KEYWORDS: bovid herpesvirus 1, glycoprotein, rabbit.

Bovid herpesvirus 1 (BHV-1) specifies 25 to 33 polypeptides, some of which are glycosylated and associated with the virus envelope and the infected cells [4, 8]. Glycoproteins of many enveloped viruses have been reported to play important roles not only in the early stages of infection [1] but also in protection against or recovery from infection as major targets of the immune response at both the humoral and cellular levels [13, 15]. BHV-1 glycoprotein gIII (designated gp87 in our previous study) functions as a major viral attachment protein [11], whereas gIV (gp71) appears to be responsible for penetration of the virus into the cell after viral attachment [6, 10]. gl (gp117) is also one of the major viral glycoproteins, and is known to cleave into 70-64- and 51-47-kDa components, although its function is still unknown [10]. All of these glycoproteins raise neutralizing antibodies and participate in antibody-dependent cell cytotoxicity and complement-mediated lysis of infected cells [3].

BHV-1 is a cause of respiratory, genital and nervous system infection in cattle [2]. Smaller laboratory animals such as mice, rats, and guinea pigs appear to be refractory to the virus [5]. The rabbit has been reported to show a variety of clinical syndromes after inoculation with BHV-1, suggesting its use as a laboratory model for the study of BHV-1 infection [7]. However, no information is available on BHV-1 proteins recognized by the rabbit immune system through the infection.

We described here a study on quantitative analysis of the antibody response against BHV-1 infection in rabbits, and a comparison with that in cattle.

The BHV-1 Los Angeles strain at 10 PFU/0.5 ml was inoculated intratracheally (IT) or intravenously (IV) into adult female rabbits. Blood samples were collected from each of the animals at one-week intervals. Sera collected from calves inoculated intranasally (IN) with the Los Angeles strain as described before [9] were kindly supplied by Dr. Y. Inaba (National Institute of Animal Health, Ibaraki, Japan). A radio-labeled-immunoprecipitation assay (RIPA) was carried out as described previously [11] except that Protein A-Sepharose beads were coated with anti-rabbit immunoglobulin or anti-bovine immunoglobulin antibodies. An enzyme-linked immunosorbent assay (ELISA) was also done using horseradish peroxidase conjugated anti-rabbit IgG antibodies. Virus neutralization assay was carried out by the 50% plaque reduction methods without complement [10].

The fluorographic antibody responses of rabbits IT-inoculated (A) and IV-inoculated (B) with the Los Angeles strain are shown in Fig. 1. IT-inoculated rabbits appeared to recognize mainly gl glycoprotein complex (gla, glb, and glc). From one to three weeks post-infection (PI), only gl complex was precipitated by the test sera. At 4 weeks PI, gIII and gIV became slightly detectable and the amount of gl precipitated was much greater than before. On the other hand, in the IV-inoculated rabbits, antibodies seemed to be produced mainly against gIII and gIV. Although gl complexes were precipitated by sera obtained at one and two weeks PI, little enhancement of the signals was found by those obtained later. The other two rabbits inoculated by the intratracheal or intravenous route showed similar responses to their counterparts (data not shown).

Figure 2 shows the antibody response of the IN-inoculated calf against BHV-1. The calf appeared to show a response similar to that in the IT-inoculated rabbit. Only gl complex was detected using serum obtained at one week PI, and then gIII and gIV were detected successively by sera obtained later. It seemed that gl complex was mainly precipitated at the final stage as well. The other two calves IN-inoculated with the Los Angeles strain showed similar patterns of antibody response (data not shown).

In order to assess the participation of rabbit antibodies which recognized individual glycoproteins in virus neutralization, each serum collected at 4 weeks PI was examined for its neutralizing activity. Sera R1 and R2, which were

![Fig. 1. Antibody response of BHV-1-infected rabbit. Sera obtained from intratracheally (A) or intravenously (B) inoculated rabbits were examined by RIPA. Lane a, serum obtained prior to infection; b, 1 week PI; c, 2 weeks PI; d, 3 weeks PI; e, 4 weeks PI.](image-url)
Table 1. Antibody titers of sera from BHV-1-infected rabbits and estimates of immunoprecipitate intensities in RIPA

<table>
<thead>
<tr>
<th>Animal</th>
<th>route</th>
<th>Antibody titer</th>
<th>Intensity in RIPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>IT</td>
<td>2,048</td>
<td>+++ + +</td>
</tr>
<tr>
<td>R2</td>
<td>IT</td>
<td>4,096</td>
<td>+++ + + +</td>
</tr>
<tr>
<td>R3</td>
<td>IV</td>
<td>128</td>
<td>+ +++ +++ +</td>
</tr>
<tr>
<td>R4</td>
<td>IV</td>
<td>8,192</td>
<td>+ +++ +++ +</td>
</tr>
</tbody>
</table>

Fig. 2. Antibody response of BHV-1-infected calf. Sera obtained from an intranasally-inoculated calf were examined by RIPA. Lane a, serum obtained prior to infection; b, 1 week PI; c, 2 weeks PI; d, 3 weeks PI; e, 4 weeks PI.

obtained from the IT-inoculated rabbits and reacted mainly with gl complex, exhibited neutralizing titers of 2–8, whereas R3 and R4 from the IV-inoculated animals, which were highly reactive with glIII and glIV, possessed neutralizing titers of 64–128. On the other hand, the ELISA titers of the IV-inoculated animals were only 2 to 4 times higher than those of the IT-inoculated animals. These findings suggest that at least in the rabbit immune system, glIII and/or glIV could induce higher neutralizing titers than gl.

In the present study it was evident that the antibody response in rabbits inoculated with BHV-1 differed quantitatively according to the inoculation route, and that the antibodies produced by IT-inoculated rabbits appeared to show similar specificity to those produced by cattle. Although neither the clinical syndrome nor virus replication was observed in this study, the IT-inoculated rabbits showed a pattern of antigen recognition similar to that in the cattle. It has been reported that rabbits IV-inoculated with BHV-1 Cooper strain showed systemic infection resulting in severe necrosis of the liver and adrenal glands and that IT-inoculated animals developed respiratory disease [7]. The Los Angeles strain was also reported to cause microscopic inflammatory foci in the liver and adrenal glands [6a]. This seems to be a difference in the target organ might influence the antigen recognition pattern in rabbits.

The present results also suggest that glIII and/or glIV is highly immunogenic with respect to induction of neutralizing antibodies in rabbits infected with BHV-1. Sera from convalescent cattle collected randomly from isolated outbreaks have been shown to have anti-glIII and glIV reactivities that are correlated with neutralizing titer [14]. Babiuk and his colleague, using individual glycoproteins to immunize animals, suggested that the immunogenic potential for raising neutralizing antibodies could show the order glV > glII > gl in cattle [3], whereas gl induced higher neutralizing titers than glIII in rabbits [16]. Immunization resulting from infection probably makes rabbits induce higher neutralizing titers with glII and/or glIV. Previous studies showed independently that monoclonal antibodies against glV exhibited higher neutralizing titers than other antibodies [10, 14].

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REFERENCES