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Evaluation of a protease activation mutant of Sendai virus as a potent live vaccine

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ABSTRACT

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A protease activation mutant of Sendai virus, TR-5, was investigated as a candidate for a live vaccine. Vaccination with TR-5 which had been activated by chymotrypsin beforehand (active TR-5) elicited protective immunity against otherwise lethal challenge infection with wild-type Sendai virus in DBA/2, C3H and ICR strains of mice. Less of the active TR-5 was required to confer protection on mice compared with an ordinary ether-inactivated Sendai virus vaccine (split vaccine). The protective immunity elicited by TR-5 lasted longer and the booster effect was more prominent compared to the split vaccine. No seroconversion was observed with contact mice when housed in a cage with mice vaccinated with the active TR-5. The overall results show that the active TR-5 is an effective and safe live vaccine of Sendai virus in mice.

INTRODUCTION

Sendai virus infects mice, rats, hamsters and guinea pigs (Profeta et al., 1969; Bhatt and Jonas, 1974; Burek et al., 1977; Ishida and Homma, 1978; Parker et al., 1978), and outbreaks of the virus infection in animal breeding colonies sometimes result in considerable economic damage. Sendai virus replicates in the respiratory tracts and lungs of these animals and causes a spectrum of clinical diseases such as tracheitis and broncho-pneumonia (Robinson et al., 1968; Appell et al., 1970; Tashiro and Homma, 1983a). In addition, infection of pregnant animals induces resorption of the embryos (Coid and Wardman, 1971), disorders of the gestation period (Coid and Wardman, 1972), and incomplete development of their offspring (Tucker and Stewart, 1976). Enzootic infection with Sendai virus is not only an obstacle for the production of experimental animals but also causes unexpected deviation of

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the results in animal experiments. Although an ether-inactivated vaccine (Fukumi and Takeuchi, 1975; Eaton et al., 1982; Tsukui et al.,1982; Ito and Matumoto, 1986; Miyamae, 1986) and a live attenuated vaccine (Kimura et al., 1979; Iwata et al., 1990) have been evaluated in experimental animals, they have not yet been widely used.

Sendai virus possesses two kinds of glycoprotein on its envelope designated HANA and F (Mountcastle et al., 1971; Homma et al., 1975). HANA glycoprotein exerts hemagglutination and neuraminidase activities through which the virus adsorbs onto the receptors of the target cells (Tozawa et al., 1973; Scheid and Choppin, 1974). F glycoprotein is responsible for fusion of the virus envelope with the cellular membrane, which mediates entry of the viral genome into the cells (Homma and Ohuchi, 1973; Scheid and Choppin, 1974). Sendai virus is produced in the infected cells as a functionally inactive virus and can be activated by treatment with trypsin (Homma, 1971, 1972; Homma and Tamagawa, 1973). F glycoprotein is synthesized as a functionally inactive precursor and its cleavage into F_1 and F_2 subunits by trypsin and trypsin-like proteases is necessary for the virus to be infectious (Homma, 1971, 1972; Homma and Ohuchi, 1973; Scheid and Choppin, 1974; Ohuchi and Homma, 1976). Thus Sendai virus can grow in multiple steps when trypsin-like proteases are present in the environment, as is the case in the chorioallantoic sac of fertile eggs (Muramatsu and Homma, 1980) and the lungs of mice (Tashiro and Homma, 1983b). However, we have succeeded in obtaining a trypsin-resistant mutant of Sendai virus, TR-2, by growing the wild type virus in tissue culture cells in the presence of chymotrypsin instead of trypsin. The F glycoprotein of TR-2 can be cleaved by chymotrypsin but not by trypsin (Tashiro and Homma, 1983a) and the virus replicates in multiple steps only in the presence of chymotrypsin. Later, TR-2 was shown to be a cleavage site mutant (Itoh et al., 1987). Since the bronchial epithelium of the lungs of mice which is the target cell of Sendai virus has protease(s) that cleaves only F glycoprotein of the wild-type virus but not of TR-2, the progeny virus of TR-2 is no longer infectious, and replication is limited to only a single step (Tashiro and Homma, 1983a).

