally with indicated amount of virus in a 1-ml inoculum at each site.
[b] Mean of the peak virus titers for the animals in each group irrespective of sampling day. SE, standard error.
[c] Virus titrations were performed on LLC-MK2 cells at 32°C. The limit of detection was 1.0 log_{10} $TCID_{50}$/ml.
[d] Nasopharyngeal swab samples were collected on days 1 to 10 and 14 postinfection. The titers on day 0 were ≤0.5 log_{10} $TCID_{50}$/ml.
[e] Trachael lavage samples were collected on days 2, 4, 6, 8, 10, and 14 postinfection. The titers on day 0 were ≤0.5 log_{10} $TCID_{50}$/ml.
[f] Serum HAI titer is expressed as the mean reciprocal log_2 ± standard error. The titer on day 0 was ≤1.0.
[g] Animals were challenged with 6.0 log_{10} $TCID_{50}$ of HPIV1 in a 1 ml inoculum i.n. and i.t.
[h] Nasopharyngeal swab and tracheal lavage samples were collected on days 0, 2, 4, 6, and 8 postchallenge. The titers on day 0 were ≤0.5 log_{10} $TCID_{50}$/ml.

DISCUSSION

Although MPIV1 was not satisfactorily attenuated for nonhuman primates, it did provide a high level of protection against HPIV1 challenge, albeit one that was slightly less protective than that induced by HPIV1. It was somewhat surprising that the high level of serum antibodies induced by MPIV1 lacked detectable HAI or neutralizing activity against HPIV1. The extent of amino acid sequence relatedness between MPIV1 vs HPIV1 in F and HN (68 and 72%, respectively) is only marginally lower than that between BPIV3 vs HPIV3 (79 and 75%, respectively). However, BPIV3, but not MPIV1, induces antibodies that efficiently neutralize its homologous HPIV. Nonetheless, immunization of animals with MPIV1 induced a high level of resistance to HPIV1 challenge. We suggest that this protection was mediated by cellular immunity. The greater protective efficacy of HPIV1 compared to MPIV1 against HPIV1 challenge likely reflects the combined contribution of cellular immunity and neutralizing antibodies. Cellular immunity is known to play a role in combating PIV1 infections (Hou *et al.,* 1992; Tao *et al.,* 1999).

Although Sendai virus is often referred to as MPIV1, this species designation is based on its permissiveness during laboratory infection rather than evidence from nature. Indeed, there is a lack of virologic or serological evidence of MPIV1 in wild mouse populations (Ishida and Homma, 1978), and the natural host or hosts of MPIV1 remain unknown. There are two known lineages of MPIV1 (Fujii *et al.,* 2001; Itoh *et al.,* 1997; Sakaguchi *et al.,* 1994; Wang *et al.,* 1994). The MPIV1 used in the present study is a recombinant virus derived from the Z strain of MPIV1 (Garcin *et al.,* 1997) that was isolated in the early 1950s, subsequent to an epidemic of pneumonitis of newborn infants in Sendai, Japan (Ishida and Homma, 1978; Kuroya and Ishida, 1953). The Z strain of MPIV1 is highly related by nucleotide sequence analysis (about 99% sequence identity) to the Harris (H) and Fushimi strains, and thus these "strains" probably represent a single Z/H/Fushimi strain that has been passaged extensively in eggs in various laboratories since the 1950s (Giorgi *et al.,* 1983; Homann and Neubert, 1989; Shioda *et al.,* 1983). Z/H/Fushimi egg passage strains are only moderately virulent for mice with a lethal $dose_{50}$ (LD_{50}) of about 10^4 PFU.

The second lineage consists of two highly virulent, low egg-passage viruses ($LD_{50} < 10^2$), the Ohita M and Hamanatsu strains, which were isolated in Japan from separate severe epidemics in laboratory mice in separate locations (Itoh *et al.,* 1992; Kiyotani *et al.,* 1990). Passage of the Hamanatsu strain in eggs readily attenuates the virus for mice such that the virulence of the egg-passaged Hamanatsu strain resembles that of the Z/H/Fushimi strain, indicating that adaptation to replication in eggs attenuates the virus for mice (Kiyotani *et al.,* 2001). Both the highly virulent Ohita M and Hamanatsu strains have now been sequenced, and they are 99.2% identical to each other, and 89% identical to the Z/H/Fushimi strains (Fujii *et al.,* 2001; Itoh *et al.,* 1997).

