Analysis of T Helper Cell Response to Glycoprotein H (gpUL75) of Human Cytomegalovirus: Evidence for Strain-Specific T Cell Determinants

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and Michael Mach

The proliferative response of helper T cells against glycoprotein H (gH; gpUL75) of human cytomegalovirus (HCMV) was determined in T cell lines from 5 healthy HCMV-seropositive donors. A differential response in two lines was noted when gH from strain AD169 or Towne was used as antigen. T cell–reactive domains between aa 15 and 510 were identified using β-galactosidase fusion proteins containing overlapping fragments of gH, and they were confirmed with synthetic peptides as stimulating antigen. T cell proliferation was observed with antigens containing aa 34–51, 111–142, 284–302, 324–342, and 454–510 of gH. None of the determinants stimulated all donors. The T cell epitope defined by aa 34–51 is located in close proximity to a strain-specific dominant B cell epitope; however, no strain dependence for this T cell determinant was observed. In contrast, the dominant T cell response against aa 284–302, which was observed in three T cell lines, was strain specific.

Human cytomegalovirus (HCMV) is an important pathogen in immunocompromised persons: Allograft recipients can have severe and sometimes fatal infections in the posttransplant period [1], and among the human immunodeficiency virus (HIV)–infected population, whose lives have been extended by more effective therapies for other opportunistic infections, it has become increasingly necessary to treat HCMV [2]. In addition, HCMV is the most common cause of congenital viral infections and results in a large and varied range of clinical manifestations [3].

Both cellular and humoral immune responses are likely to be involved in the control of HCMV infections. Indirect evidence for the importance of the cellular immune response to HCMV has been provided by studies in transplant patients, in whom the presence of HCMV-specific T cells correlates with favorable outcome of the infection [4, 5]. The significance of the humoral immune response is supported by studies showing that passively transferred anti-HCMV antibodies can modulate the severity of HCMV disease in congenitally infected babies and organ allograft recipients [6–8]. In a recent vaccination trial, protection from reinfection correlated with the level of neutralizing antibodies [9]. In addition, in the closely related murine cytomegalovirus system, protection from a lethal viral challenge can be mediated by cytotoxic T cells and by neutralizing antibodies [10–12].

Although HCMV isolates from different persons are never identical with respect to the restriction endonuclease patterns of the entire genomes, strain variations have been considered to be of little consequence for the host [13, 14]. However, in recent years, there has been an accumulation of evidence suggesting that strain-specific differences might contribute to the clinical course of HCMV infection. For example, in kidney transplant recipients, reinfection with a genetically different donor virus is associated with a higher risk of developing severe HCMV disease than is reactivation of the endogenous virus [15]. Likewise, survival rates of bone marrow transplant recipients with HCMV infection have been linked to specific genotypes in the envelope glycoprotein B (gB; gpUL55) [16].

Although the underlying mechanisms for the different clinical outcomes of HCMV infections are unexplained, strain-specific immune responses might play an important role in situations in which reinfections occur and the immune response against de novo antigens is impaired. Moreover, infection with multiple HCMV strains has also been demonstrated in HIV-infected patients [17, 18]. Strain-specific recognition by HCMV-specific antibodies is a well-known phenomenon and was described nearly 2 decades ago with polyclonal sera [19]. Antibodies against envelope glycoproteins could be particularly important since they neutralize virus. Thus far, immunoglobulins reacting with gB (gpUL55) and glycoprotein H (gH; gpUL75) of HCMV have been characterized in some detail, and cross-reactivity and strain specificity have been demonstrated for both antigens [20–22].
Strain-specific immune responses might also hamper development of an effective vaccine. Reinfection with genetically different viruses has been observed in immunocompetent HCMV-seropositive persons [19, 23, 24]. Live attenuated HCMV strains have been tried as vaccines with limited success and have prompted the search for antigens that can be included in a subunit vaccine. gB has been proposed as a candidate and has been used in a small number of vaccines [25, 26]; however, humoral and cellular immune responses against gB are not induced in all persons during natural infection [27–29].