In addition, preliminary data indicate that TR-2 has potential as a vaccine (Tashiro and Homma, 1985). Based on these results, the present study was conducted to show the possible use of a plaque purified TR mutant (TR-5) as a candidate for the live vaccine of Sendai virus in comparison with the conventional ether-inactivated vaccine. This trial is the first for the use of a cleavage site mutant as an attenuated live vaccine.

MATERIALS AND METHODS

Animals

Earlier study of vaccination with a protease activation mutant TR-2 was in ICR mice (Tashiro and Homma, 1985). To investigate whether or not pro-

tective immunity could be elicited in other strains of mice, DBA/2 and C3H mice as well as ICR mice were vaccinated with the active TR-5. Three-week-old female mice of the DBA/2NCrj (DBA/2), C3H/HeNCrj (C3H) and Crj:CD-1 (ICR) strains were purchased (Charles River Japan, Inc.) and maintained in a barrier system in our laboratory for another week before use. Sampling tests revealed that these animals were negative for hemagglutination inhibiting (HI) antibodies to Sendai virus.

Viruses

Wild-type virus. The Fushimi strain of Sendai virus was used. Egg-grown Sendai virus was adapted to DBA/2 mice by consecutively passaging the infected lung homogenates three times according to the method described previously (Tashiro and Homma, 1985). This virus was propagated once in fertile eggs and the allantoic fluid-grown virus was used for the experiment. The lethal dose was determined by inoculating serially 10-fold diluted viruses into 6-week-old DBA/2, C3H and ICR mice and the 50% lethal dose (LD_{50}) was calculated by the method of Reed and Muench. One LD_{50} for each of the 6-week-old DBA/2, C3H and ICR mice corresponded to 4.9×10^5, 1.3×10^6 and 1.9×10^8 plaque forming units (PFU), respectively.

Trypsin-resistant mutant, TR-5. TR-5 is a plaque-purified clone of the trypsin resistant mutant TR-2 (Tashiro and Homma, 1985). Although TR-5 had an amino acid substitution (Val→Met) at residue 172 of the F protein, it could not otherwise be distinguished from TR-2 (Itoh et al., 1987; Itoh and Homma, 1988). TR-5 grown in the chorioallantoic cavity of fertile eggs in the presence of 10 μg of chymotrypsin per egg was inoculated to LLC-MK_2 cells at a multiplicity of 8 PFU/cell and the cells were incubated in a serum-free MEM in the absence of chymotrypsin. After 60 h, the culture fluid was harvested and clarified by low speed centrifugation. The titer of the supernatant was 256 HAU/ml. This preparation was designated inactive TR-5. The inactive TR-5 was activated by treatment with chymotrypsin at a concentration of 30 μg/ml at 37°C for 10 min, followed by the addition of soy bean trypsin inhibitor to terminate enzymic action (Tashiro and Homma, 1983a). This virus was designated active TR-5 and the infectivity was 2.1×10^8 PFU/ml in the presence of 5 μg of chymotrypsin per ml.

Split vaccine. The split vaccine was prepared by the following method described by Ito and Matumoto (1986). The egg-grown Sendai virus was clarified by low speed centrifugation, and the supernatant was further centrifuged at 80 000 g for 2 h to sediment the virus. The pellet was suspended in distilled water to which was then added Tween 80 to give a final concentration of 0.125%. The mixture was treated with the same volume of ether for 20 min

at room temperature. After the treatment, the ether layer was removed, and the residual ether in the remaining fluid was removed with an evaporator. The resulting fluid had a titer of 320 000 HAU/ml with no detectable infectivity, and was used as the split vaccine.

