Immune Responses in Mice Immunized Intranasally With Sendai Virus

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Abstract: Mice were immunized 3 times intranasally (i.n.) at intervals of 2 weeks with a formalin-inactivated Sendai virus (SV) whole virus with and without whole formalin-inactivated *Bordetella pertussis* (Bp) as adjuvant. The antibody response to SV was measured using the hemagglutination inhibition (HI) test in serum, and the IgA and IgG antibodies were evaluated using enzyme linked immunosorbent assay in saliva and serum. It was demonstrated that repeating immunizations with SV plus Bp could induce HI serum antibodies, IgA and serum IgG antibodies. However, in mice immunized subcutaneously with SV alone, high serum IgG antibodies and HI titers were induced only. In contrast, the animals immunized i.n. with SV alone had significantly lower titers to SV than those of the other groups.

Key Words: Adjuvant, intranasal, Sendai virus, pertussis

Introduction

Sendai virus (SV) is a murine parainfluenza virus type 1 (MPIV1) (parainfluenza family), which is formed in mice, rats, hamsters and guinea pigs. It causes a typical respiratory infection and has often been used as a model for respiratory virus infections (1-5). It has also been demonstrated serologically that this infection occurs fairly often in pigs (6). The natural hosts of SV have not been clearly identified, but the virus is referred to here as MPIV1 because it infects mice efficiently, causes disease, and spreads readily to uninfected animals (4).

Although SV 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 serologic evidence of MPIV1 in wild mouse populations (2), and the natural host (or hosts) of MPIV1 remains unknown.

Several studies on animals have shown that vaccines consisting of inactivated parainfluenza virus, or components of the virus, may also be immunogenic when given intranasally (i.n.) (7-9).

Non-replicating mucosal vaccines containing killed microbes, or parts thereof, may represent an alternative to vaccines maintaining live attenuated microorganisms that themselves carry some risk of causing disease. Results from animal studies, however, suggest that this kind of vaccine based on components of microbial origin may be effective only if so-called mucosal adjuvants are added (10).

It has recently been shown that whole meningococci, as well as formalin-inactivated *Bordetella pertussis* (Bp), are able to enhance both systemic and mucosal antibody responses to the nasal viral vaccine that is weakly immunogenic in itself (11).

The secretion of vaccine-specific IgA-antibodies onto the mucosal surfaces, being the result of local mucosal stimulation, has been proposed as the most important advantage of mucosal vaccines (12,13). Non-replicating nasal vaccines may be thus developed as an alternative to corresponding vaccines for injection such as parainfluenza 1 virus (14).

The aim of the present study was the assessment of the efficacy of intranasal immunization of mice with formalin-inactivated whole SV, with and without Bp as mucosal adjuvant, and the results were compared with separate groups of mice immunized subcutaneously (s.c.) without adjuvant. The efficiency of the systemic and

mucosal immunospecific response was evaluated by measurement of antibodies to SV, i.e. IgG and HI in serum as well as IgA in saliva.

Materials and Methods

Groups of BALB/c mice, 6 inbred females in each, 7 to 8 weeks old and weighing 18 ± 2.0 g, were obtained from the breeding unit of the Institute of Immunology, Vilnius University (Lithuania). The animals were kept in a corridor system under conditions of a semibarrier type in type T-3 cages (Velaz, Prague, Czech Republic), 6 animals per cage.

Chips of deciduous trees, sterilized at 120 °C for 20 min, were used for bedding. The bedding was changed twice weekly. Temperature was maintained at 20 ± 2 °C. Illumination was provided by daylight lamps for 12 h per day. Ventilation was provided by the circulation of unfiltered air (0.5 m^3/s). Relative humidity and noise were maintained at 55 ± 5% and 50 dB, respectively.

Food granules were produced by a manufacturer in Alytus (Lithuania). The chow contained gross energy at 13.6 MJ/kg, crude protein (20%), crude oil (6.2%), and crude fiber (3.9%). The feed was balanced in terms of amino acids and vitamins. Water was provided ad libitum.

SV strain Fushimi was obtained from the Institute of Virology (Moscow, Russia) and was grown, purified by ultracentrifugation at 20,000 x g for 2 h at 4 °C and inactivated as described previously (15).