While the lack of gB-specific neutralizing antibodies in some human sera is unexplained, absence of proliferative responses of T helper (Th) cells has been linked to particular HLA class II alleles [29, 30]. Therefore, a subunit vaccine composed of gB alone may not be sufficient to induce a protective immune response in all persons. gH has been proposed as a logical second candidate antigen for strategies involving active or passive immunoprophylaxis [31]. This envelope protein induces a significant portion of neutralizing antibodies during natural infection, and in a recent investigation, we obtained evidence for Th cell responses against gH [32].

In the present study, gH-specific T cell lines from 5 healthy human donors were generated, and reactive domains were identified.

Material and Methods

Cells and viruses. HCMV AD169 was propagated on human foreskin fibroblasts in MEM (GIBCO, Glasgow, Scotland) supplemented with 5% fetal calf serum (GIBCO), glutamine (100 mg/L), and gentamicin (350 mg/L). Propagation and gradient purification were done as described [33]. Autographa californica nuclear polyhedrosis virus and recombinant baculoviruses were grown on Spodoptera frugiperda 158 cells (Sf158) in TC 100 insect medium (GIBCO) with 10% fetal calf serum, glutamine, and gentamicin. 

Recombinant baculovirus-expressed gH. The recombinant baculovirus for gH strain AD169 (Bac-gH/AD) was constructed as described [32], and the recombinant baculovirus—expressing gH strain Towne (Bac-gH/TO) was provided by Chiron (Emeryville, CA). To generate lysates of baculovirus-infected cells, 2 × 10^7 Sf158 cells were infected with Bac-gH/AD with an MOI of 10 for 48 h. Infected cells were collected, washed with PBS, and lysed by three freeze-thaw cycles followed by sonification. The cell lysate was stored in PBS at −20°C until use. The protein concentration of the lysate was determined in a bicinchoninic acid (BCA) assay (Pierce, Oud-Beijerland, Netherlands), and 4 μg/mL was used in proliferation assays.

Peptide synthesis and purification. A set of peptides (16–19 mer, 9 overlapping residues) representing aa 134–510 of gH strain AD169 (gH1–37) and various other peptides were synthesized on a multiple automatic peptide synthesizer (Zinsser Analytic, Frankfurt, Germany) using Fmoc-butyloxycarbonyl-based resins. All peptides were cleaved from the resin and deprotected in trifluoroacetic acid with scavengers, precipitated in ether, and lyophilized. All peptides were purified by high-performance liquid reverse-phase chromatography on a 5-μm column (Pep-S; Pharmacia) using a gradient of 10%–80% acetonitrile in 0.05 M trifluoroacetic acid. Peptides were lyophilized and dissolved in H2O–5% dimethyl sulfoxide (DMSO), with a final concentration of DMSO of <0.5%. For proliferation assays, each peptide was used at 20 μg/mL.

Lymphocyte proliferation assay for peripheral blood mononuclear cells (PBMC). PBMC were separated from fresh blood by Ficoll-Paque (Pharmacia) density centrifugation, and 10^5 cells/well were cultured in a 96-well round-bottom plate in RPMI 1640 supplemented with 10% autologous serum, glutamine, and gentamicin. Gradient-purified, heat-inactivated (45 min at 56°C) HCMV particles (0.5 μg/mL) were added as antigen. The cultures were incubated for 7 days at 37°C; cellular proliferation was determined by adding 1 μCi of [3H]-thymidine (Amersham, Braunschweig, Germany) to each well for the last 16 h. Cells were harvested on glass fiber filters with a PHD cell harvester (Cambridge Technology, Watertown, MA), and incorporated radioactivity was measured in scintillation fluid in a beta counter. Data are expressed as the mean of triplicate cultures in counts per minute (cpm). Values >3-fold higher than background of unstimulated cultures were considered positive responses. The stimulation index (SI) was calculated as the quotient of cpm from stimulated cultures to cpm from unstimulated cultures; values >3 were considered positive.

