Human Immune Responses to Major Human Cytomegalovirus Glycoprotein Complexes

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Sera from both human cytomegalovirus (HCMV)-seropositive adults and infants with congenital HCMV infection recognized two major HCMV glycoprotein complexes. However, proliferative responses of peripheral blood mononuclear cells to these complexes varied among seropositive adults and were not detected in any of the infants. Thus, these glycoproteins alone may not be sufficient to develop a subviral HCMV vaccine.

Human cytomegalovirus (HCMV), like other members of the herpesvirus family, is a relatively common human pathogen and has the ability to produce primary infection ranging from subclinical, asymptomatic illness to rapidly progressive systemic disease with significant morbidity and mortality (13, 16, 31). The most serious manifestations of HCMV occur in association with immunodeficiency states, including transient, generalized immunodeficiency in patients with HCMV mononucleosis (28, 29), in an HCMV-specific cell-mediated immune defect in infants with congenital HCMV infection (10, 11), in immunocompromised patients such as organ and bone marrow transplant recipients (21), and in patients with acquired immunodeficiency syndrome (17, 25).

To develop an effective subunit HCMV vaccine, it is essential to know which protein(s) can induce protective immune responses in humans.

Because the glycoproteins of HCMV are not only present in the envelopes of virions but are also highly expressed on the surfaces of infected cells involved in productive viral infection (5, 20), they are likely to be accessible to the immune system. We have previously described two families of HCMV envelope glycoprotein complexes which are biochemically and immunologically distinct (14). One family of complexes, which we have designated gc-I for purposes of discussion, contains glycoproteins of 130,000, 93,000, and 50,000 to 52,000 daltons, associated by disulfide bonds, which are immunoprecipitated by several monoclonal antibodies (McAbs) and thus appear to be immunologically related. These complexes apparently contain the glycoprotein which was first classified gA by Pereira et al. (20) and was subsequently designated gB by Cranage et al. (5) on the basis of homology of the gene encoding this glycoprotein with the gene encoding gB of herpes simplex virus type 1. A separate set of complexes, first described by Kari et al. (14) and designated gc-II, contains a predominant glycoprotein of 50,000 to 52,000 daltons; antigenically related glycoproteins of 90,000 and >200,000 daltons may represent either multimeric forms of the 50,000- to 52,000-dalton glycoprotein or distinct glycoproteins sharing a common antigenic determinant. The genes encoding glycoproteins contained within gc-I and gc-II have now been identified, and their mRNA translation products have been immunoprecipitated by our gc-I and gc-II-specific McAbs (M. F. Stinski, University of Iowa, Ames, personal communication), providing definitive evidence for the HCMV specificity of these glycoprotein complexes. Both families of glycoprotein complexes seem to be abundant in the Towne strain HCMV envelopes, since they account for a high proportion of the glycosylated proteins determined by incorporation of [3H]glucosamine.

The significance of HCMV glycoproteins in eliciting immune responses beneficial to the human host remains unknown. Immunogenic determinants of these glycoproteins appear to be biologically important, since McAbs which recognize these determinants neutralize HCMV in vitro (4, 14, 22, 23). However, ongoing HCMV replication after primary infection and symptomatic reactivation of the latent genome is associated with lack of cellular immunity, whether or not antibody is present (2, 8, 18, 27). Therefore, it seems apparent that HCMV-specific cellular immunity plays a significant role in recovery from HCMV infection. In this report, we present the results of human humoral and cellular immune responses to these two major HCMV glycoprotein complexes in normal adults and in infants with congenital HCMV infection.

HCMV whole viral antigen was obtained as heat-inactivated sucrose-gradient-purified virus pelleted from the supernatant of Towne HCMV-infected fibroblasts. Glycoprotein complexes gc-I and gc-II were purified from detergent extracts of Towne HCMV virions by anion-exchange high-pressure liquid chromatography, as previously described (14). Although ion-exchange high-pressure liquid chromatography provided significant purification of the glycoprotein complexes obtained from either virus-infected cells or purified virus, there was still 5 to 10% cross-contamination between peaks containing gc-I and gc-II, on the basis of immunoprecipitation results (14). Therefore, before being used to study both humoral and cellular immunity, gc-I was immunoprecipitated with McAb 9E10 (a gc-II-specific McAb) to remove contaminating gc-II, and gc-II was immunoprecipitated with McAb 41C2 (a gc-I-specific McAb) to remove contaminating gc-I.