Here we report that a recombinant version of the egg-adapted Z strain of MPIV1 that is only moderately virulent in laboratory mice replicates as efficiently as HPIV1 in the monkey and chimpanzee models of human respiratory infection. The observation that field strains of

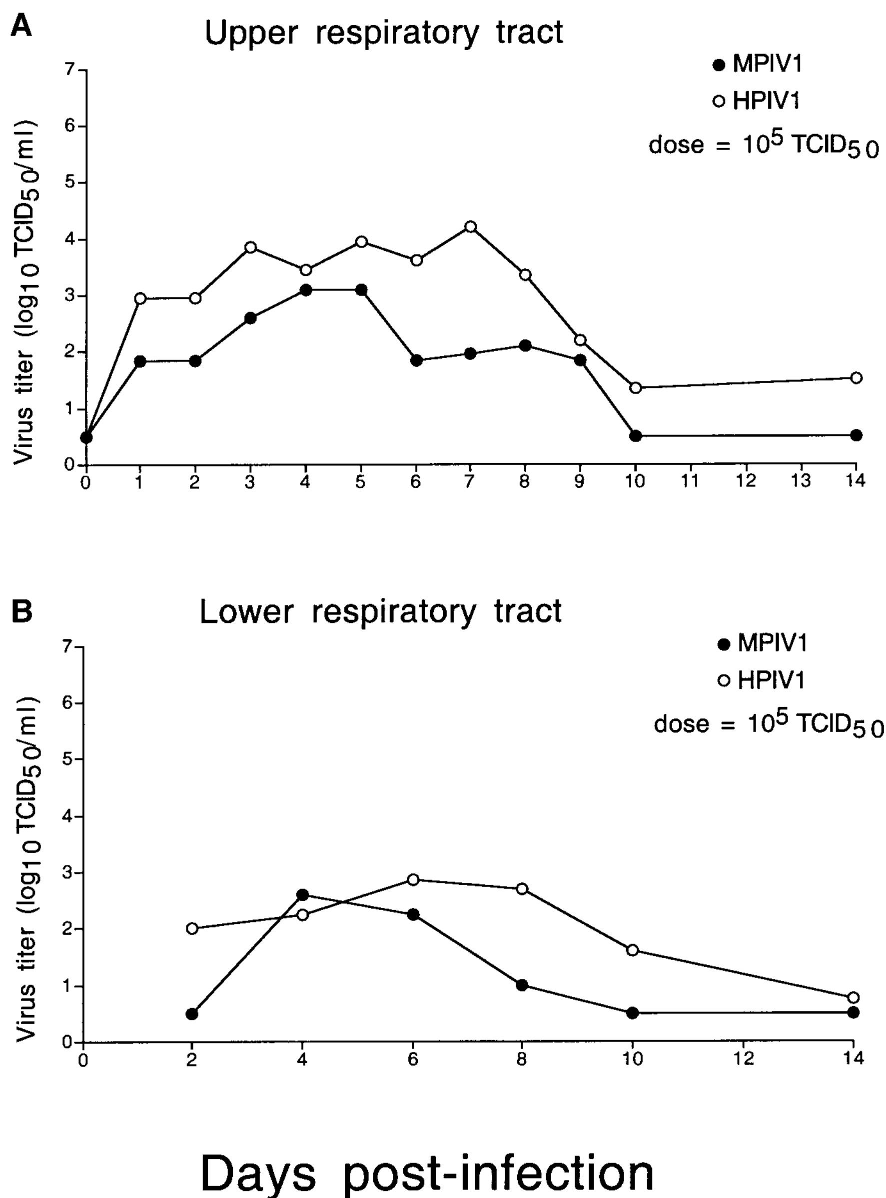

FIG. 2. The level of replication of MPIV1 and HPIV1 in the upper and lower respiratory tract of chimpanzees. Mean daily virus titers in nasopharyngeal swab (A) or tracheal lavage (B) specimens obtained on the indicated day postinoculation from animals infected i.n. and i.t. with 10^5 $TCID_{50}$ of the indicated virus: ●, MPIV1 n = 2; or ○, HPIV1 n = 2. Limit of detection = 1.0 $\log_{10}$ $TCID_{50}$/ml.