Pertussis vaccine used as a source of Bp was obtained from the State Institute of Sera, Copenhagen, Denmark. Commercial vaccine contained formalin - inactivated whole Bp. Prior to use the Bp was concentrated by centrifugation at 7000 x g at 10 °C for 20 min. The formalin-inactivated whole SV and Bp, as bacterial adjuvant, were mixed immediately before immunization.

The study consisted of 4 treatment groups with 6 mice in each group. The treatment groups were as follows:

Group 1, SV and phosphate buffered saline pH 7.2 (PBS). An immunogen mixture containing 28 μg of SV in PBS was used for immunization.

Group 2, SV and Bp. An immunogen mixture containing 28 μg of SV and 20 μg of Bp in PBS was used for immunization.

Group 3, SV and PBS. An immunogen mixture containing 5 μg of SV in PBS was administered s.c.

Group 4, Placebo (PBS). PBS was used for immunization.

All groups were immunized 3 times at intervals of 2 weeks. During the intranasal immunizations (Groups 1,2 and 4) the upper part of the nose was held down to minimize the possibility of the mixture being swallowed or entering the trachea directly. The immunogen mixture (30 μl) was delivered slowly with a micropipette into the nares so that the mouse could sniff it in. For subcutaneous immunization, 0.2 ml of PBS containing the SV was s.c. injected into a dorsal site on the mice. Animals were not anesthetized during immunization.

Samples of saliva and serum were collected as described previously (16) on days 28 and 42 after the first immunization.

SV-specific IgA antibodies in saliva and SV-specific IgG antibodies in serum were analyzed using enzyme linked immunosorbent assay (ELISA) as described previously (16). The antibody titers were expressed as the reciprocal of the highest dilution of serum or saliva in which optical density (492 nm) was 2-fold higher than that of the negative samples. The titers were converted to a base-2 logarithmic scale.

Anti-SV virus antibody titers in serum samples were measured using HI as described previously (15). The antibody titers were expressed as the reciprocal of the highest dilution of serum at which complete inhibition of hemagglutination was seen and the titers were converted to a base-2 logarithmic scale.

The protein content of inactivated SV and Bp was measured as described earlier (17).

We performed the experiment on mice after having received permission No. 0086 from the Ethics Committee on the Use of Laboratory Animals of the State Food and Veterinary Service.

The means of the IgA, IgG, and HI antibody titers were compared using 2-tailed Student's t-test. All values were expressed as mean ± standard deviation and were considered statistically significant at $P < 0.05$.

Results

The efficacy of experimental formulations was evaluated by measuring IgA, IgG and HI antibody titers in

mice on days 28 and 42 after the first immunization. After intranasal immunization immunospecific anti-SV IgA antibodies were recorded (Figure 1). However, there was no difference in responses between the SV plus Bp and SV alone groups. The responses after intranasal immunization with SV alone were significantly lower than responses obtained with intranasal immunization with SV plus Bp on day 42 after the first immunization. Notably, the IgA titers were significantly increased on days 28 and 42 after the first immunization in each of the experimental groups of mice.
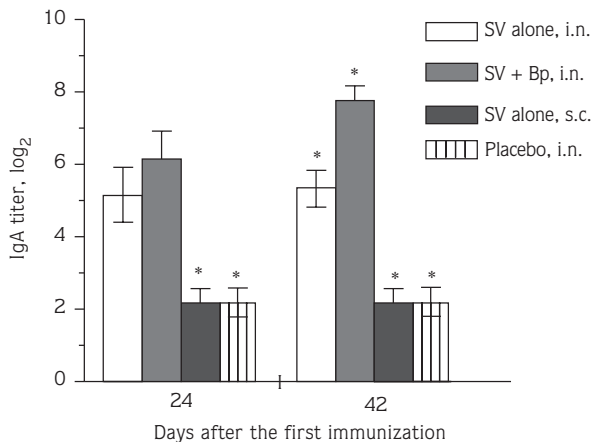

Figure 1. Saliva IgA antibody responses in mice after intranasal and subcutaneous immunizations with SV alone or admixed with Bp.
Asterisks indicate groups the values of which significantly differ from those of others on the same day.

The IgG titers induced with subcutaneous immunization with SV alone were significantly higher than those obtained with intranasal immunization with either SV alone or SV plus Bp on days 28 and 42 after the first immunization (Figure 2), while the IgG titers to SV after immunization with SV plus Bp were 1.24 and 1.34-fold higher, respectively, than those of mice injected i.n. with SV alone on days 28 and 42 after the first immunization.