Nine healthy HCMV-seropositive adult volunteers (donors A1 to A9), five seronegative adult volunteers (donors A11 to A15), and four infants aged 5 to 9 months with congenital HCMV infection (donors I1 to I4) were included in lymphocyte proliferation studies. The lymphocyte proliferation assay has been reported previously (9). Whole HCMV Towne strain virions and individual glycoprotein peaks were used at concentrations of 1 and 0.1 μg per well, respectively. The experiments were performed at least twice for each donor to ensure consistency of the responses; representative results are shown (Table 1). Among the nine
TABLE 1. Lymphocyte proliferative responses to whole HCMV antigen and major glycoprotein complexes

<table>
<thead>
<tr>
<th>Donor</th>
<th>Anti-HCMV titer (complement fixation/indirect immunofluorescence)*</th>
<th>Amt of $[^3H]$thymidine incorporatedb withc:</th>
<th>No antigen</th>
<th>Whole HCMV</th>
<th>gc-I</th>
<th>gc-II</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Seropositive</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>A1</td>
<td>32/40</td>
<td>2,863 ± 1,092</td>
<td>2,610 ± 1,092</td>
<td>63,083 ± 11,786</td>
<td>54,018 ± 9,744</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>64/80</td>
<td>2,874 ± 761</td>
<td>84,211 ± 8,387</td>
<td>26,609 ± 9,936</td>
<td>28,862 ± 9,008</td>
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</tr>
<tr>
<td>A3</td>
<td>256/160</td>
<td>3,383 ± 159</td>
<td>81,374 ± 37,083</td>
<td>18,644 ± 2,750</td>
<td>13,020 ± 5,556</td>
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<tr>
<td>A4</td>
<td>32/80</td>
<td>5,487 ± 1,133</td>
<td>55,204 ± 32,325</td>
<td>46,670 ± 5,754</td>
<td>32,214 ± 5,984</td>
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<tr>
<td>A5</td>
<td>256/160</td>
<td>6,975 ± 2,767</td>
<td>50,211 ± 8,387</td>
<td>22,814 ± 1,697</td>
<td>23,871 ± 1,918</td>
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<tr>
<td>A6</td>
<td>32/40</td>
<td>1,307 ± 464</td>
<td>77,382 ± 27,846</td>
<td>12,838 ± 1,945</td>
<td>12,739 ± 4,190</td>
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<tr>
<td>A7</td>
<td>64/80</td>
<td>826 ± 265</td>
<td>53,232 ± 15,264</td>
<td>4,316 ± 416</td>
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<td>A8</td>
<td>128/80</td>
<td>669 ± 423</td>
<td>47,433 ± 1,866</td>
<td>928 ± 387</td>
<td>634 ± 281</td>
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<td>A9</td>
<td>256/160</td>
<td>1,906 ± 349</td>
<td>25,563 ± 10,248</td>
<td>536 ± 273</td>
<td>600 ± 232</td>
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<tr>
<td>I1</td>
<td>128/640</td>
<td>250 ± 77</td>
<td>3,761 ± 3,264</td>
<td>326 ± 32</td>
<td>261 ± 39</td>
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</tr>
<tr>
<td>I2</td>
<td>256/640</td>
<td>884 ± 328</td>
<td>2,945 ± 2,765</td>
<td>537 ± 142</td>
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<tr>
<td>I3</td>
<td>32/160</td>
<td>523 ± 202</td>
<td>1,170 ± 366</td>
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<td></td>
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<tr>
<td>Seronegative</td>
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<td></td>
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<tr>
<td>A11</td>
<td>&lt;4/&lt;10</td>
<td>408 ± 171</td>
<td>542 ± 317</td>
<td>172 ± 76</td>
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<td>A12</td>
<td>&lt;4/&lt;10</td>
<td>939 ± 782</td>
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<td>383 ± 67</td>
<td>184 ± 16</td>
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<td>A13</td>
<td>&lt;4/&lt;10</td>
<td>5,327 ± 2,282</td>
<td>6,097 ± 3,602</td>
<td>1,001 ± 209</td>
<td>792 ± 284</td>
<td></td>
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<tr>
<td>A14</td>
<td>&lt;4/&lt;10</td>
<td>5,185 ± 740</td>
<td>2,357 ± 2,042</td>
<td>1,656 ± 540</td>
<td>664 ± 29</td>
<td></td>
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<tr>
<td>A15</td>
<td>&lt;4/&lt;10</td>
<td>888 ± 296</td>
<td>364 ± 266</td>
<td>259 ± 48</td>
<td>148 ± 23</td>
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</table>