MPIV1 are much more virulent in mice than the egg-passaged recombinant Z strain tested here raises the possibility that MPIV1 strains that are transmitted to humans directly from a natural host might exhibit even greater virulence in nonhuman primates than the egg-adapted strain. Given these unexpected findings, and the fact that some of the original patient isolates were known not to have been passaged in mice (Ishida and Homma, 1978), field strains of this virus could well have been responsible for the outbreak of fatal pneumonitis in newborn infants in Sendai, Japan, in 1952.

The findings from the present study also indicate that it would be risky to use wild-type MPIV1 as a Jennerian vaccine for HPIV1. Essential characteristics for candidate Jennerian vaccines are attenuation of replication, immunogenicity, and protective efficacy against their human counterparts. For example, BPIV3 had been previously shown to be attenuated for replication in both the upper and the lower respiratory tract of rhesus monkeys (Coelingh *et al.,* 1988) and in the lower respiratory tract of chimpanzees (Clements *et al.,* 1991). Therefore, before studies were initiated in humans with BPIV3, an attenuation phenotype had been demonstrated for two primate species. The MPIV1 used in the present study was indeed immunogenic and protective against HPIV1 but, unexpectedly, did not appear to be sufficiently attenuated in the nonhuman primates tested. Based on the information derived from the present study, it might be expected

TABLE 2

Replication of MPIV1 and HPIV1 in the Upper and Lower Respiratory Tract of Chimpanzees

Immunizing virus (dose; $TCID_{50}$)[a]	Number of animals	Mean peak virus titer[b,c] (log_{10} $TCID_{50}$/ml ± SE)		Serum HAI antibody titer (mean recip. log_2 ± SE) for HPIV1 on day 28[f]	Serum HAI antibody titer (mean recip. log_2 ± SE) for MPIV1 on day 28[f]
		NP swab[d]	Tracheal lavage[e]		
MPIV1 (10^5)	2	3.6 ± 0.1	2.9 ± 0.2	≤1.0 ± 0.0	6.3 ± 0.3
HPIV1 (10^5)	2	4.5 ± 0.3	3.0 ± 0.3	5.3 ± 0.3	2.3 ± 1.3[g]

[a] Chimpanzees were i.n. and i.t. with indicated amount of virus in a 1 ml inoculum at each site.
[b] Mean of the peak virus titers for the animals in each group irrespective of sampling day. SE, standard error.
[c] Virus titrations were performed on LLC-MK2 cells at 32°C. Limit of detection was 1.0 log_{10} $TCID_{50}$/ml.
[d] Nasopharyngeal swab samples were collected on days 1 to 10 and 14 postimmunization. The titers on day 0 were ≤0.5 log_{10} $TCID_{50}$/ml.
[e] Tracheal lavage samples were collected on days 2, 4, 6, 8, 10, and 14 postinfection. The titers on day 0 were ≤0.5 log_{10} $TCID_{50}$/ml.
[f] Serum HAI titer is expressed as the mean reciprocal log_2 ± SE. The titer on day 0 was ≤1.0.
[g] One animal developed antibodies to MPIV1 (mean recip. log_2 titer = 3.5), whereas the other did not (mean recip. log_2 titer ≤ 1.0).

that MPIV1 would replicate to a high level in humans and could, therefore, also cause disease. Similarly, use of MPIV1 as a vector to immunize humans against additional pathogens may require attenuation of the MPIV1 vector. The suggestion that considerable caution should be exercised before proceeding with an evaluation of MPIV1 in humans is offered with the knowledge that many nonhuman paramyxoviruses and myxoviruses, including Menangle virus (Bowden *et al.,* 2001; Chant *et al.,* 1998), Hendra virus (Murray *et al.,* 1995; Selvey *et al.,* 1995), Nipah virus (Chua *et al.,* 2000; Paton *et al.,* 1999), Newcastle Disease virus (Hanson and Brandly, 1958), and avian influenza A viruses (Alexander and Brown, 2000; Subbarao and Katz, 2000), are fully capable of causing disease in humans following interspecies transfer. Thus, not all animal viruses can serve as "Jennerian" vaccines, but rather only that subset that is restricted for replication, or otherwise attenuated, in the human host.

