Novel Immunogenicity of Oka Varicella Vaccine Vector Expressing Hepatitis B Surface Antigen

Tomoko Kamiyama, Hitoshi Sato, Terumi Takahara, Seiji Kageyama, and Kimiyasu Shiraki

Recombinant Oka (ROka) varicella vaccine expressing hepatitis B surface antigen (HBsAg) and subunit HBsAg vaccine (SHV) were used as primary and booster HBsAg vaccines in 3 combinations (SHV-SHV, SHV-ROka, and ROka-SHV) in guinea pigs. Immune responses to HBsAg and varicella-zoster virus gE:gI were evaluated. The 3 combinations induced similar levels of the lymphocyte proliferation response to HBsAg. Of the 3 combinations, SHV-SHV induced the strongest antibody response to an “a” loop of HBsAg and to the whole HBsAg. Its ratio of antibody titer to this loop versus HBsAg was significantly higher than that in SHV-ROka, suggesting the supplementary recognition of the conformational epitope of HBsAg in SHV-ROka. SHV-ROka induced delayed-type hypersensitivity (DTH) to the HBsAg and gE:gI produced in infected cells. Thus, ROka induced DTH to HBsAg and enhanced recognition of the conformational epitope. ROka varicella vaccine may serve as a novel vaccine vector to induce a Th1-type immune response.

A total of ~200 million people worldwide are thought to be carriers of hepatitis B virus (HBV), which causes acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Vaccination with blood-derived and genetically engineered subunit HBV surface antigen (HBsAg) vaccine (SHV) is used to prevent HBV infection in high-risk groups. Oka varicella vaccine has been successfully used to confer active immunity without major adverse reactions in immunocompromised patients [1], and it is approved for general use in the United States. Oka varicella vaccine induces immunity to varicella-zoster virus (VZV) infection, which is characterized as a humoral antibody response and cell-mediated immunity, especially delayed-type hypersensitivity (DTH), by the cutaneous reaction with the varicella skin test antigen [2]. Oka varicella vaccine has been used to express foreign genes, such as Epstein-Barr virus glycoprotein [3] and herpes simplex virus glycoprotein D [4]. We have constructed and characterized a recombinant Oka varicella vaccine (ROka) that expresses HBsAg [5, 6]. The immunogenicity of ROka is similar to that of SHV in inducing antibody to HBsAg in guinea pig models of VZV infection [2, 5].

In this study, using HBsAg as an immunologic marker, we characterized the immunogenicity of Oka varicella vaccine as a vaccine vector. HBsAg of both ROka and SHV was derived from the same HBsAg gene (adr subtype) [5, 6]. We compared the humoral and cell-mediated immune responses to HBsAg induced by ROka and by SHV. Antibody induced by the immunization groups was compared by using glycosylated and nonglycosylated HBsAg and its constituent 16 species of the peptides. The discrepancy of antibody to HBsAg and the linear “a” loop epitope [7] suggested the enhanced recognition of the conformational epitope of HBsAg [8] by ROka immunization. We also characterized an inverse relationship between reduction of antibody production and induction of DTH, representing the macrophage–Th1 cell–type response by the Oka varicella vaccine vector.

Materials and Methods

Vaccine. SHV for human use was supplied by the Research Foundation for Microbial Diseases of Osaka University, Suita, Japan. SHV contains 10 μg of HBsAg derived from yeast with alum. ROka was prepared from infected human embryonic lung cells, as described elsewhere [5]. Guinea pigs were immunized subcutaneously with 1 dose of SHV or 15,000 pfu of ROka.

Antigens. Nonglycosylated HBsAg derived from yeast was supplied by the Research Foundation for Microbial Diseases, Osaka University. Glycosylated HBsAg derived from the sera of HBV carriers [9] was prepared from an HBsAg-positive control kit (International Reagent, Kobe, Japan) for the reversed passive hemagglutination (RPHA) test [5]. VZV gE:gI was purified by ap-
The erythematous area was measured at 8, 24, and 48 h, and its
response to HBsAg and gE:gI were evaluated 4 weeks after the 2d
vaccination.