Vaccination procedures

The active TR-5 was inoculated intranasally once to mice of each strain under mild anesthesia. In some of the experiments, aerosol inhalation with the active TR-5 was done as follows: eight mice each in a closed box were sprayed with 15 ml of the active TR-5 suspension by a nebulizer under a constant pressure at 1 kg cm^{-2} for 25 min and then transferred to a new cage for further experiments. The vaccination with the inactive TR-5 was carried out in the same manner as the active TR-5 except that the aerosol inhalation experiment was omitted. The split vaccine was inoculated intraperitoneally twice at an interval of two weeks.

Assay of primary vaccination effects

Both the vaccinated and non-vaccinated mice received intranasal challenge with 20 LD_{50} of the wild-type virus at certain intervals after the primary vaccination. The mice were observed for clinical signs and weighed daily since change in body weight has been shown to be an excellent indication of the severity of Sendai virus infection in mice (Tashiro and Homma, 1983a, 1985).

As another indicator for vaccination effects, serum HI antibody titers were determined by a micomethod as previously described (Tozawa et al., 1973).

Assay of booster effect

Twelve months after the primary vaccination with the active TR-5, the mice were boosted once by intranasal inoculation of the same virus. In the case of the split vaccine, previously vaccinated mice were boosted twice by intraperitoneal administration of the same vaccine at an interval of two weeks. After two months, the mice were challenged intranasally with 20 LD_{50} of the wild-type virus and observed for clinical signs and change in body weight as described above.

Contact infection

Mice were inoculated intranasally with 5 HAU of the active TR-5 or the wild-type virus under mild anesthesia. After 24 h, three infected mice inoculated with the active TR-5 were housed in a cage together with three uninfected contact mice which were used as indicators of the contact infection. In the case of the wild-type virus, an infected mouse was housed in a cage with five contact mice. After 7 days, the contact mice were transferred to new cages. Four weeks later, the sera were collected and HI antibody titers determined to assess contact transmission.

TABLE 1

Summary of the experimental schedule

Vaccines:
Active TR-5 (Live vaccine candidate with cleaved F glycoprotein)
Inactive TR-5 (with uncleaved F glycoprotein)
Split-vaccine (Ether-inactivated vaccine)
Mouse strains:
DBA/2
C3H
ICR
Sets of experiments:
Protection after primary vaccination.
Serum antibody production after primary vaccination.
Protection after booster vaccination.
Vaccination by aerosol inhalation.
Contact transmission of the virus.

Summary of the experimental schedules

For convenience, experimental schedules for evaluation of the live vaccine candidate are summarized in Table 1.

RESULTS

Protective immunity elicited by primary vaccination with the active TR-5 in various strains of mice

As shown in Fig. 1, the vaccination of DBA/2 mice with 0.5 HAU (a) and 0.005 HAU (b) of the active TR-5 protected the mice against challenge infection with the wild-type virus, although most mice exhibited loss of body weight within a week after the challenge. The protective immunity elicited by 0.005 HAU of the active TR-5 was comparable to that elicited by 1600 HAU of the split vaccine (c). However, vaccination with 0.5 HAU of inactive TR-5 (e) conferred only slight protection on mice. The active TR-5 itself did not cause any clinical signs and body weight increased steadily until the day of the challenge infection.

ICR mice were about 400-times more resistant to wild-type virus than were DBA/2 mice (see Materials and Methods). The protection profile, as demonstrated by daily change in the body weight, of ICR mice vaccinated with 0.5 HAU of the active TR-5 was comparable to that of DBA/2 mice vaccinated with 0.005 HAU of the same vaccine (data not shown).

The protective effect of the vaccination with the active TR-5 in C3H mice was also assessed. As little as 0.0005 HAU was enough to elicit a protective immunity against lethal challenge with the wild-type virus (data not shown).