In contrast, all animals immunized s.c. with SV alone had significantly higher HI titers to SV than did those injected i.n. with either SV alone or SV plus Bp on day 28 after the first immunization. Furthermore, HI titers induced after 2 or 3 intranasal immunizations with SV alone were significantly lower than those of the other experimental groups of mice (Figure 3). The animals that received SV plus Bp demonstrated significantly higher HI
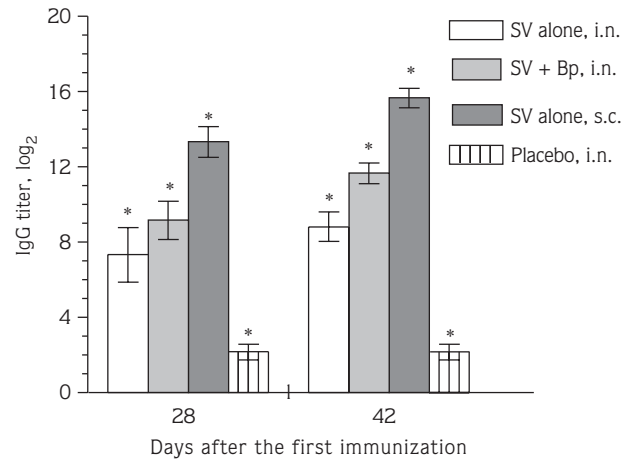

Figure 2. Serum IgG antibody responses in mice after intranasal and subcutaneous immunizations with SV alone or admixed with Bp.
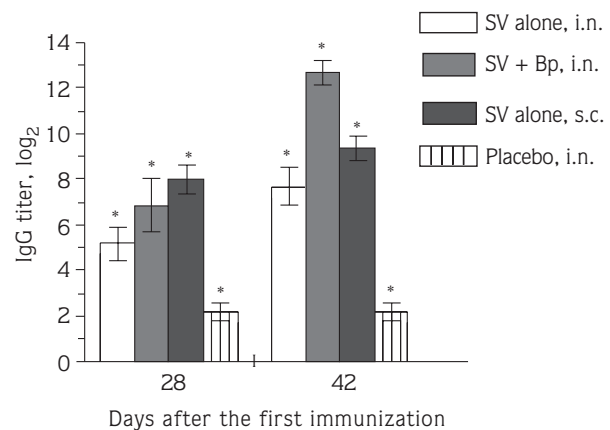Asterisks indicate groups the values of which significantly differ from those of others on the same day.

Figure 3. Serum HI antibody responses in mice after intranasal and subcutaneous immunizations with SV alone or admixed with Bp.
Asterisks indicate groups the values of which significantly differ from those of others on the same day.

titers to SV than mice immunized either i.n. or s.c. with SV alone on day 48 after the first immunization (Figure 3). After the third immunization in all experimental groups of mice, HI titers were significantly increased, 11.5, 1.85, and 1.2-fold, respectively.

Discussion

We demonstrated that repeated intranasal immunizations with SV plus Bp could also prime the immune system for both mucosal and systemic antibody

responses. Thus, the high titers to SV in saliva in Groups 1 and 2, indicate that induction of antibody responses took place in the upper airway mucosa. Furthermore, the animals immunized either i.n. or s.c. with SV alone showed high serum IgG antibodies and HI antibody titers. Induction of both systemic and mucosal antibody responses is a desirable characteristic of i.n. delivered vaccines (18).

The assumption that Bp may influence immune responses to antigens delivered on the mucosa by interfering with immunological processes below the mucosal surfaces is supported by the observation that preparations of whole-cell pertussis vaccines have a potent adjuvant effect when injected with other vaccine antigens (19).

These findings suggest that repeated immunizations with whole virus admixed with bacteria-derived components as mucosal adjuvants may thus allow for immunological memory to develop as well as formation of the protection mechanism against viral infection in mice (20).

Dissemination of the mucosal and systemic responses among mice receiving the SV plus Bp vaccine i.n. was marked. The discrepancy among the responses of these groups of mice may be due to the difference in physical form and composition of adjuvants, i.e. whole virus and Bp have different bioadhesive sites for absorption on the epithelial airway cells (21). Therefore, a modulation of these immunocompetent cells induced diverse levels of immunospecific responses.

Our results suggest that Bp, which is bacterium-derived and was used as an adjuvant for animal nasal vaccine, is a suitable candidate adjuvant for further studies.

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