ENHANCEMENT OF ANTIBODY RESPONSE TO SENDAI VIRUS IN RABBITS BY USE OF VARIOUS ADJUVANTS

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Abstract

Three different adjuvants were compared to assess their efficacy. Rabbits were twice injected subcutaneously with monthly interval and after 14 d the animals were again immunized twice intramuscularly with 2-week intervals with Sendai virus in combination with either saponin, ginseng, or Al(OH)₃. The efficacy of the adjuvants was evaluated by measurement of immunospecific IgG antibodies at days 51 and 65 after the first immunization. We found that the animals inoculated with SV plus saponin or Al(OH)₃ induced equal levels of IgG, while the rabbits immunized with SV plus ginseng showed lower titres than those in other experimental groups. Addition of CFA into the immunization mixture had no effect on enhancement of the immune response in animals at day 65 after the first immunization.

Key words: rabbit, immunization, adjuvants, IgG.

The majority of laboratory animals are used as models of man in biomedical research. A significant proportion however, of the animals are used simply to produce biological reagents. The use of animals for antibody production may be associated with ethical problems and the immunization schemes can be more or less aggressive depending on the site of administration, frequency of boosting, and type of immunopotentiating adjuvant.

Adjuvants are generally added to an antigen solution to activate the immune system non-specifically and to retain the antigen so that it is released slowly from the injection site. One of the most popular experimental adjuvants is Freund’s complete adjuvant (CFA) (4), which is an oil-based reagent containing heat killed and dried Mycobacterium tuberculosis in a mixture of paraffin oil and mannide monooleate oil. The bacterial components of CFA often cause severe side effects such as local necrosis, ulceration, fistulous tracts and disseminated granulomas (2, 7). For this reason, Codes of Practice in Canada, the USA and the Netherlands restricted researchers in using Freund’s adjuvant (FA).

Experimental studies in animals have revealed an array of diverse products which could function as an adjuvant and could probably be used as an alternative to FA.

It was showed that saponins extracted from other sources, e.g. from the bark of the tree Quillaja saponaria (molina), have been used as an adjuvant in the content of the vaccines (11). Ginseng, dry extracts prepared from the Panax ginseng C.A. Mayer-root contain immunomodulators named ginsenosides, which enhance in the pig the antibody response to viral antigen (10). The aim of the present study was comparison of the level of the immune response in rabbits to Sendai virus (SV), as a model immunogen, in combination with several adjuvants.

Material and Methods

Animals and their maintenance. Groups of Chinchilla rabbits, four females in each, 3 months old, weighing 2.0 ± 0.2 kg, were obtained from the breeding unit of the Institute of Immunology, Vilnius University (Lithuania). Animals were kept in a corridor system under conditions of semibarrier type in cages of type T/K (Velaz, Praha, Czech Republic), one animal per cage.

Chips of deciduous trees, sterilized at 120°C for 20 min, were used for bedding. The bedding was changed twice weekly. The temperature was maintained at 16 ± 2°C. Illumination was realized by daylight lamps during 12 h day. Ventilation was carried out by circulation of unfiltered air (0.5 m³/s). Relative humidity and noise were maintained at 55 ± 5%, and 50 dB, respectively.

Food granules were produced at a manufacture of Alytus (Lithuania). It consisted of gross energy 8.5 MJ/kg, crude protein (16.2%), crude oil (3.0%), and crude fibre (15.2%). The feed was balanced on amino acids and vitamins. Water was provided ad libitum.

Preparation of viral antigen. 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 (1). For preparation of mixture for immunization of animals the viral suspension was used containing 400 µg in 1 ml of phosphate-buffer saline, pH 7.2 (PBS).

