Antibodies against the gH/gL/UL128/UL130/UL131 Complex Comprise the Majority of the Anti-Cytomegalovirus (Anti-CMV) Neutralizing Antibody Response in CMV Hyperimmune Globulin

Ashley E. Fouts, Pamela Chan, Jean-Philippe Stephan, Richard Vandlen, and Becket Feierbach

Departments of Infectious Diseases, Biochemical and Cellular Pharmacology, and Protein Chemistry, Genentech, Inc., South San Francisco, California, USA

Anti-cytomegalovirus (anti-CMV) hyperimmune globulin (HIG) has demonstrated efficacy in preventing CMV disease in solid-organ transplant patients as well as congenital disease when administered to pregnant women. To identify the neutralizing component of cytomegalovirus hyperimmune globulin (CMV-HIG), we performed serial depletions of CMV-HIG on cell-surface-expressed CMV antigens as well as purified antigens. Using this approach, we demonstrate that the major neutralizing antibody response is directed at the gH/gL/UL128/UL130/UL131 complex, suggesting little role for anti-gB antibodies in CMV-HIG neutralization.

The mechanism by which naturally acquired immunity confers protection against cytomegalovirus (CMV) infection is unknown, but antibody response is likely to play an important role. Nigro et al. reported that in a nonrandomized prospective study, passive immunization with anti-CMV hyperimmune globulin (CMV-HIG) showed promising results for the treatment and prevention of congenital infection (8). In addition, preconception maternal immunity to CMV provides considerable protection against vertical transmission, likely due to the presence of neutralizing antibodies (3). Administration of CMV-HIG has also been shown to be effective in preventing CMV disease in solid-organ transplant (SOT) patients (14, 15, 20). Many studies have shown that human sera can react with a broad spectrum of CMV proteins from either purified virus or virus-infected cells (1, 2, 10, 13). However, binding to viral components does not necessarily confer protection. CMV uses two different entry mechanisms to infect fibroblasts, epithelial cells, endothelial cells, and macrophages. Fibroblast entry is mediated by glycoprotein complexes gB, gH/gL, gO, and gM/gN, whereas entry into epithelial cells, endothelial cells, and macrophages requires the addition of the gH/gL/UL128/UL130/UL131 complex (5, 6, 12, 17–19). While it has been reported that the anti-gB response correlates with neutralizing activity, these studies have been restricted to study of fibroblast entry using fibroblast-tropic strains lacking a functional locus containing genes encoding UL128 to UL131 (UL128-131). In addition, these studies drew conclusions from a small number of seropositive individuals, whose neutralizing immune responses may not be representative of the population as a whole (2, 7). More recently, several studies have suggested a positive correlation between a highly potent neutralizing antibody response and the presence of antibodies in serum against the UL128-131 gene products (4, 16).

With the aim of reconciling these different observations and identifying the neutralizing component of CMV-HIG, we performed serial depletions of CMV-HIG on cell-surface-expressed CMV antigens as well as purified antigens and evaluated the neutralizing potency of depleted CMV-HIG. In this study, we used CMV-HIG (Cytogam; CSL Behring) pooled from >1,000 infected individuals. Due to the size of the pool, we believe that this IgG preparation captures the anti-CMV immune response of the infected population. Our results showed that the most significant portion of the neutralizing activity derives from antibodies against the gH/gL/UL128/UL130/UL131 complex and not gB.

Our key goal was to assess the potency contribution of specific anti-CMV antibody populations on different cell types. Consistent with others, we have found that CMV-HIG has a much higher potency on epithelial cells, endothelial cells, and monocyte-derived macrophages than on fibroblasts (Fig. 1)(4, 16). Epithelial cells and fibroblasts (ARPE-19 and MRC-5, respectively) (American Type Culture Collection [ATCC], Manassas, VA) and P0 human umbilical vein endothelial cells (HUVECs) (Lonza, Basel, Switzerland) were grown using the manufacturer’s suggested conditions. Human monocyte-derived macrophages (MDM) were generated from monocytes isolated from a human donor using RosetteSep human monocyte enrichment cocktail (Stemcell Technologies, Vancouver, Canada) and differentiated into macrophages by overnight treatment with lipopolysaccharides (LPS). In this invitro neutralization assay, CMV-HIG was mixed with the low-passage-number clinical CMV strain VR1814 (11) such that the final virion concentration resulted in approximately one infectious virus per cell (i.e., multiplicity of infection [MOI] of 1). The MOIs for the different cell types were ascertained by determining the titer of the same viral stock on each cell type (e.g., epithelial infected cells were infected with an MOI that was determined from the titer on epithelial cells). Infection proceeded for 18 h at 37°C prior to fixation and staining for the immediate-early (IE) nonstructural antigen using an anti-IE antibody (Mab810; Millipore) and Hoechst nuclear counterstain. In this assay, CMV-HIG was greater than 100-fold more potent (comparing 50% effective concentrations [EC50s]) at blocking viral entry into epithe-
ing 0.5% bovine serum albumin (BSA) and was serially incu-
lad to 40
CMV-HIG (Cytogam) (50 mg/ml stock concentration) was di-
or gH/gL/UL128/UL130/UL131 (Fig. 2).

