Temperature-sensitive parainfluenza type 1 vaccine virus directly accesses the central nervous system by infecting olfactory neurons

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Immunohistochemical investigation showed that intranasal inoculation of mice with a temperature-sensitive (ts) mutant of parainfluenza type 1 vaccine virus resulted in infection of some olfactory neurons as well as respiratory epithelial cells. It also disclosed the presence of viral antigens in glomeruli of the olfactory bulb but not in the secondary neurons (mitral and tufted cells). Polymerase chain reaction demonstrated the persistence of virus-specific nucleic acids in the olfactory bulb. These observations lead to the conclusion that parainfluenza virus, even with a ts phenotype, gains access to the central nervous system by infecting olfactory neurons.

Human parainfluenza viruses are common respiratory pathogens and are responsible for serious respiratory infections including pneumonia and croup in young children (Heilman, 1990). It has been well established that live vaccines have advantages over inactivated or subunit vaccines in inducing local and serum antibodies as well as cytotoxic T lymphocytes (Chanock & Murphy, 1980; Crowe, 1995). Major efforts to develop live attenuated vaccines against respiratory virus infections have focused on the isolation of temperature-sensitive (ts) and cold-adapted (ca) mutants (Clements et al., 1991; Belshe et al., 1992; Crowe, 1995; Ray et al., 1995). Generally, ts lesions allow virus growth only in the upper respiratory tract and bring about growth suppression in the lung because of the higher temperature (Chanock & Murphy, 1980).

We isolated a highly attenuated ts mutant of HVJ-pB strain of parainfluenza type 1 virus from persistently infected BHK cells (Kimura et al., 1975). In in vitro studies, the ts mutant does not multiply at temperatures > 36 °C because of a defect in viral matrix protein synthesis (Kimura et al., 1979a). Experimental infection of mice with this ts mutant induced both humoral and cellular immunity (Iwata et al., 1990). The mutant virus successfully protected animals against challenge with a virulent wild-type homologous virus (Kimura et al., 1979b; Iwata et al., 1990; Tagaya et al., 1995). More recently we have shown that the wild-type parainfluenza type 1 virus infects olfactory neurons of immunocompetent mice after intranasal inoculation (Mori et al., 1995). Here we demonstrate that even the ‘attenuated ts’ mutant of parainfluenza virus can infect olfactory neurons. To our knowledge this is the first report to describe the direct transmission of a vaccine virus to the central nervous system via the olfactory route.

The ts mutant of HVJ-pB was isolated from a virus carrier culture (Kimura et al., 1975). The ts mutant was cloned and was propagated by allantoic inoculation of 10-day-old embryonated chicken eggs with 0-2 ml of 10⁻⁴-diluted seed virus. Infected eggs were incubated at 32 °C for 3 days. For virus isolation from infected mice, homogenized tissues were placed on LLCMK2 cell monolayers and incubated at 32 °C in the

<table>
<thead>
<tr>
<th>Tissue</th>
<th>7 days p.i.</th>
<th>56 days p.i.</th>
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<tr>
<td></td>
<td>vRNA</td>
<td>mRNA</td>
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<tr>
<td>Olfactory bulb</td>
<td>5/5±</td>
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<tr>
<td>Brain†</td>
<td>0/5</td>
<td>0/5</td>
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<tr>
<td>Lung</td>
<td>5/5±</td>
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* No samples from mock-infected animals tested positive by PCR. ND, Not done.
† Whole brain except the olfactory bulb.
‡ Significant by Fisher’s exact probability test (P < 0.005).

Table 1. PCR detection of parainfluenza type 1 virus genomic and messenger RNAs in the olfactory bulb of mice intranasally infected with the ts vaccine virus
Fig. 1. Immunohistochemical detection of parainfluenza type 1 virus in the nerve and respiratory tissues after intranasal inoculation with the ts vaccine virus. Viral proteins were detected in some olfactory neurons (arrows) at 7 days p.i. (a). They were also found, as specific brown grains, in glomeruli (gl) of the olfactory bulb (b). Several respiratory epithelial cells were positive for viral antigens at 3 days p.i. (c), while the lung was totally negative (d). Bar marker represents 20 μm.

Presence of trypsin (10 μg/ml). Male C3H/HeJ mice (Clea Japan) aged 5 weeks were purchased and were acclimatized for 1 week before use. Mice had fresh water and autoclaved food and were kept at 23 °C under bioclean conditions throughout the experiments. Mice were mildly anaesthetized with diethyl ether and were inoculated in the right nostril with 10^8 p.f.u. of the ts mutant virus in 25 μl PBS. At intervals, mice were anaesthetized and perfused with 4% paraformaldehyde. The nose, brain and lung were post-fixed with the same fixative at 4 °C for 24 h, and the nose was then decalcified in 4% EDTA at room temperature for 5 days. Immunohistochemistry using hyperimmune serum to parainfluenza type 1 virus was performed as described previously (Mori et al., 1995). A group of three mice was used for each time point. Tissue sections treated with preimmune serum were used as a negative control. Tissues from uninfected mice were also used as an additional control.

