Varicella-zoster virus gH:gL contains a structure reactive with the anti-human gamma chain of IgG near the glycosylation site

Tomonori Yokoyama,1,2 Satoko Ayabe,2 Huminori Miyagi,2 Toru Sugano,2 Akira Otsu,2 Hitoshi Sato,1 Seiji Kageyama,1 Takao Fujii2 and Kimiyasu Shiraki1

1Department of Virology, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan
2Teijin Institute for Biomedical Research, Asahigaoka 4-3-2, Hino, Tokyo 191, Japan

Varicella-zoster virus (VZV) glycoproteins were purified from infected cells using monoclonal antibodies and the gH:gL was found to react with antibodies to the γ chain of human IgG (h-IgG), whereas gE:gl and gB did not. When gH:gL was captured by concanavalin A, it lost reactivity with the anti-h-IgG γ chain (anti-h-γ-IgG). gH:gL reacted with anti-h-γ-IgG in an ELISA assay and gave a Kd value of $216 \times 10^{-10}$ M in a Biacore assay. The $K_d$ value of the human monoclonal antibody to gH (TI-57) used for the purification of gH:gL was $4.45 \times 10^{-10}$ M. Virus pretreated with anti-h-IgG was five times more resistant to neutralization with TI-57. Although the nature of the binding was not clear, gH:gL bound to anti-h-γ-IgG. If this interaction results from immunological similarity between gH:gL and h-IgG, it may cause immune evasion in the pathogenesis of VZV infection.

Herpes simplex virus and varicella-zoster virus (VZV) gH:gL complexes play an important role in entry of the virus particle into cells (Duus et al., 1995; Forrester et al., 1992; Fuller et al., 1989; Shiraki et al., 1997). A monoclonal antibody to gH did not react with gH in a Western blot but did react with a VZV-infected monolayer, whereas monoclonal antibodies to gE:gl and gB reacted with both types of antigen (Sugano et al., 1991). The results also indicated that the antibody to gH, which has neutralizing activity, recognizes a conformational epitope (Forghani et al., 1994; Montalvo & Grose, 1986). The monoclonal antibody to gH neutralized VZV and blocked cell-to-cell spread (Forghani et al., 1994; Montalvo & Grose, 1986; Sugano et al., 1991). The immune response to gH:gL was first recognized in the study of gB, gE:gl and gH:gL in guinea-pigs infected with the Oka varicella vaccine (Sato et al., 1998). Thus, gH:gL plays an important role in virus infection and immune response. We have characterized the structural reactivity of gH:gL with antibodies to the γ chain of human IgG (h-IgG) in this study. This may indicate a role for gH:gL in immune evasion in the pathogenesis of VZV infection.

Human embryonic lung cells were grown and maintained in Eagle’s minimum essential medium supplemented with 10% and 2% foetal bovine serum (FBS), respectively. The VZV strains used were the Oka vaccine and Kawaguchi strains (Shiraki et al., 1982). Cell-free virus stocks were prepared by freezing and thawing followed by sonication in SPGC medium (PBS supplemented with 5% sucrose, 0.1% sodium glutamate and 10% FBS) (Shiraki & Takahashi, 1982; Shiraki et al., 1982, 1997). The monoclonal antibodies used were clone 9 and clone 8 (Okuno et al., 1983), which recognize gE and gB, respectively, and TI-57, which recognizes gH (Sugano et al., 1991). Clone 9 and clone 8 are mouse monoclonal antibodies, and TI-57 is a human monoclonal antibody.

Glycoproteins were purified by affinity chromatography with monoclonal antibodies as described previously (Shiraki & Takahashi, 1982; Shiraki et al., 1997; Sato et al., 1998; Kamiyama et al., 2000). Briefly, Oka varicella vaccine-infected cells were lysed, and the lysate was applied to affinity columns of gB, gE and gH prepared using monoclonal antibodies. The buffer in each purified fraction was replaced with PBS by ultrafiltration followed by removal of IgG with protein G-Sepharose 4F (Pharmacia). Purified glycoproteins were analysed by SDS–PAGE and stained with silver according to the manufacturer’s instructions (Daichi Kagaku).

The specificity of reactivity of gH:gL with anti-h-IgG was determined by ELISA. Individual wells of ELISA plates were coated with 0.5 μg of gE:gl, gB or gH:gL, followed by washing and blocking the wells. Then horseradish peroxidase-conjugated anti-whole h-IgG and antibodies specific for the γ, κ or λ chain of h-IgG (Tago Immunochromes) or the γ chain of h-IgG (Dako and ICN) were used in the ELISA. 1-Step TMB Substrate (Pierce) was used as the enzyme substrate for determination of the enzyme reaction.

In order to examine the possibility of TI-57 contaminating the purified gH:gL preparation, two control experiments were designed.
Table 1. Reactivity of glycoproteins with anti-h-IgG in the ELISA assay

Data are expressed as the mean (n = 3) of A values ± SD. NT: Not tested.