Generation of HCMV- and gH-specific T cell lines. For primary stimulation, freshly isolated PBMC (2 × 10^5 cells/well in a 96-well round-bottom plate) were incubated for 5 days with 0.5 μg/mL purified HCMV particles or 1.5 μg/mL lysate of Bac-gH/AD–infected cells in RPMI 1640 with 10% autologous serum. After 5 days, medium containing 10 U/mL human interleukin (IL)-2 (gift of Bioest, Dreieich, Germany) was added for the next 9 days, with an exchange of medium every 3 days. T cell cultures were restimulated by incubating 2 × 10^5 irradiated (3000 rad) PBMC and 2 × 10^5 prestimulated T cells with HCMV particles
or lysate of Bac-gH/AD–infected cells as antigen in normal medium for 5 days followed by incubation with medium containing IL-2 for the next 9 days. Proliferation of the generated T cell lines was assayed by incubating $2 \times 10^5$ irradiated PBMC and $2 \times 10^4$ T cells with the appropriate antigens for 4 days. Proliferation was measured by $[^{3}H]$thymidine incorporation for the last 16 h, as described above.

Characterization of cell surface phenotype. Cell surface phenotypes of the generated T cell lines were determined by fluorescence flow cytometry. For each T cell line, $3 \times 10^5$ Ficoll-purified cells were incubated (30 min at 4°C) with 50 µL of hybridoma culture supernatant of MAbs OKT3 (anti-CD3), OKT4 (anti-CD4), and OKT8 (anti-CD8) (American Type Culture Collection, Rockville, MD). The cells were washed three times in PBS–3% bovine serum albumin, and bound antibodies were detected with fluorescein isothiocyanate–conjugated rabbit anti–mouse immunoglobulin (Dako, Hamburg, Germany) for 60 min at 4°C. Cells were washed three times in PBS–3% bovine serum albumin, resuspended in PBS–1% formaldehyde, and tested for cell surface staining by flow cytometry (EPICS; Coulter Electronics, Luton, UK).

Determination of HLA class II restriction. A set of blocking MAbs against monomorphic determinants on human HLA-DR, -DQ, and -DP molecules was used to identify the antigen-presenting HLA class II molecules. MAb L243 (anti–HLA-DR), TÜ22 (anti–HLA-DQ), and B7/21 (anti–HLA-DP) were provided by one of us (H.K.), MAb 111 (anti-HIV/rev), provided by C. Aepinus (Institut für Klinische und Molekulare Virologie, Erlangen), was used as a control [36]. The antibodies were used in PBS at a concentration of 5 µg/mL and were added 90 min before addition of the T cells to antigen-pulsed, irradiated PBMC at 4°C. The relative inhibition of the antigen-dependent proliferation of the T cells was calculated as follows: % inhibition = $100 - \left[\frac{(cpm_{\text{antigen}} + \text{anti-HLA MAb} - cpm_{\text{no antigen}}) - (cpm_{\text{antigen}} + \text{control MAb} - cpm_{\text{no antigen}})}{cpm_{\text{antigen}} + \text{control MAb} - cpm_{\text{no antigen}}} \times 100\right]$.

HCMV serology. Each blood donor’s serum was tested for HCMV antibodies with an ELISA kit (Enzygnost anti-CMV/IgG; Behring, Marburg, Germany). Antibodies specific for gB and gH were determined in immunoblots using recombinant baculovirus as antigen.

HLA typing. The HLA haplotype for each blood donor was determined in the HLA diagnostic laboratory at the Institut für Klinische Rheumatologie und Immunologie, Erlangen, using commercially available antibodies.

Results

T cell proliferative response against HCMV particles. The proliferative response of PBMC against purified HCMV parti-
specific T cell lines were generated as described above using 1.5μg H. To investigate this differential response further, gH–baculoviruses expressing gH of either the AD 169 (Bac-gH/AD) or Towne (Bac-gH/TO) strain of HCMV. T cell lines restimulated with irradiated PBMC and HCMV particles for 5 days and then incubated in IL-2-containing medium for 9 days. The prestimulated T cells were tested in a 4-day proliferation assay with a lysate of insect cells infected with recombinant baculovirus expressing gH of strain 1AD169 or TTowne. Cellakes were determined for 10 HCMV-seropositive and 3 HCMV-seronegative persons by use of a standard lymphocyte proliferation assay. Freshly isolated PBMC were incubated for 7 days with gradient-purified HCMV particles, and the proliferative response was measured by [3H]thymidine incorporation during the last 16 h of the assay. Antigen was used at an optimum concentration for maximum proliferative responses (0.5 μg/mL; data not shown). PBMC from HCMV-seropositive donors had SIs of 20–161 compared with unstimulated controls; PBMC from HCMV-seronegative donors had no proliferative response. The 5 donors who showed the highest response in three consecutive experiments (data not shown) were selected for further experimentation (see below). It should be noted that donors J3 and J4 had identical HLA haplotypes, as defined by serologic typing (table 1).

