MUCOSAL AND HUMORAL IMMUNITY IN MICE IMMUNIZED INTRANASALLY WITH MURINE PARAINFLUENZA VIRUS TYPE 1 IN COMBINATION WITH ADJUVANTS

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Abstract

Mice were immunized twice intranasally with a formalin-inactivated whole Sendai virus (SV) vaccine at a dose of 2.8 µg in combination with either cholera toxin (CT) or whole formalin-inactivated Bordetella pertussis (Bp). The mice induced not only serum antibodies, but mucosal IgA and serum IgG responses as well. All mice immunized subcutaneously with SV alone induced high serum IgG antibodies and haemagglutination inhibition antibody titres. In contrast, the animals immunized intranasally with SV alone had significantly lower responses to SV than the other ones.

Key words: mice, murine parainfluenza virus, vaccine, intranasal immunization, mucosal adjuvant.

Material and Methods

Animals. Groups of BALB/c mice, 6 inbred females in each, 7 to 8 weeks old, weighing 16-20 g, were obtained from the breeding unit of Vaisa, the experimental base of the Institute of Immunology, Vilnius University (Lithuania).

Virus. Fushimi SV strain, obtained from the Institute of Virology (Moscow, Russia), was used. It was grown, purified by differential centrifugation, and inactivated as described previously (1, 4).

Vaccine formulation. Three different vaccines were prepared. The first vaccine contained the SV alone. The other two vaccines consisted of the SV in combination with either CT or Bp, respectively, as mucosal adjuvants.

Adjuvant. CT was purchased from Calbiochem-Novabiochem (San Diego, CA). The B. pertussis vaccine used as a source of Bp was obtained from the State Institute of Sera, Copenhagen, Denmark. A commercial vaccine contained formalin inactivated whole bacteria Bordetella pertussis. Prior to use, the Bp was concentrated by centrifugation at 7000 x g at 10°C for 20 min. The formalin-inactivated whole Sendai virus and bacterial adjuvants were mixed immediately before immunization.

Immunization. Each of the three groups of six mice was immunized intranasally (i.n.) with 30 µl of one
Fig. 1. Saliva IgA antibody response in mice after i.n. and s.c. immunizations with SV alone or admixed with either CT or Bp. Asterisks indicate groups whose values differ significantly from those of the others at the same day.

Fig. 2. Serum IgG antibody response in mice after i.n. and s.c. immunizations with SV alone or admixed with either CT or Bp. Asterisks indicate groups whose values differ significantly from those of the others at the same day.

Fig. 3. Serum HI antibody response in mice after i.n. and s.c. immunizations with SV alone or admixed with either CT or Bp. Asterisks indicate the groups whose values differ significantly from those of the others, at the same day.
of the prepared vaccines. The vaccines contained 2.8 µg of the total protein. The same dose of SV was used in combination with 4.0 µg of CT or 20 µg of Bp, respectively. Furthermore, one group of animals was immunized subcutaneously (s.c.) using 5.0 µg (measured as total protein) of the SV vaccine alone, and served as positive control. The second group of mice was immunized i.n. with placebo (30 µl of phosphate buffered saline). All the mice were immunized two times at four-week intervals. During the i.n. immunization the upper part of the nose was held down to minimize the possibility that the vaccine would be swallowed or enter the trachea directly. Antigen solution was administered slowly with a micropipette into the nares so that the mouse could sniff it in. The animals were not anesthetized during immunization.

**Collection of samples.** Samples of saliva and serum were collected four weeks after initial immunization and two weeks after the second i.n. dose. Saliva samples were collected as described earlier (11). Blood was obtained from the lateral femoral vein and kept in heparinized capillary tubes (Virtex, Herlev, Denmark). Then it was separated and stored at -20°C until it was analysed.

**Quantitation of antibody response.** SV-specific IgA antibodies in saliva and SV-specific IgG antibodies in serum were analysed by the ELISA as described earlier (23). The antibody titres were expressed as the reciprocal of the highest dilution of serum or saliva, the optical density (492 nm) which was 2-fold higher than that of the negative samples. The titres were converted to a base-2 logarithmic scale.

