Induction of humoral and cell-mediated immunity to hepatitis B surface antigen by a novel adjuvant activity of Oka varicella vaccine

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Oka varicella vaccine induces humoral and cell-mediated immunity to varicella-zoster virus (VZV), even in immunocompromised hosts. This vaccine showed novel adjuvant activity against co-inoculated hepatitis B surface antigen (HBsAg). Either a mixed inoculation of HBsAg with heat-inactivated Oka varicella vaccine at one site or a separate inoculation of HBsAg and live vaccine at different sites induced an antibody response but failed to induce delayed type hypersensitivity (DTH) to HBsAg. In contrast, immunization of HBsAg mixed with live vaccine induced DTH and an enhanced antibody response to HBsAg. The adjuvant activity of Oka varicella vaccine was similar in terms of antibody production to that of alum adjuvant. A T helper cell-dominant immunity to VZV and HBsAg continued for 1 year. Oka varicella vaccine combined with a foreign antigen may serve as a novel polyvalent vaccine for the infectious diseases for which cell-mediated immunity is beneficial.

Oka varicella vaccine has been developed to confer active immunity to immunocompromised children and its further application has lead to its approval for general use in the United States (Takahashi et al., 1974; Arvin & Gershon, 1996; Vazquez et al., 2001). Oka varicella vaccine can be applied to immune individuals to boost immunity to varicella-zoster virus (VZV) (Baba et al., 1980; Berger et al., 1984; Levin et al., 1994) and now to elderly people to prevent zoster and post-herpetic neuralgia (Takahashi et al., 1998, 2001; Berger et al., 1998; Levin et al., 1998). T helper cell (Th1)-dominant immunity has been characterized by the VZV skin test, represented by delayed type hypersensitivity (DTH) (Kamiya et al., 1977; Asano et al., 1981; Shiraki et al., 1984; LaRussa et al., 1985), cytokine production and cytotoxicity (Diaz et al., 1989; Jenkins et al., 1998). Based on this attenuation and immunogenicity, we have developed a recombinant varicella vaccine expressing either the hepatitis B surface antigen (HBsAg) (Shiraki et al., 1991) or the human immunodeficiency virus Env antigen (Shiraki et al., 2001). Immunization of the recombinant Oka varicella vaccines induces cell-mediated immunity to the expressed antigens (Shiraki et al., 2001; Kamiyama et al., 2000) and a recombinant vaccine expressing herpes simplex virus (HSV) glycoprotein D alleviates genital HSV infection (Heinemann et al., 1995).

Some vaccinees respond to the VZV skin test on day 4 after vaccination and patients infected with VZV respond once the rash appears (Asano et al., 1985). The cutaneous reaction to gH : gL appears in guinea pigs on day 3 after Oka varicella vaccine inoculation (Sato et al., 1998). Oka varicella vaccine replicates near the inoculation site and then the immune response, which might be created mainly by dendritic cells and infiltrating lymphocytes, results in the induction of DTH (Finkelman, 1995; Abbas et al., 1996; Moser & Murphy, 2000). We hypothesized that the immune response induced by Oka varicella vaccine may recognize HBsAg to create the Th1-dominant immune response, as assessed by DTH, after its co-immunization with HBsAg. Thus, we have immunized HBsAg with Oka varicella vaccine and observed a Th1-dominant immune response to HBsAg in the experimental model, confirming the novel adjuvant activity of Oka varicella vaccine.

HBsAg and alum-conjugated HBsAg vaccine (10 µg) derived from yeast were supplied from the Research Foundation for Microbial Diseases of Osaka University, Suita, Japan. VZV gE : gL was purified by the application of an Oka varicella vaccine-infected cell lysate to an affinity column coupled with a monoclonal antibody against gE (Kamiyama et al., 2000; Shiraki et al., 1997, 2001). Oka varicella vaccine was prepared from infected human embryonic lung cells (Kamiyama et al., 2000; Sato et al., 1998; Shiraki et al., 1984, 2001). The mock vaccine was prepared similarly but without infection. The dose of Oka varicella vaccine was 1 × 10⁴ p.f.u. for immunization. The dose for the heat-inactivated vaccine was also 1 × 10⁴ p.f.u.; heat inactivation was at 56°C for 30 min.
Male guinea pigs (Hartley strain) weighing about 280 g (Sankyo Labo Service) were immunized subcutaneously with various vaccines (Sato et al., 1998; Shiraki et al., 1984). For the HBsAg dose-finding experiment, Oka varicella vaccine and 0, 1, 10, 20 or 40 μg of HBsAg were mixed and five guinea pigs from each group were immunized. After 3 weeks, humoral and cell-mediated immune responses were evaluated by ELISA and the skin test to gE:gl and HBsAg.