Generation of gH-specific T cell lines. To characterize gH-specific T cell responses, short-term HCMV-specific T cell lines were generated from the 5 donors with the highest responses. Cell lines were established because reproducible responses against various gH-specific antigens could be produced in the 7-day assay format in only 1 donor (data not shown). Therefore, PBMC were isolated and stimulated with purified HCMV particles for 5 days and then incubated in IL-2-containing medium for 9 days. The prestimulated T cells were restimulated with irradiated PBMC and HCMV particles for 5 days, and IL-2 was added for 9 days. Reactivity of the HCMV-specific T cell lines was then tested in a 4-day proliferation assay with a lysate of insect cells infected with recombinant baculoviruses expressing gH of either the AD169 (Bac-gH/AD) or Towne (Bac-gH/TO) strain of HCMV. T cell lines J1, J2, and J3 responded to Bac-gH/AD, whereas Bac-gH/TO induced proliferation in only J1 (table 1).

The lack of reactivity of lines J2 and J3 with Bac-gH/TO suggested the possibility of a strain-specific T cell response to gH. To investigate this differential response further, gH-specific T cell lines were generated as described above using 1.5μg/mL Bac-gH/AD. The lines were then tested in a 4-day proliferation assay with a set of purified β-galactosidase fusion proteins representing the entire open-reading frame of gH strain AD169 (figure 1A). Control antigens included lysates from A. californica nuclear polyhedrosis virus–infected insect cells (Bac-Wt) and the β-galactosidase fusion partner.

The lines exhibited specific recognition patterns that differed with respect to the protein fragment recognized and the level of the proliferative response (table 2). Since some cells proliferated in response to the purified β-galactosidase fusion proteins, all SIs were calculated using this as the control proliferation. Two types of antigen-specific responses could be differentiated quantitatively: those that resulted in an SI of ≤10 and those with an SI of ≥20. This pattern of response was constant over a number of experiments with different cell lines and antigen preparations (data not shown); therefore, we arbitrarily divided the data into high (SI ≥20) and low (SI ≤10) proliferative responses. Both types of responses were induced by each antigen. The highest levels of stimulation were with fusion proteins PN86 and SN86, containing aa 142–508 with an overlap between residues 278 and 358. Both antigens stimulated a high response in three T cell lines (J1, J2, and J3). AP86 and NH86 induced high responses in J1 and J4 and in J2, respectively.

We conclude from this series of experiments that crude lysates of recombinant baculovirus–infected insect cells could stimulate gH-specific T cells in PBMC from HCMV-seropositive donors and that T cell determinants can be identified using purified bacterial fusion proteins. T cell–reactive determinants were distributed over the entire molecule. Of interest, 2 donors sharing the same HLA class II haplotype exhibited a different recognition pattern for gH.

Fine specificity of the T cell proliferative response against aa 15–142 of gH. Next, we wanted to define the proliferative response to individual determinants between aa 15 and 142 of gH in T cell lines that exhibited a high response to fusion.

### Table 1. Proliferative response of HCMV-specific T cell lines against gH of HCMV strains AD169 and Towne.

<table>
<thead>
<tr>
<th>Donor</th>
<th>HLA type *</th>
<th>Bac-gH/AD ‡</th>
<th>Bac-gH/TO ‡</th>
<th>Bac-WT §</th>
<th>No antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>J1</td>
<td>DR15;DR17;DR52;DQ6;DQ2</td>
<td>4341 (5)</td>
<td>4289 (5)</td>
<td>954</td>
<td>208</td>
</tr>
<tr>
<td>J2</td>
<td>DR11;DR4;DR52;DR53;DQ7</td>
<td>2542 (6)</td>
<td>1115 (3)</td>
<td>451</td>
<td>292</td>
</tr>
<tr>
<td>J3</td>
<td>DR11;DR7;DR52;DR53;DQ7;DQ2</td>
<td>16,521 (7)</td>
<td>5090 (2)</td>
<td>2486</td>
<td>350</td>
</tr>
<tr>
<td>J4</td>
<td>DR11;DR7;DR52;DR53;DQ7;DQ2</td>
<td>4281 (2)</td>
<td>4161 (2)</td>
<td>2118</td>
<td>337</td>
</tr>
<tr>
<td>J5</td>
<td>DR3;DR13;DR52;DQ2;DQ6</td>
<td>5062 (3)</td>
<td>1222 (1)</td>
<td>2034</td>
<td>348</td>
</tr>
</tbody>
</table>