**Haemagglutination inhibition.** SV antibody titres in serum samples were measured by haemagglutination inhibition (HI) as described previously (1). The titre was expressed as the reciprocal of the highest dilution of serum at which complete inhibition of haemagglutination was seen and the titres were converted to a base-2 logarithmic scale.

**Total protein estimation.** The protein content of inactivated SV was calculated as described earlier (14).

**Statistical analyses.** The mean of the IgA, IgG and HI antibody titres were compared using two-tailed Student’s t-test. All the values were expressed as mean ± standard deviation and were considered to be statistically significant at P < 0.05.

**Results**

**Mucosal antibody response after immunization with SV in combination with adjuvants.** Mucosal antibody responses have been observed in mice immunized i.n. and s.c. (Fig. 1). Antibody titres in animals immunized i.n. with the SV alone were significantly lower than in those receiving the same antigen with either CT or Bp at days 28 and 42 after the first immunization. On the other hand, the responses to SV plus CT injection did differ significantly from the responses to similar virus with Bp at day 42 after the first immunization. Notably, the IgA titres were significantly increased within 28 to 42 d after the first immunization in each of the experimental groups of mice.

**The IgG antibody response after immunization with SV together with adjuvants.** The IgG titres in serum to SV after immunization with either CT or Bp adjuvanted SV-groups were higher than those in mice injected with the SV alone, at days 28 and 42 after the first immunization (Fig. 2). The mice immunized s.c. had significantly higher antibody titres than did those immunized i.n. at days 28 and 42 after the first immunization. On the other hand, there was no difference in IgG responses among the groups immunized with SV mixed with adjuvants at days 28 and 42 after the first immunization. In fact, the mice immunized s.c. had significantly higher antibody titres than mice that received SV with adjuvants, either CT or Bp, at days 28 and 42 after the first immunization. Furthermore, the IgG titres were significantly increased within 28 to 42 d after the first immunization in each of the experimental groups.

**The serum HI antibody response after immunization with SV in combination with adjuvants.** The serum HI antibody response to SV in mice immunized i.n. and s.c. is shown in Fig 3. Animals that were immunized s.c. with SV alone demonstrated a significantly higher HI antibody titre to SV than those immunized i.n. at day 28 after the first immunization. Animals that received i.n. injection of SV alone had significantly lower HI antibody titres to SV than mice immunized with SV in combination with either CT or Bp. At day 42 after the first immunization the HI antibody titres were significantly lower in mice immunized i.n. with SV alone than those in other experimental animals. On the other hand, only the antibody titres induced by i.n. immunization using SV plus Bp were significantly higher than the antibody response induced after s.c. immunization with SV alone. Of interest, the antibody titres were significantly increased within 28 to 42 d in each of the experimental groups of animals.

**Discussion**

In this study, we have demonstrated that i.n. immunization with SV in combination with either CT or Bp vaccines could also prime the immune system for both mucosal and systemic booster antibody responses to later repeated i.n. immunizations. Thus, the high titres to SV in saliva indicate that the induction of antibody responses had taken place in the mucosa of the upper respiratory tract. Furthermore, the animals immunized either i.n. or s.c. with SV alone showed high serum IgG and HI antibody titres. Induction of both systemic and mucosal antibody responses is a desirable characteristic of intranasally administered vaccines (10). These findings suggest that repeated immunization with the whole virus in combination with bacteria-derived components as mucosal adjuvants may allow immunological memory to develop as well as to form the protection mechanism against viral infection (15).
In the present work, we found out the significant dissemination of the mucosal and systemic responses among mice receiving i.n. SV vaccines with the CT adjuvant. The discrepancy among responses in these mice may be due to the difference in physical form and composition of the adjuvants, i.e. whole virus and Bp may have different bioadhesive sites for absorption on the epithelial cells of the respiratory tract (24). Therefore, a modulation of these immunocompetent cells induced various levels of immunospecific responses.

Our results suggest that non proliferating vaccines based on bacterium-derived particles may be effective when given i.n. and that they may be used in conjunction with similar vaccines.

This work is a necessary step prior to the use of the vaccine formulations. The nasal mode of immunization has the prospect to increase the efficacy of vaccines and also to develop an active nasal parainfluenza vaccine.

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