Lymphocyte proliferation assay. Mononuclear cells from
spleens were separated by use of ficoll-hypaque density gradients
and resuspended in complete medium (RPMI 1640 supplemented
with 10% heat-inactivated fetal bovine serum and
5 × 10^5 M 2-mercaptoethanol) for lymphocyte proliferation [11]. In brief, 10 μL
of purified HBsAg (100 μg/mL) or gE:gI (100 μg/mL) was plated onto
each well of a 96-well plate, and 50 μL of mononuclear cells
(4 × 10^5 cells/mL) was added. The mixtures were cultivated in triplicates for 5 days and were pulsed during the final 18 h with 5 μCi
well of [3H] thymidine (84 Ci/mmol; Moravek Biochemicals, Brea,
California) for the determination of incorporated radioactivity. The results
were expressed as a stimulation index by dividing the radioactivity
of a stimulated culture by that of its unstimulated counterpart.

Cutaneous reaction. Guinea pigs were injected intradermally at
3 sites on their backs with 0.1 mL of HBsAg (1 μg) and gE:gI
(3.2 μg) after hair was removed with a chemical depilatory [2, 11].
The erythematous area was measured at 8, 24, and 48 h, and its
area was regarded as an ellipse and expressed by the following formula:
area = π × [(long diameter × short diameter)/4].

Antibody assay. Sera were tested for HBsAg and gE:gI by PHA
test (International Reagent) and ELISA, respectively [5, 11]. Sera
were diluted 100 times with PBS containing 2% skim milk and were
applied to each well, which had been treated with 0.5 μg of gE:gI,
to determine the antibody titer to gE:gI in the ELISA. Then
anti-guinea pig IgG goat IgG conjugated with peroxidase (whole
molecule; Organon Teknika/Cappel, Durham, NC) was distributed
to the wells, and the reaction was visualized by use of the substrate
solution of the hepatitis A total immunoglobulin–EIA kit (Denka
Seiken, Niigata, Japan). Antibody to HBsAg was measured by the
ELISA, using HBsAg derived from the sera of HBV carriers (gly-
cosylated HBsAg) and yeast (nonglycosylated HBsAg) [6, 9].
HBsAg was coated in wells at a concentration of 1 μg/50-μL well,
and a further reaction was done as described above.

Peptide ELISA. To determine the antigenic epitope of HBsAg, 16 species of peptides constituting the whole part of HBsAg were
prepared in 96-well plates of the Multipin noncleavable peptide
synthesis kit (Chiron, San Diego) in accordance with the manu-
facturer’s instructions. The antibody titer to each peptide was
measured by an ELISA assay.

Table 1. Antibody titer to varicella-zoster virus (VZV) gE:gI and hepatitis B surface antigen (HBsAg) in 3 immunization groups and 1 placebo group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHV-SHV</th>
<th>SHV-ROka</th>
<th>ROka-SHV</th>
<th>PBS-PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody to VZV gE:gI</td>
<td>0.002 ± 0.000a</td>
<td>0.565 ± 0.029b</td>
<td>0.231 ± 0.023</td>
<td>0.004 ± 0.001c</td>
</tr>
<tr>
<td>Antibody to HBsAg</td>
<td>19.4 ± 0.60</td>
<td>16.2 ± 0.49c</td>
<td>14.2 ± 0.80d</td>
<td>0.00 ± 0.00e</td>
</tr>
<tr>
<td>Glycosylated (human)</td>
<td>0.248 ± 0.019</td>
<td>0.174 ± 0.026f</td>
<td>0.132 ± 0.024g</td>
<td>0.00 ± 0.007h</td>
</tr>
<tr>
<td>Nonglycosylated (yeast)</td>
<td>0.966 ± 0.011</td>
<td>0.662 ± 0.040i</td>
<td>0.637 ± 0.072j</td>
<td>0.00 ± 0.000k</td>
</tr>
<tr>
<td>“a” loop peptide (111–131)</td>
<td>0.847 ± 0.087</td>
<td>0.351 ± 0.094l</td>
<td>0.382 ± 0.130m</td>
<td>0.00 ± 0.015n</td>
</tr>
<tr>
<td>Ratio of antibody to HBsAg and peptide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human/yeast</td>
<td>0.257 ± 0.020</td>
<td>0.259 ± 0.026o</td>
<td>0.202 ± 0.015p</td>
<td></td>
</tr>
<tr>
<td>Peptide/yeast</td>
<td>0.879 ± 0.097</td>
<td>0.506 ± 0.104q</td>
<td>0.545 ± 0.124r</td>
<td></td>
</tr>
<tr>
<td>Peptide/human</td>
<td>3.471 ± 0.363</td>
<td>1.978 ± 0.324s</td>
<td>2.665 ± 0.490t</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Antibody titer is expressed as mean ± SE of 5 guinea pigs. SHV, subunit HBsAg vaccine; ROka, recombinant Oka varicella vaccine; RPHA, reversed passive hemagglutination assay.