* HLA class II haplotypes were determined with commercially available antibodies.
‡ Determined by [3H]thymidine incorporation during last 16 h of 4-day proliferation assay. SI, stimulation index.
§ SD < 20%.
Lyse of insect cells infected with 4 μg/mL recombinant baculovirus–expressing gH of strain AD169 or TTowne or with 142 μg/mL Autographa californica nuclear polyhedrosis virus.
Table 2. Proliferative response of gH-specific T cell lines against prokaryotically expressed gH-derived fusion proteins.

<table>
<thead>
<tr>
<th>Donor</th>
<th>AP86&lt;sup&gt;1&lt;/sup&gt; (aa 15–142)</th>
<th>PN86&lt;sup&gt;1&lt;/sup&gt; (aa 142–358)</th>
<th>SN86&lt;sup&gt;1&lt;/sup&gt; (aa 278–508)</th>
<th>NH86&lt;sup&gt;1&lt;/sup&gt; (aa 508–743)</th>
<th>β-gal&lt;sup&gt;2&lt;/sup&gt;</th>
<th>No antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>J4</td>
<td>11,022 (22)</td>
<td>1200 (2)</td>
<td>1627 (3)</td>
<td>431 (1)</td>
<td>497</td>
<td>650</td>
</tr>
<tr>
<td>J5</td>
<td>2077 (1)</td>
<td>7445 (5)</td>
<td>6814 (5)</td>
<td>8592 (6)</td>
<td>1458</td>
<td>675</td>
</tr>
<tr>
<td>J2</td>
<td>1132 (3)</td>
<td>63,997 (199)</td>
<td>42,041 (131)</td>
<td>26,363 (82)</td>
<td>322</td>
<td>664</td>
</tr>
<tr>
<td>J3</td>
<td>4434 (5)</td>
<td>69,711 (79)</td>
<td>56,970 (67)</td>
<td>1810 (2)</td>
<td>882</td>
<td>584</td>
</tr>
<tr>
<td>J1</td>
<td>25,780 (47)</td>
<td>16,469 (30)</td>
<td>14,160 (26)</td>
<td>2601 (5)</td>
<td>551</td>
<td>730</td>
</tr>
</tbody>
</table>

<sup>1</sup> Determined by [3H]thymidine incorporation during last 16 h of 4-day proliferation assay. SI, stimulation index. SD <20%.
<sup>2</sup> gH-derived β-galactosidase fusion proteins (0.5 µg/mL).

Protein AP86. This area of the protein was interesting because it contained the highest amino acid sequence heterogeneity among HCMV isolates, including a strain-specific B cell epitope [22]. A set of nested β-galactosidase fusion proteins was tested with gH-specific lines J4 and J1. As antigen, we used fusion proteins AP86 (aa 15–142), EB86 (aa 34–110), Exo86-54 (aa 15–34), and Exo86-64 (aa 15–60) at 0.5 µg/mL (figure 1A). The truncated β-galactosidase<sub>aa 1-375</sub> served as the reference antigen for control stimulation.

Both T cell lines had distinct proliferative response patterns to the fusion proteins (table 3). Line J4 responded to protein AP86 and was negative for the remaining antigens, suggesting that the reactive determinant was located between aa 111 and 142. Line J1 had a proliferative response to proteins AP86, EB86, and Exo86-64; thus, the responding determinant was located between aa 34 and 60, a region common to all reactive proteins. The T cell determinant for line J1 was confirmed using the synthetic peptide AP1 (aa 34–51). T cells had a high proliferative response (SI, 104) to AP1 (aa 34–51) compared with unstimulated cultures. The increased level of proliferation in response to the synthetic peptide was probably due to the higher molar concentration in the AP1 (aa 34–51) assay than in the one for the fusion proteins. Taken together, the data suggest that the amino-terminal part of gH between residues 15 and 142 contains at least two T cell epitopes located between aa 34–51 and 111–142, respectively (figure 1B).