**Immunization protocol.** The study consisted of 8 treatment groups with 4 rabbits in each group. The treatment groups were as follows:

- **Group A**, SV and saponin. The viral suspension was mixed with an equal volume of solution containing saponin (Merck, Germany) 10 mg in PBS.
- **Group B**, SV and saponin with CFA. The mixture containing SV and saponin was prepared as described for Group A and then it was emulsified with an equal volume of CFA (Calbiochem, Corp, USA).
- **Group C**, SV and ginseng. The viral suspension was mixed with an equal volume of solution containing ginseng (350 mg in PBS). The ginseng used in this study was a standardized dry extract prepared from the Panax ginseng C.A. Meyer-root (KRKA, Novo, Slovenia). The extract contained the ginsenosides: 10% Rg1, in one capsule (350 mg).
- **Group D**, SV and ginseng with CFA. The mixture containing SV and ginseng was prepared as described for Group C and then it was emulsified with an equal volume of CFA (Calbiochem).
- **Group E**, SV and Al(OH)$_3$. The viral suspension was mixed with an equal volume of solution containing 0.1% (v/v) Al(OH)$_3$.
- **Group F**, SV and Al(OH)$_3$ with CFA. The mixture containing SV and Al(OH)$_3$ was prepared as described for Group E and then it was emulsified with an equal volume of CFA (Calbiochem).
- **Group G**, SV with CFA (Positive control). The mixture containing SV was emulsified with an equal volume of CFA (Calbiochem).
- **Group H**, SV and PBS (Negative control). The mixture containing SV in PBS.

All groups of animals were immunized subcutaneously at seven different sites on the back and after a month the rabbits were boosted with subcutaneous injections. After 14 d the animals were immunized twice intramuscularly at 2-week interval.

**Collection of samples.** Samples of serum were collected at days 51 and 65 after the initial immunization. Blood samples were taken from the marginal ear vein. Serum was obtained from the blood as described previously (1).

**Quantitation of antibody responses.** SV-specific IgG in serum were analysed by enzyme-linked immunosorbent assay (ELISA) as described earlier (8). In the assay the peroxidase-conjugated goat anti-rabbit IgG was used (Sigma, St. Louis, USA). The antibody titre of serum was calculated as the reciprocal of the dilution of sample that gave absorbance to half the value.

**Total protein estimation.** The protein content of inactivated SV was calculated as described previously (9).

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

**Results**

**Serum immunospecific anti-SV antibody titres.** Generally, immunospecific anti SV IgG antibodies were recorded in all groups (Fig. 1, 2), while the titres were less than log 2 3 in animals before initial immunization.

The IgG titres in the Group C were significantly lower than those in other experimental groups at day 51 after initial immunization (7 d after the third immunization) (Fig. 1). Interestingly, in the Groups A and G the same IgG titres were observed, whilst the IgG titres in the Group F were significantly higher than those in other experimental groups. IgG titres in B and D groups were, respectively, 1.1 and 1.13-fold lower than those in E Group.

In contrast, there was no significant difference in IgG titres among the Groups A, B, D, and E at day 65 after the first immunization (7 d after the fourth immunization) (Fig. 2). In fact, IgG titres in the Group C were significantly lower than those in the other experimental groups, whereas IgG titres in the Group F achieved the maximal level compared with others. The addition of the CFA in immunization mixture has no effect on enhancement of the immune response in the Groups B and D, especially at day 65 after the first immunization.

Furthermore, the IgG titres were significantly increased within 51 to 65 d after the first immunization in each of the experimental groups, except for the Group E.

In addition, no side effects occurred in the groups, which received immunization mixture without CFA (data not shown) during all time of the immunization regime.

**Discussion**

In this study, immunization of rabbits with SV in combination with various adjuvants caused specific antibodies to be present in the serum. The CFA was used as a ‘golden’ standard since this adjuvant is very effective in inducing a specific immune response to antigens.

The saponins extracted from the bark of the tree *Q. saponaria* are an interesting alternative to the aluminium salts (Al-salts). The ginsenosides extracted from *P. ginseng* C.A. Meyer-root are also saponins, triterpenoid glycosides of the dammaran series (3, 6, 12). In this paper, the saponin and ginseng demonstrated an adjuvant effect after several immunizations. It confirms earlier findings (5, 10) of studies in which ginseng and saponin were used as adjuvants. The best results were obtained when Al(OH)$_3$, and CFA were included in the immunization mixture, probably, they act synergistically.
Immunomodulators directly modify a specific immune function or have a net positive or negative effect on the activity of the immune system. Herbal drugs and their active components have been shown to be an important source of immunomodulators (12).

The present results indicate that saponin and ginseng seem to have a stimulating effect on the humoral response in rabbits.

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References