Total RNA was extracted from mouse tissue with TRIzol reagent (Life Technologies). cDNA was synthesized by Moloney murine leukaemia virus reverse transcriptase (Life Technologies) with 10 pmol sense primer (5' ACCAAACAAGAG 3'). For detection of virus-specific mRNA, 50 pmol of oligo(dT) was used as a primer. cDNA samples were amplified in a single tube by nested or seminested polymerase chain reaction (PCR) using a DNA Thermal Cycler (Perkin-Elmer) as previously described (Mori et al., 1995), with some modifications. Primers for the parainfluenza type 1 virus nucleoprotein (NP) gene were identified in the previous paper (Mori et al., 1995). The first PCR was carried out in a 20 μl solution containing 2 μl 10× Ex Taq buffer (Takara), 0.4 μl dNTPs (each 10 mM), 0.3 μl each of the external primers (5 pmol/μl), 0.5 U Ex Taq DNA polymerase (Takara), and 2 μl of the cDNA sample. After two drops of liquid paraffin were added, the tube was briefly centrifuged. The thermal cycling program was: 94 °C for 3 min, 25 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, followed by 72 °C for 5 min. For the second
round amplification, the first PCR product was mixed in the same tube with a 30 μl solution containing 3 μl 10X Ex Taq buffer, 1 μl dNTPs (each 10 mM), 1 μl each of the internal primers (50 pmol/μl), and 1-25 U Ex Taq DNA polymerase. The tube was then briefly centrifuged. The thermal cycling program consisted of: 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min, followed by 72 °C for 5 min. The final PCR product was analysed by electrophoresis on a 2% agarose gel, visualized by ethidium bromide staining and confirmed by Southern hybridization as described previously (Mori et al., 1995). A group of five mice was used at each time point. Controls included tissues from uninfected mice, reaction mixture without RNA and influenza A virus as a positive control. Significance of the number of PCR-positive samples was evaluated by Fisher’s exact probability test.

The PCR detected the genomic and messenger RNAs of the ts mutant virus in the olfactory bulb at 7 days post-infection (p.i.) (Table 1). Viral RNAs were not found in any part of the brain except the olfactory bulb. The PCR also disclosed viral persistence in the nerve tissue up to 56 days p.i., while in the lung the virus RNAs had disappeared by that time point.

Immunohistochemistry detected virus antigens in several ciliated respiratory epithelial cells lining the nasal cavity at 3 days p.i., though it could not disclose the presence of the virus at 7 days p.i. and later in these cells (Fig. 1). In some olfactory neurons the virus proteins were detected at 7 days p.i., but they were not found at 14 and 28 days p.i. Virus proteins (shown by brown grains) were also found in glomeruli of the olfactory bulb at 7 days p.i. (Fig. 1). The secondary neurons (i.e. mitral and tufted cells) and other nerve tissues of the brain were negative for viral proteins. It should be noted that the infected olfactory neurons and respiratory cells displayed no histopathological alterations. The lung showed no histopathological changes and was negative for viral antigens. No infectious virus could be isolated from the olfactory bulb. The mutant virus-infected mice displayed no appreciable respiratory and neurological symptoms.

In this study we have shown that parainfluenza virus infects olfactory neurons, even if the virus is highly attenuated for growth at a non-permissive temperature in tissue culture (Kimura et al., 1975) and in the lower respiratory tract of experimental animals (Iwata et al., 1990; Tagaya et al., 1995). Ts virus vaccine candidates have been developed with an expectation that ts lesion(s) would suppress virus growth in the lower respiratory organs (Chanock & Murphy, 1980). On the other hand, a lower temperature in the nasal cavity may allow multiplication of the ts virus not only in respiratory epithelial cells but also in olfactory neurons. The olfactory pathway is one of the most important routes of access to the central nervous system for some neurotropic viruses (Tomlinson & Esiri, 1983; Lundh et al., 1987; Lafay et al., 1991; Barnett & Perlman, 1993). In the light of this fact, more careful consideration of neurotropism should be given in the development of a safer live paramyxovirus vaccine for intranasal usage. However, this may not apply in the case of influenza virus infection, since the PR/8 and WSN strains of influenza A virus, which cause severe pneumonia in mice, could not infect murine olfactory neurons (unpublished data).

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References


