Properties of Monoclonal Antibodies Against Glycoproteins of Western Equine Encephalitis Virus

KIICHI YAMAMOTO,¹ KATSUYUKI HASHIMOTO,¹ JOE CHIBA,² AND BUNISITI SIMIZU¹†

Departments of Virology and Rickettsiology¹ and Pathology,² National Institute of Health, Shinagawa-ku, Tokyo 141, Japan

Received 3 January 1985/Accepted 13 May 1985

To analyze the biological activities of the alphavirus glycoproteins, eight different monoclonal antibodies against the two glycoproteins of western equine encephalitis virus were isolated. Five of the eight monoclonal antibodies were shown to be specific for E1 and three for E2 protein by an enzyme-linked immunosorbent assay and by radioimmunoprecipitation. Three of the five anti-E1 and all of the anti-E2 monoclonal antibodies inhibited hemagglutination by purified virions. One anti-E1 and two anti-E2 monoclonal antibodies possessed high virus-neutralizing activity.

Alphaviruses possess at least three antigens. Two surface antigens, the E1 and E2 envelope glycoproteins, are responsible for hemagglutination inhibition (HI) and neutralization (NT) (5, 6). Serological grouping of alphaviruses is based upon their cross-reactivity in HI or NT tests (2, 10), but the precise role of the two glycoproteins in those reactions is not well understood even in the case of Sindbis (SINV) virus, Semliki Forest virus, or western equine encephalitis (WEE) virus (5, 7, 8).

We have been studying the functions of alphavirus glycoproteins (McAbs) inhibit the HA activity (1, 12). Because of this ambiguity regarding HI activities of antibodies against alphavirus glycoproteins, we began a study to elucidate the HI functions of WEE virus glycoproteins by using McAbs.

We report here the preparation and characterization of McAbs of WEE virus glycoprotein. Some anti-E1 McAbs possessed HI activity, and one of these possessed HI-inhibiting activity. All of the anti-E2 McAbs had HI activity. One anti-E2 McAb reacted only with the processed E2 protein, not with the precursor protein, PE2.

### TABLE 1. Properties of McAbs to WEE virus

<table>
<thead>
<tr>
<th>Antibody</th>
<th>ELISA titer (log₁₀)</th>
<th>50% PRNT</th>
<th>HI titer</th>
<th>Specificity</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascitic fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl.2</td>
<td>4.15</td>
<td>&lt;2.00</td>
<td>&lt;100</td>
<td>240</td>
<td>E1</td>
</tr>
<tr>
<td>Cl.4</td>
<td>4.00</td>
<td>&lt;2.00</td>
<td>&lt;100</td>
<td>&lt;20</td>
<td>E1</td>
</tr>
<tr>
<td>Cl.6</td>
<td>5.54</td>
<td>&lt;2.00</td>
<td>&lt;100</td>
<td>&lt;20</td>
<td>E1</td>
</tr>
<tr>
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<td>5.08</td>
<td>&lt;2.00</td>
<td>&lt;100</td>
<td>240</td>
<td>E1</td>
</tr>
<tr>
<td>Cl.8</td>
<td></td>
<td>4.50</td>
<td>800</td>
<td>640</td>
<td>E1</td>
</tr>
<tr>
<td>Cl.1</td>
<td>&lt;2.00</td>
<td>4.28</td>
<td>&lt;100</td>
<td>360</td>
<td>E2</td>
</tr>
<tr>
<td>Cl.3</td>
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<td>4.32</td>
<td>1,600</td>
<td>480</td>
<td>E2</td>
</tr>
<tr>
<td>Cl.5</td>
<td>&lt;2.00</td>
<td>6.04</td>
<td>7,200</td>
<td>2,560</td>
<td>E2</td>
</tr>
</tbody>
</table>

Anti-whole virion antibody 5.59 5.56 5.63 >7,000 2.048 E1 and E2

* ELISA was performed with plates coated with the three antigens, purified virions, and glycoproteins E1 and E2, prepared as described previously (15). Values represent the reciprocal of 10-fold dilutions starting at 1:100 and with an optical density reading of more than 0.1 at 410 nm.

† HI titers represent the reciprocal of the highest twofold dilution inhibiting HA.

‡ This clone gave unstable readings. Its specificity was determined by immunoprecipitation as shown in Fig. 1.

### Properties

WEE virus (McMillan strain) was used in all studies for immunizing the mice for production of McAbs and for detecting antibody activity. As the source of spleen cells for hybridomas, BALB/c mice were primed with an intraperitoneal injection of purified, Formalin-inactivated virus in Freund incomplete adjuvant, followed by intravenous injection of infectious virus without adjuvant. Hybridomas that were positive by an enzyme-linked immunosorbent assay (ELISA) were cloned twice by limiting dilution, and the ascitic fluid was harvested from BALB/c.

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¹ Corresponding author.

† Present address: Department of Microbiology, School of Medicine, Chiba University, Chiba 280, Japan.