(a) Reactivity of antibodies specific for each chain of human IgG with gE:gl, gB and gH:gl in the ELISA assay

<table>
<thead>
<tr>
<th>VZV glycoproteins</th>
<th>Gamma chain</th>
<th>Kappa chain</th>
<th>Lambda chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>gE:gl</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>gB</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>gH:gl</td>
<td>1.049 ± 0.024</td>
<td>0.38 ± 0.008</td>
<td>0.126 ± 0.003</td>
</tr>
</tbody>
</table>

(b) Reactivity of gH:gl and mock gH:gl with anti-h-γ-IgG in the ELISA assay

<table>
<thead>
<tr>
<th>VZV glycoproteins</th>
<th>Gamma chain</th>
<th>Kappa chain</th>
<th>Lambda chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>gH:gl</td>
<td>0.409 ± 0.001</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Mock gH:gl</td>
<td>0.139 ± 0.003</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Control without antigen</td>
<td>0.126 ± 0.002</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

performed. In the first, uninfected cell lysate was applied to an anti-gH column and eluted as mock gH:gl, and its reactivity with anti-h-γ-IgG in the ELISA assay was compared with that of gH:gl. In the second, the reactivity of artificial mixtures of 500 ng/ml gH:gl and TI-57 at various concentrations (0.001–1000 ng/ml) with anti-h-IgG γ chain (anti-h-γ-IgG) was tested in an ELISA assay.

As concanavalin A (ConA, Sigma) reacted with all three glycoproteins (data not shown), a ConA-captured ELISA was used to investigate the reactivity of anti-h-γ-IgG with gE:gl, gB and gH:gl. The plates were coated with 0.5 µg of ConA and then coated with 1.0 µg of gE:gl, gB or gH:gl after washing and blocking the wells. Alkaline phosphatase-conjugated goat anti-h-γ-IgG (Tago) was diluted in PBS and p-nitrophenyl phosphate was used as the enzyme substrate.

The interaction between anti-h-γ-IgG and VZV glycoproteins was also investigated using a BIAcore test (Pharmacia Biosensor) based on surface plasmon resonance. The sensor tip was coated with anti-h-γ-IgG (Biosource/Tago Immunochromicals) and analytes, including gH:gl, were applied. Prior to immobilization of anti-h-γ-IgG, the carboxyl groups of the CM5 matrix were activated by derivatization with 50 mM N-hydroxysuccinimide mediatied by treatment with 200 mM N-ethyl-N′-(dimethylaminopropyl)carbodiimide for 6 min. Anti-h-γ-IgG (200 µg/ml) was dissolved in 10 mM sodium acetate, pH 4.5, and passed over the activated surface for 7 min at 5 µl/min. This method resulted in 6000–10 000 resonance units of immobilized antibody. Non-covalently associated antibody was removed with 10 mM HEPES buffer (pH 7.4) containing 3 mM EDTA, 0.15 M sodium chloride and 0.05% Tween 20, and unreacted N-hydroxysuccinimide esters on the dextran surface were blocked by treatment with 1 M ethanolamine hydrochloride for 7 min. The analyte was then passed over the immobilized antibody and binding was quantified by measuring the mass increase on the matrix surface. The binding of the analyte to anti-h-γ-IgG was monitored as a change in response during a specific injection time and was analysed by BIA evaluation software 3.0.

A neutralization test was performed based on a method described previously (Shiraki et al., 1982; Sugano et al., 1987). Briefly, 100 p.f.u. of cell-free virus was pretreated with 125 µg/ml of anti-h-IgG (Dako) or SPGC for 1 h and then with various concentrations (0–10 µg/ml) of neutralizing TI-57 for another 1 h. The mixture was inoculated into cells and the number of plaques was counted. The neutralizing activity was expressed as the dilution necessary to reduce the number of plaques by 50% (ED50).

VZV gB, gE:gl and gH:gl were purified using affinity chromatography and analysed by SDS–PAGE (data not shown). The molecular masses of glycoproteins were as reported previously (Shiraki et al., 1982, 1997; Sato et al., 1998; Okuno et al., 1983; Sugano et al., 1991; Grose, 1990). The reactivity of the three glycoproteins with human antibodies was analysed by ELISA. There was a high level of
background reactivity to gH:gL compared to gB or gE:gL, even after extensive blocking or changing the ratio of the first and second antibody combinations (data not shown). We subsequently examined the direct interaction between anti-h-γ-IgG and gH:gL bound to the wells (Table 1a). High levels of background activity were observed even using gH:gL without human sera and goat antibodies to the γ, λ and κ chains of h-IgG were tested for the determination of the reactivity with gH:gL without human serum. The order of the reaction rates was γ > κ > λ. In a separate experiment, the reactions of mock gH:gL and gH:gL with anti-h-γ-IgG were compared in order to assess possible contamination with TI-57 (h-IgG). In contrast to gH:gL, mock gH:gL did not react with anti-h-γ-IgG (Table 1b). Thus gH:gL directly reacted with anti-h-γ-IgG.

