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Immunization with Vaccinia Virus Recombinants That Express the Surface Glycoproteins of Human Parainfluenza Virus Type 3 (PIV3) Protects Patas Monkeys against PIV3 Infection

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Patas monkeys (*Eryphrocebus patas*) were immunized intradermally with two vaccinia virus recombinants that individually express the hemagglutinin-neuraminidase glycoprotein or the fusion glycoprotein of human parainfluenza virus type 3 (PIV3). These immunizations induced a high titer of PIV3 serum-neutralizing antibodies. At 1 month after immunization, monkeys were challenged intratracheally with PIV3. Subsequent virus replication was reduced in these monkeys by 3.2 $\log_{10}$ and 1.9 $\log_{10}$ (mean peak virus titers) in the upper and lower respiratory tracts, respectively, compared with control animals. The average duration of virus shedding was also reduced from 9.0 to 3.4 days in the upper respiratory tract and from 5.3 to 1.2 days in the lower respiratory tract. These findings demonstrate that a single intradermal dose of live recombinant vaccinia viruses can significantly restrict the replication of a virus which primarily infects the epithelial cells of the respiratory tract.

Human parainfluenza virus type 3 (PIV3) is one of a group of respiratory paramyxoviruses, including parainfluenza virus types 1 and 2 and respiratory syncytial virus, that account for a major proportion of the serious lower respiratory tract (LRT) disease in infants and children worldwide (2, 3). Although development of a PIV3 vaccine has been given a high priority by the World Health Organization over a period of more than 20 years, attempts at immunoprophylaxis by conventional approaches, such as the parenteral administration of live or formalin inactivated virus, have met with failure (2, 3, 7, 8, 26).

PIV3 is a large, enveloped virus that contains a single strand of negative-sense genomic RNA (21, 24). It has two surface glycoproteins, the hemagglutinin-neuraminidase (HN) protein, which is involved in virus attachment, and the fusion (F) protein, which is responsible for viral penetration as well as for syncytium formation, which is the hallmark of paramyxovirus cytopathology (3, 15, 16).

Previously, cDNAs of the complete F and HN genes were cloned, sequenced (5, 23), engineered for expression, and inserted into the thymidine kinase locus of the WR strain of vaccinia virus (VV) under the control of the early-late P7.5 promotor (22). The recombinant viruses, vaccinia-F and vaccinia-HN, expressed F or HN protein that appeared to be authentic biochemically, biologically, and immunologically. In particular, infection of cotton rats (*Sigmodon fulviventer*) with either recombinant induced a high level of PIV3-specific serum antibodies and the immunized animals exhibited significant resistance to subsequent infection by PIV3 (22).

Recombinant VV expressing a variety of viral or parasitic antigens have been described previously (9). Many of these have properties which suggest a potential usefulness in immunoprophylaxis. In our view, it is desirable to initially evaluate vaccines destined for human use in nonhuman primates, in which safety, immunogenicity, and protective efficacy can be examined before clinical trials. To date, only one such study, involving a VV recombinant expressing the surface antigen of hepatitis B virus (HBV), has been published (11). In that study, the induction of HBV-specific serum antibodies could not be demonstrated, although partial protection was observed. Successful immunization against a respiratory virus such as PIV3 poses an additional challenge because parenterally administered recombinant viruses or subunit vaccines appear to be inefficient at stimulating local immunity in the respiratory tract (17), which represents an important component of resistance to these viruses during natural exposure. In the work described here, we evaluated vaccinia-F and vaccinia-HN administered intradermally (i.d.) for the ability to induce PIV3-specific serum neutralizing antibodies and to confer resistance to PIV3 replication in the respiratory tract of a nonhuman primate.

MATERIALS AND METHODS

Viruses and cells. The coding regions for the PIV3 F and HN genes were inserted into the *Sma*I site of the VV coexpression vector pSC11 (22). Recombinant VV were isolated after transfection of the recombinant pSC11 into WR strain VV-infected cells as described previously (1, 6, 22). Construction of the VV-hepatitis B surface antigen recombinant (vaccinia-HBsAg) has been described previously (19). Purified stocks of recombinant VV were prepared from infected CV-1 monolayers (6). PIV3 strain 47885 was plaque purified and propagated in HEp-2 cells.