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mice primed with purified cloned hybridomas. The antibody subclasses of each monoclonal antibody were determined by the double immunodiffusion test with anti-mouse subclass antisera (Litton Bionetics, Inc., Kensington, Md.), and immunoglobulin G1 (IgG1), IgG2a, IgG2b, and IgG3 subclasses were detected (Table 1). Before any HI test, all ascitic fluids and antisera were treated with acetone to remove HI inhibitors (9). In some cases immunoglobulin was partially purified by half-saturated ammonium sulfate. The HI test was done by the technique of Clarke and Casals (4), with 1-day-old chicken erythrocytes instead of goose cells. For the NT test on chicken embryo fibroblast (CEF) cells, serial fivefold dilutions of heat-inactivated ascitic fluid were mixed with an equal volume of ca. 300 PFU of WEE virus, and the test was performed by the method described previously (13).

Clonal hybridomas were obtained and screened for the production of antibodies directed against purified WEE virions by ELISA. Positive hybridomas were selected, cloned, and examined for antigenic specificity by radioimmunoprecipitation with solubilized purified virions. Eight clones produced antibodies against the purified intact WEE virus (Table 1 and Fig. 1). Clones 2, 4, 6, 7, and 8 produced anti-E1 antibody, and clones 1, 3, and 5 produced anti-E2 antibody. The specificity of the anti-E1 and E2 antibodies was also shown by ELISA with purified envelope glycoproteins E1 and E2 (Table 1) and by radioimmunoprecipitation with WEE virus-infected CEF cell lysates.

We examined the reactivity of these clones with precursor proteins and mature glycopolypeptides. CEF cells were infected with WEE virus and pulse-labeled or pulse-chased. Cyttoplasmic lysates prepared from the infected CEF cells were mixed with each clone. Figure 2 shows that clones 1 and 3 precipitated both PE2 and E2 proteins, whereas clone 5 precipitated only the E2 protein. It is possible that at least one epitope hidden in PE2 protein was revealed during the cleavage of PE2 so that clone 5 McAb had access to it in the E2 protein. McAbs specific to E1 were shown to react with E1 proteins which were pulsed for 15 min and those which were pulse chased for 90 min (data not shown).

The original ascitic fluid of clone 7 McAb possessed moderate HI activity (Table 1), whereas it caused marked HL inhibition, unlike the other clones (data for clone 5 shown in Table 2). This clone 7 McAb inhibited HL activity of WEE virus by more than 90%, even at a dilution that showed no HI activity (Table 2). Clone 5 showed less inhibitory activity (75% inhibition) than clone 7, even at a dilution with the high HI titer of 100. Antibody against the whole virion showed intermediate inhibitory activity be-
tween that of clone 7 and that of clone 5. These results suggest that clone 7 McAb is a specific antibody of HL inhibition and that this McAb does not possess any NT activity. These results also confirmed our previous observation that the E1 protein is involved in HL by WEE virus (16). A similar McAb was reported for SIN virus (3).

It has been established that the E1 protein of WEE virus possesses HA and HL activities (8, 15, 16). Therefore, we expected that anti-E1 McAbs would inhibit the HA activity. Indeed, three of five anti-E1 McAbs exhibited HI activity (Table 1). However, these HI titers were not as high as their ELISA titers. On the other hand, all anti-E2 McAbs inhibited HA activity, and their HI titers were relatively high compared with their ELISA titers. Clone 5 McAb showed the strongest inhibition; the titer was more than 2,000, like that of anti-whole virion antiserum.

These results suggest that, although isolated E1 protein can cause HA, the functional viral hemagglutinin on its envelope consists of an E1-E2 complex, and a certain antibody to either protein can interfere with HA activity of the virions. Because of the stable association of E1 and E2 proteins (11), binding of anti-E2 McAb to E2 protein might inhibit HA activity by induction of conformational changes of the E1 protein. As another possible explanation of the HI activity of anti-E2 antibody, it is easy to consider that the binding of anti-E2 antibody to intact virions may sterically inhibit the interaction of the E1 protein and erythrocytes.

We selected only McAbs which were able to bind to purified whole WEE virions on the solid phase in ELISA (Table 1). From the data of the ELISA in Table 1, it was clear that non-NT McAbs could bind infectious virions without affecting virus infectivity. Therefore, it was true that these McAbs were produced against epitopes unrelated to NT activity. Among our McAbs, one anti-E1 (clone 8) and two anti-E2 (clones 3 and 5) McAbs showed reasonable NT activity for their HI or ELISA titers (Table 1). For a long time, based on the previous studies with monospecific antibodies to purified glycoproteins of SIN virus, it had tacitly been assumed that E2 antigen is involved in the NT reaction (5, 14). However, in the case of SIN and Semliki Forest viruses, it was recently reported that not only anti-E2 McAb but also some anti-E1 McAbs exhibit NT activity (1, 3, 12). These results indicate that both E1 and E2 McAbs are involved in neutralization of alphaviruses. Our data on NT activity also support the results of other laboratories showing that NT epitopes may not be restricted to the E2 protein.

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**LITERATURE CITED**