From the results of the dose-finding experiment, the amount of HBsAg was determined to be 20 μg per dose. To determine the specificity of the live, combined vaccine, the immune responses to gE:gl and HBsAg were compared among the seven immunization groups. When the live or heat-inactivated vaccine was mixed with HBsAg, the mixture was inoculated to the right back. When the live or heat-inactivated vaccine and HBsAg were inoculated separately, the live or heat-inactivated vaccine and HBsAg were inoculated on the right and left backs, respectively. Five guinea pigs were immunized under the following vaccination schemes: a mixture of live vaccine and HBsAg; a mixture of heat-inactivated vaccine and HBsAg; a mixture of mock vaccine and HBsAg; a live vaccine and HBsAg inoculated at separate sites; a heat-inactivated vaccine and HBsAg inoculated at separate sites; a mock vaccine and HBsAg inoculated at separate sites; and a control (PBS alone). Immune responses to gE:gl and HBsAg were evaluated 3 weeks after vaccination.

Guinea pigs were injected intradermally at three sites with 0.1 ml of antigen on their backs; hair was removed by chemical depilatory. HBsAg (1 μg) and gE:gl (1 μg) were used for the dose-finding experiment and HBsAg (2 μg) and gE:gl (3.2 μg) were used for the other experiments. The erythematous area was measured at 8, 24 and 48 h and the size (area) of the cutaneous reaction was determined at three sites at 24 h (Kamiyama et al., 2000; Sato et al., 1998; Shiraki et al., 2001).

Sera were tested for antibody titres to gE:gl and HBsAg by ELISA (Kamiyama et al., 2000; Sato, 1998; Shiraki et al., 2001). Sera were diluted 1:80 with PBS containing 2% skimmed milk and applied to each well treated with 1 μg HBsAg and 0.5 μg gE:gl for antibody titres to HBsAg and gE:gl, respectively. Then the anti-guinea pig IgG, goat IgG antibody conjugated with peroxidase (whole molecule, Cappel) was distributed to the wells and the reaction was visualized by HAT-EIA (Denka Seiken). Epitope analysis of HBsAg was performed using 16 species of its constituent peptides, as reported by Kamiyama et al. (2000). The statistical significances of the data were assessed using an unpaired Student’s t-test.

Fig. 1 shows the dose response effects of HBsAg in the Oka varicella vaccine mixture on the antibody response and DTH to gE:gl and HBsAg. There was no significant difference in the magnitude of antibody responses to gE:gl among the various immunization groups. Antibody responses to gE:gl were not affected by the amount of HBsAg. Antibody responses to HBsAg were significantly lower in the heat-inactivated vaccine groups than those in the live vaccine groups (P<0.05). Weak responses to gE:gl remained in the heat-inactivated vaccine groups (with HBsAg at 20 and 40 μg) until 24 h; these were thought to be Arthus reactions from the time-course of reaction. Significantly strong DTH was induced in the live vaccine groups, especially at doses of 20 and 40 μg (P<0.05). The antibody response and the magnitude of DTH to HBsAg increased similarly depending on the amount of HBsAg in the live vaccine mixture and the response was almost saturated when 20 μg HBsAg was in the mixture. Thus, live Oka varicella vaccine showed significant adjuvant activity towards HBsAg by inducing DTH and enhancing antibody production and these responses were almost saturated when 20 μg HBsAg was in the mixture.