Response to immunization and protective efficacy. PIV3 serum-neutralizing antibody titers were determined by a complement-enhanced 60% plaque reduction neutralization assay. Hemagglutination inhibition tests were performed at 25°C in V-bottomed plastic dishes with 0.5% (vol/vol) guinea pig erythrocytes, using twofold dilutions (4). Patas monkeys were housed at SEMA Inc., Rockville, Md. This facility is accredited by the American Association for the Accreditation of Laboratory Animal Care. PIV3 titers in nasal washes and tracheal lavage specimens were determined by 50%

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TABLE 1. Serum antibody response of patas monkeys to immunization with recombinant VV and challenge with PIV3[a]

Inoculum (route)	Dose	No. of monkeys	Antibody titer response to immunization and challenge[b]		
			Neutralizing[c]	HI[d] before challenge	HI postchallenge
PIV3 (i.t.)	10^7	6	7,931	1,169	3,315
Vaccinia-F + vaccinia-HN (i.d.)	10^8 of each	5	7,147	520	1,816
Vaccinia-HBsAg (i.d.)	10^8	6	10	2	1,169

[a] Animals were challenged with 10^7 $TCID_{50}$ PIV3 i.t. 28 days postimmunization.
[b] Sera were collected 28 days postimmunization and 28 days postchallenge. All titers are expressed as the reciprocal geometric mean.
[c] Measured by 60% plaque reduction neutralization assay.
[d] Dilution which inhibits erythrocyte agglutination by 4 agglutinating units of PIV3.

tissue culture infectious dose ($TCID_{50}$) assay. Assays were performed in duplicate on LLC-MK2 cells. The minimum level of detectability was $10^{1.2}$ $TCID_{50}$ per ml.

RESULTS AND DISCUSSION

Patas monkeys were chosen for this study because preliminary investigations showed that they support the replication of both PIV3 and VV (unpublished data). Monkeys used in this study were tested and shown to be free of preexisting serum antibodies to PIV3 by enzyme-linked immunosorbent assay and by the absence of hemagglutination-inhibiting (HI) antibody (data not shown). They then received a primary immunization consisting of either (i) 10^7 $TCID_{50}$ of PIV3 administered intratracheally (i.t.) (six animals), (ii) 10^8 PFU of vaccinia-F and 10^8 PFU of vaccinia-HN administered i.d. at separate sites (five animals), or (iii) 10^8 PFU of the recombinant VV vaccinia-HBsAg administered i.d. (six animals). Vaccinia-HBsAg expresses the HBV surface antigen (19) and in this study served as the negative (heterologous immunogen) control.

All monkeys receiving VV recombinants developed dermal lesions, which averaged 16 by 14 mm in size. At 28 days after immunization, serum samples were collected and each of the monkeys was challenged with 10^7 $TCID_{50}$ of PIV3 administered i.t. The i.t. route of challenge was chosen to enhance delivery of PIV3 to the LRT.

Animals immunized with vaccinia-F and vaccinia-HN developed a high titer of serum-neutralizing antibodies which was nearly identical to that of animals infected with PIV3 (Table 1). Also, monkeys infected with either PIV3 or vaccinia-F and vaccinia-HN developed a comparable titer of HI antibodies. These HI antibody responses were similar quantitatively to those described for children and young adults after natural PIV3 infection (7, 10).

The recovery of virus from immunized monkeys after challenge with PIV3 is shown in Fig. 1. The magnitude and duration of PIV3 replication in the upper respiratory tract (URT) and the LRT of animals immunized with vaccinia-F and vaccinia-HN were greatly reduced compared with control (vaccinia-HBsAg-immunized) animals and were only slightly greater than that observed for animals previously infected with PIV3. Animals immunized with vaccinia-F and vaccinia-HN exhibited a 3.2 $\log_{10}$ reduction in mean peak virus titer recovered from the URT and a 1.9 $\log_{10}$ reduction in mean peak virus titer recovered from the LRT compared with control animals. Similarly, the average duration of virus shedding in monkeys immunized with vaccinia-F and vaccinia-HN was reduced in the URT from 9.0 (control animals) to 3.4 days and in the LRT from 5.3 (control animals) to 1.2 days.

At 28 days after i.t. challenge with PIV3, sera were collected from all monkeys and HI antibody titers were determined. As shown in Table 1, all three groups exhibited an increase in HI antibody titer (2.8- and 3.5-fold for animals immunized with PIV3 or with the VV recombinants, respectively). This increase in titer probably was induced by the low levels of PIV3 replication in the respiratory tracts of the immunized animals (Fig. 1). Also, the preformed antigen in the challenge virus inoculum might have been a contributing factor. A low level of challenge virus replication in the immunized animals is not surprising, because reinfection after natural PIV3 infection is common. Clinical studies (3, 7, 8) have indicated that subsequent reinfection which may occur in the presence of significant serum HI antibodies tends to be much less severe than primary infections.