Fine specificity of the T cell proliferative response against aa 134–510 of gH. Data obtained with bacterial fusion proteins indicated that the mid region of gH contains dominant T cell determinants, since three cell lines showed a high proliferative response. A set of synthetic peptides (16–19mers, 9 overlapping amino acids) was used to investigate the proliferative response between aa 134 and 510 of gH in the gH-specific T cell lines J1, J2, J3, and J5 (figure 2). Peptide gH16 (aa 284–302) stimulated responses in lines J1, J2, J3, and J5 (figure 2). Peptide gH20 (aa 324–342) induced a high proliferation in line J5 and a weak response in J3. For lines J2, J3, and J5, a cluster of reactive peptides was identified between peptides gH33–37 (aa 454–510). The individual lines, however, exhibited characteristic patterns. The results obtained with the peptides agreed with data obtained with the fusion proteins that had been used to established reactivity of lines J1, J2, J3, and J5 with fusion proteins PN86 and SN86. PN86 and SN86 share aa 278–358, which include gH16 (aa 284–302) and gH20 (aa 324–342) (figure 1B).

Table 3. Analysis of the fine specificity of gH-specific T cell lines using a set of nested β-galactosidase fusion proteins from aa 15 to 142 of gH.

<table>
<thead>
<tr>
<th>Donor</th>
<th>AP86&lt;sup&gt;1&lt;/sup&gt; (aa 15–142)</th>
<th>EB86&lt;sup&gt;1&lt;/sup&gt; (aa 34–111)</th>
<th>Exo86-54&lt;sup&gt;1&lt;/sup&gt; (aa 15–34)</th>
<th>Exo86-64&lt;sup&gt;1&lt;/sup&gt; (aa 15–64)</th>
<th>AP1&lt;sup&gt;1&lt;/sup&gt; (aa 34–51)</th>
<th>β-gal&lt;sup&gt;2&lt;/sup&gt;</th>
<th>No antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>J4</td>
<td>8990 (19)</td>
<td>499 (1)</td>
<td>621 (1)</td>
<td>912 (2)</td>
<td>ND</td>
<td>474</td>
<td>222</td>
</tr>
<tr>
<td>J1</td>
<td>15,821 (18)</td>
<td>5959 (7)</td>
<td>605 (1)</td>
<td>4648 (7)</td>
<td>71,169 (104)</td>
<td>903</td>
<td>682</td>
</tr>
</tbody>
</table>

<sup>1</sup> Determined by [3H]thymidine incorporation during last 16 h of 4-day proliferation assay. SI, stimulation index. SD <20%.
<sup>2</sup> gH-derived β-galactosidase fusion proteins (0.5 µg/mL).
<sup>1</sup> Synthetic peptide AP1 aa 34–51 (20 µg/mL); ND, not determined.
<sup>2</sup> β-galactosidase<sub>aa 1–375</sub> (0.5 µg/mL).
Fluorescence flow cytometry of the cell surface phenotype of all T cell lines demonstrated that >90% of the cells were positive for CD3 and CD4 and negative for CD8, suggesting a major histocompatibility complex (MHC) class II restriction of the antigen response. Blocking experiments using MAbs against human HLA-DR, -DQ, and -DP molecules demonstrated a dominance for HLA-DR in presenting the identified epitopes (data not shown).

Strain-specific T cell proliferative response. When published amino acid sequences from different HCMV isolates were compared with the T cell-reactive regions, two domains of gH showing sequence heterogeneity among isolates were identified. One was the domain located on peptide AP1 (aa 34–51), for which two prototype sequences are known, represented by laboratory strains AD169 and Towne [37, 38]. The Towne sequence is characterized by a deletion of a proline...
residue at position 36 and a histidine-to-lysine exchange at position 37 (table 4). Part of this sequence has previously been identified as a strain-specific neutralizing B cell epitope [22]. The other domain was represented by peptide gH16 (aa 284–302). Heterogeneity in this area of gH included positions 285 and 296 (table 4). At both positions, the mutations consisted of nonconserved amino acid exchanges.