* HCMV antibody titers were determined in the clinical virology laboratory at the University of Minnesota and are expressed as the reciprocal of serum dilution. Complement fixation/indirect immunofluorescence titers of <4 to <10 were considered negative.

b Proliferation assays were set up using $10^6$ PBMC per well and various antigens in flat-bottomed microwell plates. The cultures were incubated for 7 days, and $[^3H]$thymidine was included for the final 16 to 24 h. Data are presented as mean counts per minute ± standard deviation from triplicate experiments.

c Whole HCMV was sucrose-gradient-purified HCMV Towne strain viral particles (2 μg per well; gc-I (0.1 μg per well) and gc-II (0.1 μg per well)) were HPLC-isolated glycoprotein complexes which were further purified by immunoabsorption with McAbs specific for gc-II and gc-I, respectively.

For other background individuals, this pattern was well. 5. At each of five individuals in HPLC-isolated glycoprotein HPLC was performed for other background individuals. This pattern was consistent. Each individual was tested on October 28, 2017 by guest http://jvi.asm.org/ Downloaded from days; $[^3H]$thymidine was included for the final 16 to 24 h. Data are presented as mean counts per minute ± standard deviation from triplicate experiments. Whole HCMV was sucrose-gradient-purified HCMV Towne strain viral particles (2 μg per well; gc-I (0.1 μg per well) and gc-II (0.1 μg per well)) were HPLC-isolated glycoprotein complexes which were further purified by immunoabsorption with McAbs specific for gc-II and gc-I, respectively.

In Table 1, results of such experiments are shown (Fig. 1). Donor A8 did not respond to either glycoprotein complex at any of the concentrations tested. Donor A4 responded to gc-I and gc-II better than did donors A3 and A6. At all concentrations of antigen tested, although peripheral blood mononuclear cells (PBMC) isolated from the three donors responded equally well to whole HCMV antigen. We cannot entirely exclude the possibility that subjects A8 and A9 may have only conformation-dependent T cells reactive with these glycoproteins. It has recently been demonstrated that some T cells recognize conformational determinants on the three-dimensional structure of the native hemagglutinin molecules of

[FIG. 1. Lymphocyte proliferative responses of representative blood donors to whole HCMV virus and glycoprotein complexes. PBMC at $10^6$ cells per well were incubated with various concentrations of whole HCMV (A), gc-I (B), and gc-II (C) for 7 days; $[^3H]$thymidine (1 μCi per well) was included in the cultures during the final 18 h of incubation. The results are expressed as mean counts per minute of triplicate cultures; background counts per minute (without antigen) were subtracted throughout.]
influenza virus (19). Thus, minor changes of HCMV glycoproteins induced during purification might account for the lack of T-cell responses in these subjects. This seems unlikely, since the protein purification was performed under nondenaturing conditions. Whether differences in lymphocyte responses to partially purified glycoproteins among individuals reflect antigenic differences among different strains of HCMV involved in the initial infection or whether different major histocompatibility complex II products may preferentially allow for restricted recognition of certain HCMV glycoproteins is also undefined.

When PBMC were isolated from infants with congenital HCMV infection aged 5 to 9 months (11 to 13), no significant HCMV-specific proliferative responses could be detected by using whole HCMV as the antigen. This result is consistent with the observation of our own laboratory (10, 11) and other laboratories (24, 26) that infants with congenital HCMV infection have a selective defect in HCMV-induced lymphocyte proliferation in the first year of life. Since these infants produced antibodies specific for HCMV (Table 1), HCMV-specific helper T cells may be present as well. We were interested to determine whether PBMC isolated from these infants were proliferative in response to either glycoprotein complex. Our results, shown in Table 1, indicated that PBMC isolated from three infants tested did not respond to either gc-I or gc-II. The mechanism(s) involved in the selective defect in cellular immune recognition in congenital HCMV infection is unclear.