![Fig. 1. Dose response effect of HBsAg in the mixture with live and heat-inactivated Oka varicella vaccine. Guinea pigs were immunized with the mixture of live and heat-inactivated Oka varicella vaccine (1 x 10^6 p.f.u.) and HBsAg at 0, 1, 10, 20 or 40 μg per dose. Immune responses were assessed by ELISA and cutaneous reaction to gE:gl and HBsAg. The size (area) of the cutaneous reaction was regarded as an ellipse and expressed by the formula: Area=π x (long diameter) x(short diameter) / 4.
Results are expressed as mean±SEM for five guinea pigs; bars indicate SEM. (a) Antibody response to gE:gl. (b) Cutaneous reaction to gE:gl. (c) Antibody response to HBsAg. (d) Cutaneous reaction to HBsAg. Bars between groups indicate a statistically significant difference. A single asterisk (*), a double asterisk (**) and a triple asterisk (***) indicate P<0.05, <0.01 and <0.001, respectively.](Image 319x450 to 548x712)
The dose of HBsAg in the Oka varicella vaccine mixture was determined to be 20 μg by the dose-finding experiment. Immune responses to gE:gl and HBsAg were compared among the seven immunization groups (Fig. 2). Antibody responses to gE:gl were induced similarly in groups immunized with both the live and the heat-inactivated vaccines but the mock vaccine- and PBS-immunized groups did not respond to gE:gl. DTH to gE:gl was induced significantly in the live vaccine groups but not in the other groups (P<0.001). Thus, the DTH response to gE:gl was dependent on immunization with live vaccine. Although antibody to HBsAg was induced in all groups immunized with HBsAg-containing vaccines, HBsAg mixed with live vaccine induced a significantly higher antibody response than the other groups (P<0.01). Also, this group alone elicited a significant DTH response to HBsAg (P<0.001). These results were confirmed by repeating the experiments. Immunization of HBsAg and live vaccine at separate inoculation sites induced DTH to gE:gl but not to HBsAg. This indicated that an immunization of HBsAg mixed together with live Oka varicella vaccine and inoculated at a single site was essential to induce DTH and enhanced antibody responses to HBsAg.

All groups immunized with HBsAg responded to the major ‘a’ loop epitope of HBsAg and the antibody titre to this epitope was proportionate to that of whole HBsAg, as assessed by epitope analysis of HBsAg with peptide ELISA (data not shown). Therefore, antibody production was quantifiably influenced but antigen recognition was not qualitatively affected by the immunization method.

Antibody decline during the year after immunization with combined vaccination was compared with HBsAg or PBS immunization alone (Fig. 3). Antibody titres to gE:gl declined gradually. Antibody titres to HBsAg were significantly higher in the combined vaccine group than in the other groups during this 1 year period. Antibody titres in the HBsAg alone group at the first month were equivalent to those of the combined vaccine group at 9 months (P<0.05) and the enhanced level of antibody titres by Oka varicella vaccine corresponded to the antibody decline seen at the 9 month period. DTH to HBsAg was observed in the combined vaccination group but not in the other immunization groups. The adjuvant effect of Oka varicella vaccine on cell-mediated and humoral immunity to HBsAg continued for at least 1 year.

We have examined the hypothesis that the local Th1-dominant immune environment where Oka varicella vaccine replicates may make it possible to recognize the co-existing foreign antigen and to induce a Th1-dominant immune response to this antigen. As we have postulated, immunization of HBsAg with Oka varicella vaccine induced DTH and enhanced antibody production to HBsAg. The local environments, including dendritic cells, lymphocytes and cytokines, may be important in inducing the immune response to HBsAg. The separate inoculations of HBsAg with live vaccine induced DTH to gE:gl but failed to induce DTH to HBsAg, suggesting the importance of the co-existence of HBsAg with live vaccine. On the other hand, immunogenicity, duration of immunity and safety of Oka varicella vaccine have been well documented, even in immunocompromised children (Takahashi et al., 1974; Arvin & Gershon, 1996). We have used HBsAg as an indicator to monitor the immune response in this study. A current alum or MF59 adjuvant has limited the potential to induce cell-mediated immunity (Singh & O’Hagan, 1999). When the adjuvant activity of Oka varicella vaccine to HBsAg was compared with alum-based HBsAg vaccine, there was no significant difference in antibody production to HBsAg (data not shown). The anti-HBsAg antibody levels of the combined vaccine group might be sufficiently high to protect against hepatitis B virus (HBV) infection (Jack et al., 1999). Successful induction and boosting of immunity to VZV by Oka varicella vaccine may support the general use of Oka varicella vaccine as a vaccine adjuvant. This indicates that Oka varicella vaccine as an adjuvant vaccine may be a
new concept in the development of a novel combined vaccine that may be able to confer a Th1-dominant immune response even in immune individuals.

Patients with chronic hepatitis B who are treated with lamivudine might need cell-mediated immunity to HBV to recover from their carrier state; otherwise, they need prolonged treatment until complete elimination of HBV (Lau et al., 2000). A Th1-dominant immune response is important for virus clearance and recovery from chronic hepatitis B (Rossol et al., 1997). The results with this combined vaccine suggested the possibility of improving the immune status in patients with chronic hepatitis B.

This is the first report that demonstrates the adjuvant activity of VZV using Oka varicella vaccine for inducing a cell-mediated immune response by simply mixing the candidate antigen with Oka varicella vaccine. Therefore, the utilization of Oka varicella vaccine as a novel adjuvant vector may contribute to a new vaccine development for the infectious diseases in which a Th1-dominant immune response may play a beneficial role.

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REFERENCES