MATERIALS AND METHODS

Cells and viruses

LLC-MK2 cells (ATCC CCL 7.1) were maintained in OptiMEM I (Life Technologies, Gaithersburg, MD) supplemented with 5% FBS and gentamicin sulfate (50 μg/mL), or in EMEM supplemented with 10% FBS, gentamicin sulfate (50 μg/ml), and 2 mM glutamine. The biologically derived HPIV1/WASH/20993/1964 isolate used in this work was confirmed previously to be virulent in human volunteers (Murphy *et al.,* 1975) and was propagated in LLC-MK2 cells as described previously (Tao *et al.,* 1998). Recombinant wild-type Sendai virus, strain Z (referred to here as MPIV1), was recovered from cDNA as described previously (Garcin *et al.,* 1997) and was confirmed to be moderately virulent for mice (Garcin *et al.,* 1997). It was passaged once in LLC-MK2 cells in OptiMEM I (Life Technologies) that was supplemented with gentamicin sulfate (50 μg/ml) and porcine-derived trypsin (1.2 μg/ml; BioWhitaker, MD).

Evaluation of viruses in African green monkeys and chimpanzees

African green monkeys (*C. aethiops*) and chimpanzees (*P. troglodytes*) that were negative for serum HAI antibody to HPIV1 were inoculated simultaneously by the i.n. and i.t. routes with 1 ml per site of L15 medium containing 10^4 or 10^6 $TCID_{50}$ of virus, as described previously (Durbin *et al.,* 2000). Nasopharyngeal (NP) swab samples were collected on days 1 through 10 and 14 postinfection, and tracheal lavage (TL) samples were collected on days 2, 4, 6, 8, 10, and 14 postinfection. Virus present in the NP and TL specimens was quantified by titration on LLC-MK2 cell monolayers at 32°C, as previously described (Durbin *et al.,* 2000), and the mean peak virus titer obtained was expressed as log_{10} $TCID_{50}$/ml.

African green monkeys were challenged i.n. and i.t. with 10^6 $TCID_{50}$ of wild-type HPIV1 28 days after immunization, and NP and TL samples were collected on days 2, 4, 6, and 8 postchallenge. HPIV1 present in the samples was quantified as described above. The pre- and postimmunization serum HAI or virus neutralizing antibody titer to HPIV1 or Sendai virus was measured as described previously (Durbin *et al.,* 2000; van Wyke Coelingh *et al.,* 1985).

ACKNOWLEDGMENTS

We thank Robert Chanock for thoughtful review of the manuscript, and Ernie Williams, Fatimeh Davoodi, and Tammy Tobery for technical assistance. This research is part of a continuing program of research and development with Wyeth-Lederle Vaccines and Pediatrics through CRADA Grants AI-000030 and AI-000087. This work was partially supported by a grant from the Swiss National Science Fund.

REFERENCES

Alexander, D. J., and Brown, I. H. (2000). Recent zoonoses caused by influenza A viruses. *Rev. Sci. Tech.* **19**(1), 197–225.

Bailly, J. E., McAuliffe, J. M., Skiadopoulos, M. H., Collins, P. L., and Murphy, B. R. (2000). Sequence determination and molecular analy-

sis of two strains of bovine parainfluenza virus type 3 that are attenuated for primates. *Virus Genes* **20**(2), 173–182.

Bowden, T. R., Westenberg, M., Wang, L. F., Eaton, B. T., and Boyle, D. B. (2001). Molecular characterization of Menangle virus, a novel paramyxovirus which infects pigs, fruit bats, and humans. *Virology* **283**(2), 358–373.

Chanock, R. M., Murphy, B. R., and Collins, P. L. (2001). Parainfluenza viruses. *In* "Fields Virology" (D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman, and S. E. Strauss, Eds.), Vol. 1, 4th ed., pp. 1341–1379. 2 vols. Lippincott, Williams, & Wilkins, Philadelphia, PA.

Chant, K., Chan, R., Smith, M., Dwyer, D. E., and Kirkland, P. (1998). Probable human infection with a newly described virus in the family Paramyxoviridae. The NSW Expert Group. *Emerg. Infect. Dis.* **4**(2), 273–275.