Results

Immune response to VZV gE:gI. We compared the immune
response to gE:gI among 4 immunization groups (table 1, figure
1). Because SHV-SHV and PBS-PBS immunization groups were
not immunized with VZV, no immune response to gE:gI was
observed. SHV-ROka and ROka-SHV immunization induced
similar lymphocyte proliferation responses and cutaneous re-
actions to gE:gI (figure 1). The cutaneous reaction continued
48 h after inoculation and represented typical DTH over the
time course. As shown in table 1, SHV-ROka and ROka-SHV
immunization induced significantly higher antibody titers against gE:gI than
ROka-SHV immunization (P < .01). This difference may be
caused by the decline in antibody titer 4–8 weeks after im-
munization with ROka.

Immune response to HBsAg. We compared the immuno-
genicity of ROka and SHV (table 1, figure 1). The PBS-PBS
combination did not induce any immune response to HBsAg. The 3 immunization combinations induced a similar level of
lymphocyte proliferation (figure 1C). The cutaneous reaction to HBsAg in the SHV-SHV and ROka-SHV groups showed a typical Arthus-type reaction over the time course, and both immunization groups, in contrast to the SHV-ROka group, failed to induce reactivity at 24 and 48 h (P < .01). SHV-ROka immunization induced a strong cutaneous reaction to HBsAg at 24 and 48 h, and the reaction profile during the time course was similar to that of gE:gI observed in ROka immunization. Thus, only SHV-ROka immunization induced the cutaneous reaction characteristic of DTH against HBsAg.

Antibody response to HBsAg. Table 1 shows the results of antibody production to HBsAg and its constituent peptides. Antibody to HBsAg assessed by the RPHA, using serum-derived HBsAg, was significantly higher in the SHV-SHV group than in the other combinations (P < .01). Glycosylated and non-glycosylated HBsAg were used as test antigens to measure antibody to HBsAg; by ELISA of the combinations, SHV-SHV immunization induced the strongest antibody production to glycosylated (P < .05) and nonglycosylated HBsAg (P < .01), consistent with the RPHA result.

All groups immunized with HBsAg vaccines responded to the major “a” loop epitope of HBsAg; little response to the other peptides was observed. There was no significant difference in the profile of the antibody response to each peptide among the 3 HBsAg immunization groups. However, antibody titer to the major “a” loop epitope of HBsAg was highest in the SHV-SHV group (P < .05) and was comparable in the ROka-SHV and SHV-ROka groups.

There was no significant difference in the ratio of antibody to nonglycosylated and glycosylated HBsAg among the 3 groups. However, the ratio of antibody to the “a” loop peptide versus the nonglycosylated or glycosylated HBsAg was smallest in the SHV-ROka immunization group and was significantly lower in the SHV-ROka group than in the SHV-SHV group (P < .05). This suggests that antibodies in the SHV-ROka immunization group may recognize an epitope other than the
A Th1-type response is important in viral clearance and recovery from chronic hepatitis B [15]. ROka induced a Th1-dominant immune response to HBsAg. Therefore, this recombinant vaccine may help improve the immune status to HBV in persons with chronic hepatitis B. However, because the Th1 response is also important in the pathogenesis of liver cell injury, liver function should be carefully monitored. Altogether, vaccination with this recombinant varicella vaccine may improve the immune status of patients with chronic hepatitis B and thereby have a beneficial effect in reducing hepatitis activity.

This study revealed that the Oka varicella vaccine vector induced a VZV-type immune response to HBsAg, especially a Th1-type response, as assessed by reduced antibody production and DTH induction. The Oka varicella vaccine vector may induce a Th1-type response to the foreign antigen. This response may be beneficial in improving the clinical status of patients such as those with chronic hepatitis B. Furthermore, the induction of cellular immunity by this live recombinant vaccine may be useful for future vaccine development, especially for human immunodeficiency and hepatitis C viruses, in which the humoral immunity produced by the traditional approach of the pure protein vaccine is not effective.

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References