106 to 6

these are consistent
iments. Both soluble gB and gH/gL bound antibodies that recog-
ize nonconformational and conformational epitopes, sug-
gesting that these proteins were folded properly (data not
shown). Purified recombinant proteins (1 to 2 mg of soluble gB
or gH/gL) were chemically coupled to Sterogene ALD Super-
flow resin via sodium cyanoborohydride (0.2 ml of 1 M solu-
tion) and dialyzed against PBS overnight. The individual resins
were loaded into columns and extensively washed with PBS to
remove any unbound protein. Two milligrams of CMV-HIG
in a volume of 800 µl) was loaded onto the individual col-
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Experiments show little role for anti-gB antibodies in CMV-HIG neutralization. One caveat of this approach is that depletion was incomplete. The remaining potency on epithelial cells may be due to gH/gL/UL128/UL130/UL131 antibodies that recognize only epitopes present on the virus, and thus, cannot be captured on cells. Additionally, the remaining potency on fibroblasts may be accounted for by antibodies against gO or gM/gN. Ultimately, this study may help provide a basis for developing new vaccines and immunotherapies for CMV infection.

**TABLE 1** Depletion of CMV-HIG, assayed on surface-expressed protein, for neutralization on epithelial cells

<table>
<thead>
<tr>
<th>CMV protein(s) expressed on the cell surface</th>
<th>% depletion of CMV-HIG</th>
<th>gH/gL lysate</th>
<th>gH/gL/128/130/131 lysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (mock depletion)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gB</td>
<td>91</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>gH/gL</td>
<td>0</td>
<td>67</td>
<td>73</td>
</tr>
<tr>
<td>gH/gL/128/130/131</td>
<td>5</td>
<td>68</td>
<td>79</td>
</tr>
</tbody>
</table>

*Percent depletion of specific antibody populations in CMV-HIG (normalized to the values for mock depletion). Percentages are transformed from ELISA data. Maxisorb (Nunc) plates were coated with lysate made from epithelial cells (ARPE-19) expressing gB, gH/gL, or gH/gL/UL128/UL130/UL131. The plates were blocked and incubated with depleted CMV-HIG prior to washing and detection using anti-human IgG (Fc fragment-specific) antibody conjugated to horseradish peroxidase (HRP) (Jackson Laboratory).*

**FIG 2** Epithelial cells express adenovirus-encoded CMV glycoproteins on their surfaces. ARPE-19 cells were transduced with adenovirus encoding either gB or gH/gL (at a multiplicity of infection [MOI] of 150) or cotransfected with adenoviruses encoding gH/gL (at an MOI of 100) or UL128/130/131 (at an MOI of 150). After 3 days, the cells were dissociated using Accutase, stained with the following primary antibodies: anti-gH (α-gH) (MSL-109), anti-gB (α-gB) (ITC-88), affinity-purified rabbit anti-UL130 (SPWFTLTANQPSPPWSKLTPKPHDC), or affinity-purified rabbit anti-UL131 (TAEKNDYRVP HYWDACSRLPQTRTRYK). The cells were stained with the appropriate secondary antibody conjugated to allophycocyanin (APC) (Jackson ImmunoResearch). Fluorescence of individual cells was measured using FACSFlow (BD Biosciences) and analyzed using FlowJo software (Tree Star). Adenoviral vectors were constructed to contain CMV gB, gH and gL, or UL128, UL130, and UL131. gH and gL were cloned into a pRK tk Neo vector separated by a “self-cleaving 2A peptide” (15a). The entire transcript was then cloned into pShuttle-UBC-TO-EV and recombined into pAd-pl-DEST using the ViraPower system (Invitrogen). UL128, UL130, and UL131 were amplified from cDNA and cloned into pRK tk Neo vector, each gene separated by a “self-cleaving 2A peptide.” The entire coding sequence of UL128, UL130, and UL131 and that of gB were cloned into adenoviral vectors and stocked by Vector Biolabs.

**FIG 3** Neutralization of CMV VR1814 by CMV-HIG, depleted of specific CMV glycoprotein antigens. To evaluate neutralization potency, depleted CMV-HIG was serially diluted and incubated with virus at an MOI of 1 for 1 h prior to addition to epithelial cells (ARPE-19) or fibroblasts (MRC-5) (B). At 18 h postinfection, cells were fixed, stained, and analyzed by immunofluorescence for anti-IE antibody and Hoechst stain. The concentration of CMV-HIG (in micrograms per milliliter) is shown on the x axes of the graphs.
TABLE 2 Depletion of CMV-HIG, assayed on purified protein, for neutralization on fibroblast cells

<table>
<thead>
<tr>
<th>CMV protein(s)</th>
<th>% depletion of CMV-HIG</th>
<th>Purified gB</th>
<th>Purified gH/gL</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (mock depleted)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gB</td>
<td>95</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gH/gL</td>
<td>0</td>
<td>84</td>
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*Percent depletion of specific antibody populations in CMV-HIG (normalized to the values for mock depletion). Percentages are transformed from ELISA data. Maxisorb (Nunc) plates were coated with soluble gB or gH/gL protein. The plates were blocked and incubated with depleted CMV-HIG prior to washing and detection using anti-human IgG (Fc fragment-specific) antibody conjugated to horseradish peroxidase (HRP) (Jackson Laboratory).

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REFERENCES