Chua, K. B., Bellini, W. J., Rota, P. A., Harcourt, B. H., Tamin, A., Lam, S. K., Ksiazek, T. G., Rollin, P. E., Zaki, S. R., Shieh, W., Goldsmith, C. S., Gubler, D. J., Roehrig, J. T., Eaton, B., Gould, A. R., Olson, J., Field, H., Daniels, P., Ling, A. E., Peters, C. J., Anderson, L. J., and Mahy, B. W. (2000). Nipah virus: A recently emergent deadly paramyxovirus. *Science* **288**(5470), 1432–1435.

Clements, M. L., Belshe, R. B., King, J., Newman, F., Westblom, T. U., Tierney, E. L., London, W. T., and Murphy, B. R. (1991). Evaluation of bovine, cold-adapted human, and wild-type human parainfluenza type 3 viruses in adult volunteers and in chimpanzees. *J. Clin. Microbiol.* **29**(6), 1175–1182.

Coelingh, K., Winter, C. C., Tierney, E. L., London, W. T., and Murphy, B. R. (1988). Attenuation of bovine parainfluenza virus type 3 in nonhuman primates and its ability to confer immunity to human parainfluenza virus type 3 challenge. *J. Infect. Dis.* **157**(4), 655–662.

Cook, K. M., and Chanock, R. M. (1963). *In vivo* antigenic studies of parainfluenza viruses. *Am. J. Hyg.* **77**, 150–159.

Durbin, A. P., Elkins, W. R., and Murphy, B. R. (2000). African green monkeys provide a useful nonhuman primate model for the study of human parainfluenza virus types-1, -2, and -3 infection. *Vaccine* **18**(22), 2462–2469.

Fujii, Y., Kiyotani, K., Yoshida, T., and Sakaguchi, T. (2001). Conserved and non-conserved regions in the Sendai virus genome: Evolution of a gene possessing overlapping reading frames. *Virus Genes* **22**(1), 47–52.

Garcin, D., Itoh, M., and Kolakofsky, D. (1997). A point mutation in the Sendai virus accessory C proteins attenuates virulence for mice, but not virus growth in cell culture. *Virology* **238**(2), 424–431.

Giorgi, C., Blumberg, B. M., and Kolakofsky, D. (1983). Sendai virus contains overlapping genes expressed from a single mRNA. *Cell* **35**(3 Pt. 2), 829–836.

Gorman, W. L., Gill, D. S., Scroggs, R. A., and Portner, A. (1990). The hemagglutinin-neuraminidase glycoproteins of human parainfluenza virus type 1 and Sendai virus have high structure-function similarity with limited antigenic cross-reactivity. *Virology* **175**(1), 211–221.

Hanson, R. P., and Brandly, C. A. (1958). Newcastle Disease. *Ann. NY Acad. Sci.* **70**, 585–597.

Heikkinen, T., Thint, M., and Chonmaitree, T. (1999). Prevalence of various respiratory viruses in the middle ear during acute otitis media. *N. Engl. J. Med.* **340**(4), 260–264.

Homann, H. E., and Neubert, W. J. (1989). Cloning and sequencing of the polymerase gene (P) of Sendai virus (strain 6/94). *Nucleic Acids Res.* **17**(23), 10102.

Hou, S., Doherty, P. C., Zijlstra, M., Jaenisch, R., and Katz, J. M. (1992). Delayed clearance of Sendai virus in mice lacking class I MHC-restricted CD8+ T cells. *J. Immunol.* **149**(4), 1319–1325.

Hurwitz, J. L., Soike, K. F., Sangster, M. Y., Portner, A., Sealy, R. E., Dawson, D. H., and Coleclough, C. (1997). Intranasal Sendai virus vaccine protects African green monkeys from infection with human parainfluenza virus-type one. *Vaccine* **15**(5), 533–540.

Ishida, N., and Homma, M. (1978). Sendai virus. *Adv. Virus Res.* **23**, 349–383.

Itoh, M., Isegawa, Y., Hotta, H., and Homma, M. (1997). Isolation of an avirulent mutant of Sendai virus with two amino acid mutations from a highly virulent field strain through adaptation to LLC-MK2 cells. *J. Gen. Virol.* **78**(Pt. 12), 3207–3215.