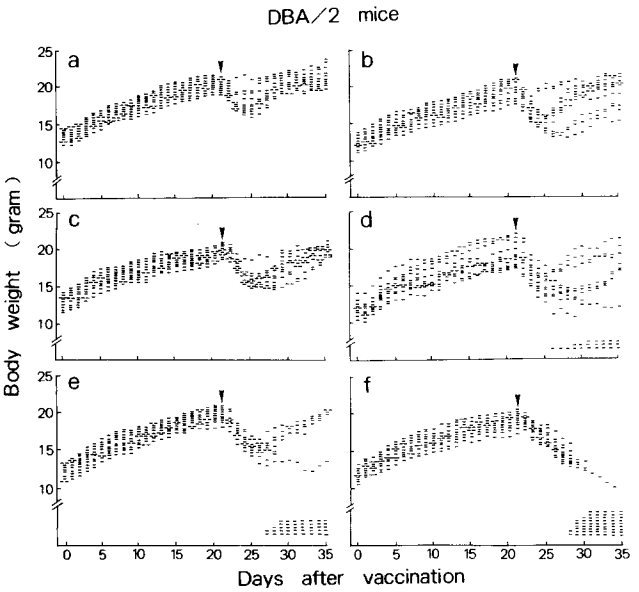

Fig. 1. Protective effects of primary vaccination with the active and the inactive TR-5 as well as the split vaccine against lethal Sendai virus infection in DBA/2 mice. Three-week-old mice were inoculated with either the active (a: 0.5 HAU, b: 0.005 HAU) or the inactive TR-5 (e: 0.5 HAU) intranasally or with the split vaccine (c: 1600 HAU, d: 160 HAU) intraperitoneally. Control mice (f) received nothing. After 3 weeks, all mice were challenged intranasally with 20 LD_{50} of the wild-type virus, and observed for clinical signs and body weight changes. Each dot represents the body weight of each mouse. Dead mice are plotted at the bottom. Arrow heads indicate the time of challenge infection with the wild-type virus. The differences in the survival ratios between the control (f) and test mice (a to e) are statistically significant (a, b and c) $P<0.001$; (d) $P<0.01$; (e) $P<0.05$.

Duration of the protective immunity elicited by primary vaccination

The following experiment was carried out to examine how long the vaccination-elicited protective immunity persisted. DBA/2 mice vaccinated with the active TR-5 or the split vaccine were challenged with the wild-type virus 6, 8 and 10 months later (Fig. 2). Six months after the primary vaccination, all of the vaccinated mice survived the lethal challenge (a, b), although some of the mice vaccinated with the split vaccine showed moderate to severe clinical signs. The protective effect elicited by the vaccination, however, declined to some extent during the 6 months when compared with the results shown in Fig. 1. The protection conferred by active TR-5 8 months after vaccination (d) remained at almost the same level as that observed at 6 months, whereas the protective effect of the split vaccine declined rapidly (e). At the end of 10 months, vaccination with the active TR-5 still conferred a certain degree of protection (g), 4 out of 7 mice being protected from the lethal challenge, while protection was no longer observed with the split vaccine-treated mice (h).