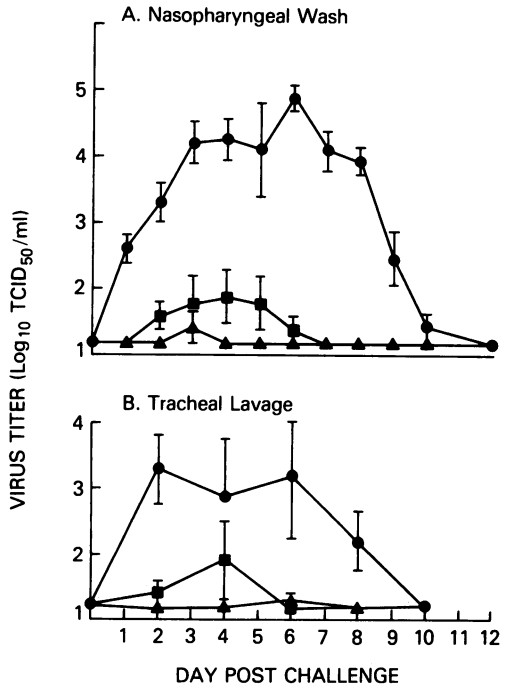

FIG. 1. VV recombinants expressing the PIV3 F or HN surface glycoproteins induce resistance to infection by PIV3. Patas monkeys were immunized as indicated in the text with PIV3 (▲) (six animals), vaccinia-F and vaccinia-HN (■) (five animals), or vaccinia-HBsAg (●) (six animals) and, at 28 days postimmunization, were challenged with 10^7 $TCID_{50}$ of PIV3 i.t. Nasopharyngeal washes (panel A) were collected daily for 10 days and again on day 12 after challenge. Tracheal lavage specimens (panel B) were collected on alternate days for 10 days after challenge.

Respiratory illness was not observed in any of the monkeys after immunization or challenge, precluding direct conclusions on the ability of the immunizations to prevent or ameliorate PIV3 disease. However, it is worth noting that a similar reduction in the level of replication of other respiratory viruses such as influenza and parainfluenza virus type 1 in humans is associated with attenuation of illness (12, 18). For respiratory paramyxoviruses, immunity induced by either immunization or natural infection often is incomplete and reinfection is common. The realistic goal of a successful PIV3 vaccine, then, is not to prevent infection but to induce sufficient immunity to greatly reduce viral replication and thereby prevent serious disease.

The results from this study demonstrate that the i.d. administration of VV recombinants that express the surface glycoproteins of PIV3 induced high titers of PIV3-specific serum-neutralizing antibodies and induced significant resistance to challenge by PIV3 in both the URT and the LRT of patas monkeys. On the basis of previous results (13, 22), it was not surprising to find substantial restriction of PIV3 replication in the LRT of patas monkeys in the presence of a high titer of serum-neutralizing antibodies. A more unexpected finding was that immunization with vaccinia-F and vaccinia-HN induced substantial immunity in the URT of these monkeys. In a prior study, in which vaccinia-F or vaccinia-HN was administered i.d. to rodents before challenge, the URT was not as well protected against PIV3 challenge as the LRT (22). In addition, analogous protection studies involving VV recombinants expressing the surface glycoproteins of respiratory syncytial virus (13) or the influenza virus hemagglutinin glycoprotein (20) demonstrated only limited protection in the URT. However, it has been demonstrated for respiratory syncytial virus in a rodent model that very high titers of passively administered serum-neutralizing antibodies can confer partial resistance in the URT (14). Consistent with this, in humans, there is evidence for passive transudation of serum antibodies to the URT (25), and such antibodies could restrict viral replication at that site. Thus, the unexpectedly high level of immunity in the URT observed here could be a consequence of the very high titers of PIV3-specific serum antibodies induced by vaccinia-F and vaccinia-HN. Other immune mechanisms might also be involved in the protection of the URT and LRT seen in this study; for example, parenterally administered VV recombinants have been shown to induce cytotoxic T lymphocytes (27).

Conventional approaches to development of vaccines for the respiratory paramyxoviruses, such as infection with temperature-sensitive mutants or parenteral administration of live or Formalin-inactivated virus, have been unsuccessful. In contrast, the results of our study indicate that, under the appropriate conditions, a single immunization with VV recombinants expressing the PIV3 glycoproteins can induce a level of immunity in nonhuman primates that greatly restricts PIV3 replication and would be predicted to prevent serious disease in humans. Thus, recombinant DNA or subunit vaccine strategies appear to be feasible approaches to vaccine development for these important respiratory viruses.

The PIV3-VV recombinants used in this study can not be evaluated in humans because the parental VV used in their construction is a mouse-adapted, neurotropic strain. Studies are under way to construct analogous VV-PIV3 recombinants using VV strains (Wyeth and Lister) that are acceptable for human use. These will be evaluated in patas monkeys, and the results will determine whether trials in humans would be appropriate. We are also investigating the feasibility of other vector systems for use in vaccines against PIV3 disease.

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