Itoh, M., Wang, X. L., Suzuki, Y., and Homma, M. (1992). Mutation of the HANA protein of Sendai virus by passage in eggs. *Virology* **190**(1), 356–364.

Kapikian, A. Z., Vesikari, T., Ruuska, T., Madore, H. P., Christy, C., Dolin, R., Flores, J., Green, K. Y., Davidson, B. L., Gorziglia, M., and et al. (1992). An update on the "Jennerian" and modified "Jennerian" approach to vaccination of infants and young children against rotavirus diarrhea. *Adv. Exp. Med. Biol.* **327**, 59–69.

Karron, R. A., Wright, P. F., Hall, S. L., Makhene, M., Thompson, J., Burns, B. A., Tollefson, S., Steinhoff, M. C., Wilson, M. H., Harris, D. O., *et al.* (1995). A live attenuated bovine parainfluenza virus type 3 vaccine is safe, infectious, immunogenic, and phenotypically stable in infants and children. *J. Infect. Dis.* **171**(5), 1107–1114.

Kiyotani, K., Sakaguchi, T., Fujii, Y., and Yoshida, T. (2001). Attenuation of a field Sendai virus isolate through egg-passages is associated with an impediment of viral genome replication in mouse respiratory cells. *Arch. Virol.* **146**(5), 893–908.

Kiyotani, K., Takao, S., Sakaguchi, T., and Yoshida, T. (1990). Immediate protection of mice from lethal wild-type Sendai virus (HVJ) infections by a temperature-sensitive mutant, HVJpi, possessing homologous interfering capacity. *Virology* **177**(1), 65–74.

Komada, H., Kusagawa, S., Orvell, C., Tsurudome, M., Nishio, M., Bando, H., Kawano, M., Matsumura, H., Norrby, E., and Ito, Y. (1992). Antigenic diversity of human parainfluenza virus type 1 isolates and their immunological relationship with Sendai virus revealed by using monoclonal antibodies. *J. Gen. Virol.* **73**(Pt. 4), 875–884.

Kuroya, N., and Ishida, M. (1953). Newborn virus pneumonitis (type Sendai). II. Isolation of new virus possessing hemagglutinin activity. *Yokohama Med. Bull.* **4**, 217–233.

Lee, M. S., Mendelman, P. M., Sangli, C., Cho, I., Mathie, S. L., and August, M. J. (2001). Half-life of human parainfluenza virus type 3 (hPIV3) maternal antibody and cumulative proportion of hPIV3 infection in young infants. *J. Infect. Dis.* **183**(8), 1281–1284.

Lyn, D., Gill, D. S., Scroggs, R. A., and Portner, A. (1991). The nucleoproteins of human parainfluenza virus type 1 and Sendai virus share amino acid sequences and antigenic and structural determinants. *J. Gen. Virol.* **72**(Pt. 4), 983–987.

Matano, T., Kano, M., Nakamura, H., Takeda, A., and Nagai, Y. (2001). Rapid appearance of secondary immune responses and protection from acute CD4 depletion after a highly pathogenic immunodeficiency virus challenge in macaques vaccinated with a DNA prime/sendai virus vector boost regimen. *J. Virol.* **75**(23), 11891–11896.

Murphy, B. R., and Chanock, R. M. (2001). Immunization against viral diseases. *In* "Fields Virology" (D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman, and S. E. Strauss, Eds.), Vol. 1, pp. 435–468. 2 vols. Lippincott, Williams, & Wilkins, Philadelphia, PA.

Murphy, B. R., Prince, G. A., Collins, P. L., Van Wyke Coelingh, K., Olmsted, R. A., Spriggs, M. K., Parrott, R. H., Kim, H. W., Brandt, C. D., and Chanock, R. M. (1988). Current approaches to the development of vaccines effective against parainfluenza and respiratory syncytial viruses. *Virus Res.* **11**(1), 1–15.

Murphy, B. R., Richman, D. D., Chalhub, E. G., Uhlendorf, C. P., Baron, S., and Chanock, R. M. (1975). Failure of attenuated temperature-sensitive influenza A (H3N2) virus to induce heterologous interference in humans to parainfluenza type 1 virus. *Infect. Immunol.* **12**(1), 62–68.