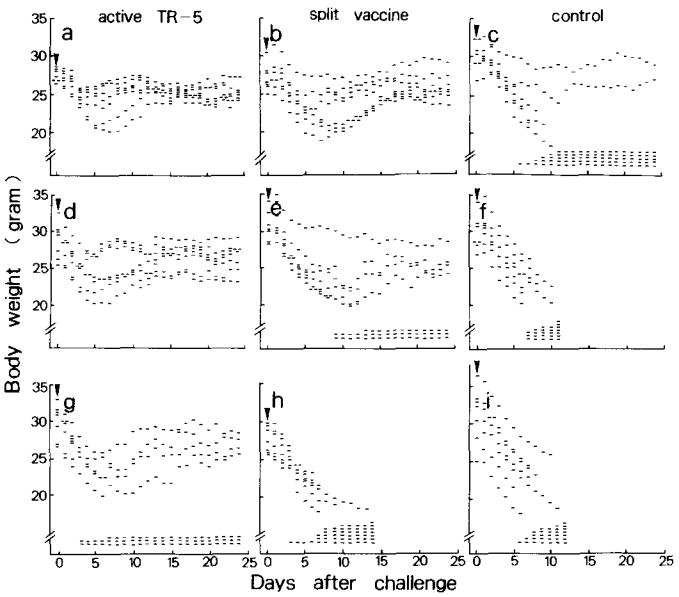

Fig. 2. Duration of protective immunity elicited by primary vaccination with the active TR-5 and the split vaccine. Four-week-old DBA/2 mice were vaccinated either intranasally with 0.5 HAU of the active TR-5 (a, d, g) or intraperitoneally with 1600 HAU of the split vaccine (b, e, h). Control mice remained unvaccinated (c, f, i). After 6(a, b, c), 8(d, e, f), and 10(g, h, i) months, the mice were challenged intranasally with 20 LD_{50} of the wild-type virus, and observed for clinical signs and body weight changes. Each dot represents the body weight of each mouse. Dead mice are plotted at the bottom. Arrow heads indicate the challenge infection with the wild-type virus.

Thus, a tendency was noted that the active TR-5 elicited longer-lasting protective immunity than the split vaccine, although the differences in the survival ratio were not statistically significant by the χ^2-test, due probably to the limited numbers of test mice.

Serum HI antibody response after primary vaccination

To assess another immunological aspect of vaccination, serum HI antibody titers of the vaccinated mice were determined. Three weeks after the vaccination of DBA/2 mice, the geometric mean values of HI titers elicited by 0.5 HAU of the active TR-5 and 1600 HAU of the split vaccine were 11 and 154 HIU/ml, respectively. The difference in the mean HI titers were statistically significant ($P<0.001$). It should be noted that despite the significant difference in the capacity to elicit serum HI antibody between the active TR-5 and the split vaccine, no difference was found between them in protection (see Fig. 1). Six months after vaccination with the split vaccine, the HI titers of mice decreased to a mean value of 32 HIU/ml. On the other hand, the HI titers of the mice vaccinated with the active TR-5 remained unchanged for

several months, and the mean HI titers 6, 8 and 10 months after the vaccination were practically the same as those elicited by the split vaccine (data not shown).

Effects of the booster vaccination

The protective immunity of the mice against lethal challenge infection declined 8 to 10 months after primary vaccination with either the active TR-5 or the split vaccine (see Fig. 2). The booster effect of these vaccines on the protective immunity was examined using DBA/2 mice. The test mice were boosted with the active TR-5 or the split vaccine 12 months after the primary vaccination and were challenged 2 months later with 20 LD_{50} of the wild-type virus. None of the mice boosted with the active TR-5 showed any clinical signs after the challenge, whereas most of the mice boosted with the split vaccine showed typical signs of Sendai virus infection and 3 out of 8 mice died of pneumonia within 2 weeks of challenge. The results again demonstrated that the active TR-5 conferred better protective immunity than the split vaccine.

Vaccination by aerosol inhalation

For the purpose of large scale vaccination, aerosol inhalation of the active TR-5 was examined. Mice receiving aerosol containing 250, 25 and 2.5 HAU of the active TR-5 as described in the Materials and Methods and were challenged 21 days later with 20 LD_{50} of the wild-type virus. All (8/8) of the mice vaccinated with the aerosol containing 250 HAU survived the lethal challenge. Aerosol vaccination with 25 and 2.5 HAU conferred partial protection against the challenge, with the survival ratios being 88% (7/8) and 50% (4/8), respectively. Thus, aerosol inhalation of the active TR-5 elicited protective immunity in a dose-dependent manner.

Absence of the virus spread from the infected mice vaccinated with the active TR-5

A contact transmission experiment was conducted as described in Materials and Methods. Two strains of mice, DBA/2 and C3H, were used. With either strain, no seroconversion occurred with the contact mice which were housed in a single cage together with the infector mice vaccinated with the active TR-5. The results suggested that the active TR-5 would not be transmitted from one mouse to another. On the contrary, all of the DBA/2 mice housed together with the wild-type virus-infected mice showed seroconversion. Contact transmission of the wild-type virus was also demonstrated among C3H mice, though less efficiently than among DBA/2 mice (data not shown).