Sera from representative donors were then used to immunoprecipitate gc-I and gc-II to determine whether their B-cell immune responses paralleled those of T cells. Sera from seropositive normal donors immunoprecipitated 20 to 29% of the counts per minute incorporated as [3H]glucosamine in gc-I, whereas sera from a seronegative donor precipitated 1% or less of the counts per minute in gc-I (data not shown). gc-I complexes precipitated by human immune sera were reduced and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The number and molecular weights of the glycoproteins precipitated were identical to those of the glycoproteins immunoprecipitated by gc-I-specific McAb 41C2 (Fig. 2A). Sera from all seropositive control donors also immunoprecipitated 5 to 12% of the counts per minute in gc-II complexes, and negative sera precipitated 1% or less of the counts per minute in gc-II (data not shown). gc-II complexes were also examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis after reduction. Again, the number and molecular weights of the glycoproteins immunoprecipitated by the human immune sera were very similar to those immunoprecipitated by the gc-II-specific McAb 9E10 (Fig. 2B). These results suggest that gc-II might be more immunogenic in humans than in mice, based on the fact that murine McAbs against gc-I have been generated in several laboratories (14, 22–24), whereas only a single McAb recognizing gc-II has been reported to date (14). Sera from infants with congenital HCMV infection showed the same immunoprecipitation patterns as sera from normal seropositive adults. These data suggest that the cell-mediated immune defect in infants with congenital HCMV infection does not affect the antibody response to the most abundant envelope glycoproteins of HCMV.

Sera from donors A1, A6, A9, A11, and I4 were tested for reactivity to HCMV antigen, gc-I, and gc-II in an enzyme-linked immunosorbent assay (Fig. 3). Again, sera from all seropositive donors recognized both gc-I and gc-II, whereas serum from the seronegative donor A11 did not react to either HCMV glycoprotein complex. The results shown in Table 1 and Fig. 3 demonstrate that there is no correlation between antibody and lymphocyte proliferative responses to these HCMV glycoproteins in humans. For example, mononuclear cells from donor A9 did not proliferate in response to either gc-I or gc-II (Table 1) whereas serum from the same donor did react with both glycoprotein complexes. Mononuclear cells of donor A1 responded to gc-I and gc-II to a greater extent than did cells from any other donor tested, yet the level of antibody against gc-I and gc-II in the serum of donor A1 was not higher than that of other donors (i.e., A6 and A9). Sera from infants with congenital HCMV infection reacted with both glycoprotein complexes, but their PBMC did not respond to either glycoprotein in a proliferation assay. The lack of correlation between B- and T-cell responses to HCMV observed in both adults and infants with congenital HCMV infection has also been demonstrated for other viruses (1, 3, 30, 32). For example, the response of B cells to influenza virus is primarily due to hemagglutinin (15, 30, 32), whereas both external and internal viral proteins play significant roles in T-cell recognition (3, 30, 32). For varicella-zoster virus, there is usually an association between antibody responses and lymphocyte proliferation with purified varicella-zoster virus proteins, but discrepancies in the detection of antibody and lymphocyte proliferation in response to the subunit antigens were observed in some individuals (1).

We believe this is the first report which investigates both human T- and B-cell responses to HCMV envelope glycoproteins. Previously, several laboratories have reported murine McAbs reactive with HCMV glycoprotein complexes identical to gc-I (4, 6, 14, 20, 22, 23), and T cells exhibiting proliferative responses to gc-I have been demonstrated in guinea pigs after immunization with HCMV (12). The immunogenicity of gc-II is completely uncharacterized, due in part to the availability of only a single McAb (9E10) so far. When the two glycoprotein complexes were studied for their in-

**FIG. 2.** Immunoprecipitation of glycoprotein complexes purified by high-pressure liquid chromatography and immunofinity methods with human immune sera. (A) gc-I complexes immunoprecipitated with human immune sera. Lanes 11, 12, and 14 contain sera from infants with congenital HCMV infections, whereas lanes A9, A1, and A4 contain sera from HCMV-positive adults. Lane A11 contains HCMV-seronegative adult serum. Numbers to the right indicate molecular weight (10^3). Lane 4IC2 shows glycoproteins from the same preparation immunoprecipitated with gc-I-specific McAb 4IC2. (B) gc-II glycoprotein complexes immunoprecipitated with human immune sera. Lane designations and molecular weight indicators are the same as in panel A. Lane 9E10 shows glycoproteins from the same preparation immunoprecipitated with gc-II-specific McAb 9E10.
volvement in cellular immune responses, we found no correlation between cellular and humoral responses. Moreover, PBMC from some individuals do not respond to either glycoprotein complex, despite significant proliferative responses to whole HCMV antigen. Therefore, it appears that HCMV proteins other than these envelope glycoproteins are important in stimulating T-cell proliferative responses; one of these has been reported to be a 64,000-dalton matrix protein (7). Although HCMV envelope glycoproteins appear to consistently induce B-cell responses (9, 12, 20, 22; this study), the lack of universal helper T-cell responses to these glycoproteins indicates that a subviral HCMV vaccine composed of these glycoproteins alone may not be sufficient to induce protective immunity.

LITERATURE CITED