Murray, K., Rogers, R., Selvey, L., Selleck, P., Hyatt, A., Gould, A., Gleeson, L., Hooper, P., and Westbury, H. (1995). A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg. Infect. Dis.* **1**(1), 31–33.

Newman, J. T., Surman, S. R., Riggs, J. M., Hansen, C. T., Collins, P. L., Murphy, B. R., and Skiadopoulos, M. H. (2002). Sequence analysis of the Washington/1964 strain of human parainfluenca virus type 1

(HPIV1) and recovery and characterization of wild type recombinant HPIV1 produced by reverse genetics. *Virus Genes* **24,** 77–92.

Paton, N. I., Leo, Y. S., Zaki, S. R., Auchus, A. P., Lee, K. E., Ling, A. E., Chew, S. K., Ang, B., Rollin, P. E., Umapathi, T., Sng, I., Lee, C. C., Lim, E., and Ksiazek, T. G. (1999). Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* **354**(9186)**,** 1253–1256.

Sakaguchi, T., Kiyotani, K., Sakaki, M., Fujii, Y., and Yoshida, T. (1994). A field isolate of Sendai virus: Its high virulence to mice and genetic divergence form prototype strains. *Arch. Virol.* **135**(1–2)**,** 159–164.

Sangster, M., Smith, F. S., Coleclough, C., and Hurwitz, J. L. (1995). Human parainfluenza virus type 1 immunization of infant mice protects from subsequent Sendai virus infection. *Virology* **212**(1)**,** 13–19.

Selvey, L. A., Wells, R. M., McCormack, J. G., Ansford, A. J., Murray, K., Rogers, R. J., Lavercombe, P. S., Selleck, P., and Sheridan, J. W. (1995). Infection of humans and horses by a newly described morbillivirus. *Med. J. Aust.* **162**(12)**,** 642–645.

Shioda, T., Hidaka, Y., Kanda, T., Shibuta, H., Nomoto, A., and Iwasaki, K. (1983). Sequence of 3687 nucleotides from the 3′ end of Sendai virus genome RNA and the predicted amino acid sequences of viral NP, P and C proteins. *Nucleic Acids Res.* **11**(21)**,** 7317–7330.

Subbarao, K., and Katz, J. (2000). Avian influenza viruses infecting humans. *Cell. Mol. Life Sci.* **57**(12)**,** 1770–1784.

Tao, T., Durbin, A. P., Whitehead, S. S., Davoodi, F., Collins, P. L., and Murphy, B. R. (1998). Recovery of a fully viable chimeric human parainfluenza virus (PIV) type 3 in which the hemagglutinin-neuraminidase and fusion glycoproteins have been replaced by those of PIV type 1. *J. Virol.* **72**(4)**,** 2955–2961.

Tao, T., Skiadopoulos, M. H., Durbin, A. P., Davoodi, F., Collins, P. L., and Murphy, B. R. (1999). A live attenuated chimeric recombinant parainfluenza virus (PIV) encoding the internal proteins of PIV type 3 and the surface glycoproteins of PIV type 1 induces complete resistance to PIV1 challenge and partial resistance to PIV3 challenge. *Vaccine* **17,** 1100–1108.

van Wyke Coelingh, K. L., Winter, C., and Murphy, B. R. (1985). Antigenic variation in the hemagglutinin-neuraminidase protein of human parainfluenza type 3 virus. *Virology* **143**(2)**,** 569–582.

Wang, X. L., Itoh, M., Hotta, H., and Homma, M. (1994). A protease activation mutant, MVCES1, as a safe and potent live vaccine derived from currently prevailing Sendai virus. *J. Virol.* **68**(5)**,** 3369–3373.

Whitehead, S. S., Hill, M. G., Firestone, C. Y., St. Claire, M., Elkins, W. R., Murphy, B. R., and Collins, P. L. (1999). Replacement of the F and G proteins of respiratory syncytial virus (RSV) subgroup A with those of subgroup B generates chimeric live attenuated RSV subgroup B vaccine candidates. *J. Virol.* **73**(12)**,** 9773–9780.