A ConA-captured ELISA was employed to investigate the reactivity of anti-h-γ-IgG with each of the three VZV glycoproteins immobilized on the plate by the glycan. In this assay, gE:gL, gB and gH:gL did not react with anti-h-γ-IgG (data not shown). This indicates that the anti-h-γ-IgG binding site of gH:gL may be located near a glycosylation site.

The interaction of gH:gL or TI-57 with anti-h-γ-IgG was characterized further using the BIAcore assay. Of the three glycoprotein complexes, gH:gL alone reacted with anti-h-γ-IgG (Kd = 2.16 × 10−7 M), whereas the Kd value of TI-57 was 4.45 × 10−10 M. Both reactivities were dose-dependent. As gH:gL was purified using h-IgG (TI-57), the possibility of contamination of gH:gL with TI-57 was examined in the ELISA and BIAcore assays by testing artificial mixtures of gH:gL and TI-57 as shown in Fig. 1. More than 91% contamination of TI-57 in the purified gH:gL was detectable as a dose–response reaction with anti-h-γ-IgG under the baseline reactivity of gH:gL (91% and less of TI-57) as shown in Fig. 1(a). Therefore we postulated that the maximum level of contamination of gH:gL with TI-57 might be 91%. The artificial mixtures of TI-57 and gH:gL were also analysed for reactivity with anti-h-γ-IgG in the BIAcore assay (Fig. 1b). More than 0.25% contamination of TI-57 in the purified gH:gL was easily detected in the BIAcore assay, and the reaction profile and Kd values were similar to TI-57, and completely different from those of gH:gL alone. Thus 0.25% contamination of TI-57 was detectable in the BIAcore assay, whereas even 91% contamination was not detectable in the ELISA. The BIAcore assay was therefore more sensitive than the ELISA assay in detecting contaminating TI-57, and the Kd values of the mixtures were specific to contaminating TI-57 and different from gH:gL. This indicated that the baseline reactivity of gH:gL observed with 91% and less of TI-57 in the ELISA assay was the reactivity of gH:gL itself with anti-h-γ-IgG and was not influenced by the presence of TI-57. These results excluded the possible contribution of contaminating TI-57 in gH:gL in the ELISA and BIAcore assays and confirmed specific reactivity between gH:gL with anti-h-γ-IgG. The two control experiments using the mock gH:gL and the artificial mixtures of TI-57 and gH:gL excluded the possibility of the influence of contaminating TI-57 in gH:gL.

Because gH:gL reacted with anti-h-IgG, the interaction of TI-57 and anti-h-IgG with cell-free virus was assessed by a neutralization assay in which cell-free virus was treated with anti-h-IgG and then neutralized with TI-57 (Fig. 2). Anti-h-IgG pretreatment of cell-free virus modified the dose–response curve of neutralization with TI-57. The ED50 values of untreated and anti-h-IgG-pretreated viruses were 30 ng/ml and 144 ng/ml of TI-57, respectively. Pretreatment with anti-h-IgG thus rendered the virus five times less susceptible to neutralization. This result suggests two possibilities. One is that anti-h-IgG treatment of the virus functionally suppressed
neutralization with TI-57, possibly by interfering with binding of TI-57 to the neutralizing epitope of gH. Another is that anti-h-IgG captured TI-57 before it could bind to cell-free virus, resulting in interference with neutralization.

The glycomoieties of VZV glycoproteins were characterized in terms of their reactivity with lectins and glycosidases (Matsui et al., 1994). ConA capturing all three glycoprotein complexes. When the glycomoieties of gH was fixed on ConA, gH:gL did not react with anti-h-IgG. ConA interfered with binding of anti-h-IgG to gH:gL and this suggests that the binding sites of gH:gL to ConA and anti-h-IgG are located close to each other.

Anti-h-IgG failed to neutralize the infectivity of VZV, suggesting that binding of anti-h-IgG to gH:gL does not inhibit the function of gH:gL, in contrast to that of neutralizing TI-57. Pretreatment of the virus with anti-h-IgG rendered it five times less susceptible to neutralization with TI-57. The interpretation of this finding is, however, not simple. Further experiments are needed to ascertain whether interaction of the virus particle with anti-h-IgG might interfere with the binding and subsequent neutralization by TI-57.

Amino acid sequence similarity between gH and the γ chain of h-IgG was not observed by analysis with the software Gene Works 2.5.1. Radiolabelled gH was not immunoprecipitated with anti-h-IgG (Dako) and protein G–Sepharose (data not shown). Although the nature of the binding was not clear, these results suggested that binding between anti-h-IgG and gH:gL might be conformational. If this reactivity between gH and anti-h-IgG is due to an immunological or antigenic similarity between gH and h-IgG, immune recognition of gH might be impaired in the infected hosts. This study suggests a new concept for potential immunological escape/evasion in VZV infection.

We thank Ms Tomoko Sagawa for helpful discussion and Ms Jacqueline Brown for her editorial assistance.

References


Received 26 April 2000; Accepted 25 October 2000