DISCUSSION

We have studied the possibility of the use of the protease activation mutant of Sendai virus TR-5 as a live vaccine. One of the important aspects to be studied was whether or not TR-5 could confer protection beyond the strain barrier of mice, since the immunological response to an antigen as well as the susceptibility to a virus are known to be dependent largely on the genetic background of the host. The present study has revealed that the active TR-5 efficiently elicited protective immunity against potentially lethal challenge with the wild-type virus both in DBA/2 and C3H mice. These two strains of mice showed better immunological responses to the vaccination with the active TR-5 than ICR mice (Fig. 1). The difference in the induction of the protective immunity might be due to the difference in susceptibility to infection with the live vaccine, as the susceptibility of the ICR strain to the wild-type virus was shown to be different from the other strains as described in the Materials and Methods.

It was shown that less of the active TR-5 was required compared to the split vaccine to induce the same degree of protective immunity in mice (Fig. 1). Furthermore, the protective immunity elicited by the active TR-5 lasted longer than that induced by the split vaccine (Fig. 2). The booster effect was also more prominent with the active TR-5 than with the split vaccine. Thus, the active TR-5 appears to have many advantages as a vaccine compared to the ordinary split vaccine.

Although the split vaccine efficiently produced serum HI antibody in mice, the protective immunity against the lethal challenge was weak compared to the active TR-5, indicating that serum HI antibody titer does not necessarily correlate with the degree of protective immunity. In this connection, Nedrud et al. (1987) demonstrated a strong correlation between protection against Sendai virus infection and local immunity, i.e., antiviral IgA and IgG antibodies in the upper and the lower murine respiratory tracts, respectively. The protective effects of anti-viral IgA antibodies in the respiratory tract against infections with Sendai virus (Mazanec et al., 1987) and influenza A virus were also demonstrated (Liew et al., 1984; Clements et al., 1986). Furthermore, higher nasal IgA antibody titers were found in humans vaccinated with a live vaccine than in those vaccinated with an inactivated vaccine (Johnson et al., 1985; Clements and Murphy, 1986). We previously observed that intranasal inoculation of the active TR-2 from which TR-5 originated induced secretory IgA antibody production in the respiratory tract of mice (Tashiro and Homma, 1985). Thus, it is likely that intranasal vaccination with active TR-5 induced local immunity, both secretory IgA and other classes of immunoglobulins, which protects mice against infection with wild-type Sendai virus. We have also reported the induction of a T cell-mediated immune response in mice inoculated intranasally with the active TR-2, but not in mice

inoculated intraperitoneally with the split wild-type virus (Tashiro et al., 1988).The involvement of T cell-mediated immunity may account for the better booster effect elicited by active TR-5 than that by split vaccine.

The attempt to transmit active TR-5 from vaccinated to unvaccinated mice failed, indicating that the contact infection with the active TR-5 seldom occurs. These observations are consistent with our previous findings that infectious progeny virus could not be recovered from the lungs of mice infected with active TR-2 and that virus replication was limited to a single cycle (Tashiro and Homma, 1983a, 1985). Also, repeated attempts to recover the trypsin sensitive revertant by successive blind passages of lung homogenates to uninfected mice have so far failed, despite the fact that such a revertant could easily be obtained in tissue culture by passaging TR-5 in the presence of trypsin (Itoh and Homma, 1988).The additional advantage of TR-5 as a live vaccine candidate is that the mechanism of attenuation of TR-5 has been fully studied; the resistance of TR-5 to the trypsin activation is due to the amino acid change from arginine to isoleucine at residue 116 of the F glycoprotein which is the cleavage site for trypsin. In addition, the cleavage site mutation together with resistance to trypsin could be markers that easily distinguish vaccine virus from wild-type virus.

The active TR-5 vaccine is a candidate for a novel live vaccine having a mutation at the cleavage site.

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