This sequence heterogeneity could potentially result in a strain-specific T cell response against these domains. This assumption was tested in lines J1 and J2. T cell lines were generated by stimulation of PBMC with fusion proteins followed by peptides representing strain AD169. Lines were tested with synthetic peptides representing either AD169 or the variant sequences. The proliferative response was identical when peptides representing the amino-terminal domain between aa 34 and 51 (API and API-TO) were compared (table 4). In contrast, when gH16 variant peptides were analyzed, there was a strain-specific proliferative response. Only peptide gH16/AD169 could induce a T cell response in gH-specific T cell lines (table 4).
Table 4. Strain-specific response of gH-specific T cell lines.

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Line J2</th>
<th>Line J1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gH16¹</td>
<td>gH16-TO²</td>
</tr>
<tr>
<td>20</td>
<td>21,728</td>
<td>2109</td>
</tr>
<tr>
<td>4</td>
<td>6582</td>
<td>4050</td>
</tr>
<tr>
<td>0.8</td>
<td>3813</td>
<td>2395</td>
</tr>
<tr>
<td>0.2</td>
<td>1700</td>
<td>2540</td>
</tr>
<tr>
<td>0</td>
<td>1395</td>
<td>1395</td>
</tr>
</tbody>
</table>

*Proliferative responses of gH/strain AD169-specific T cell lines were determined by [3H]thymidine incorporation during last 16 h of 4-day proliferation assay.

¹ Synthetic peptides gH16 (KAQLNRHSYLKDSDFLDAA) and API (LDPHAFHLLLNTYGRPIR) of strain AD169.
² Synthetic peptides gH16-TO (KDQLNRHSYLKDPSDFLDAA) and API-TO (LDKAFHLLLNTYGRPIR) of strain Towne.
³ Synthetic peptide gH16-94 (KTQLNRHSYLKDPSDFLDAA) of clinical isolate (20 µg/mL).

**Discussion**

Analysis of HCMV genomes from patient samples invariably leads to identification of individual strains, with no 2 isolates being identical [13]. Potential consequences of strain variations for the clinical course of HCMV infections are only beginning to be unraveled [15, 16, 31, 39]. Reactivation of previously acquired infections and reinfection occur in transplant recipients and HIV-infected persons. Immune suppression with an associated limited capacity to react to de novo antigens might constitute a situation that is impossible for the immune system to control. In addition, vaccines based on antigens from a single virus strain may fail to induce protective responses against heterologous virus strains.

A central step in mounting an efficient immune response is the activation of antigen-specific Th cells [40]. We analyzed the Th cell response to gH in 5 healthy HCMV-seropositive donors. The experiment design approximated a situation that occurs during reactivation of latent virus or during reinfection (i.e., a primed immune system encounters free virus or virus-infected cells). According to our data, the Th cell response to gH of HCMV was limited to a small number of epitopes. Under our experimental conditions, T cell epitopes that were cross-reactive for all 5 donors could not be detected. It is possible that we could not stimulate the entire repertoire of gH-specific T cells with antigen derived from strain AD169. A complete stimulation could be achieved only by using antigen from homologous virus strains, the isolation of which is usually impossible from healthy HCMV-seropositive persons.

The situation is comparable to that for envelope proteins from other virus systems, such as influenza or HIV, which also lack broadly cross-reactive T cell epitopes [41–44]. The immunodominant domains of gH seem to reside in the middle region of the molecule between aa 284 and 510. A high proliferative response to single and multiple determinants was seen in three of the five T cell lines. The occurrence of multiple, overlapping T cell determinants is not uncommon [45, 46]. The amino- and carboxy-terminal portions of the protein induced a comparable response in two and one T cell line, respectively.

Strain specificity might play an important role for recognition of gH-specific Th cells. When full-length gH was used, a strain-specific response was noted in two (J2 and J3) of five HCMV-specific T cell lines. These two lines reacted in a dominant fashion with gH16 (aa 284–302), a region of gH that is not conserved among HCMV strains. This dominance was not secondary to repeated stimulation of T cells, since donor J3, the only donor for which a gH-specific T cell proliferation could be established in standard 7-day proliferation assays, showed the same specificity without restimulation of T cells (data not shown). Mutations in the coding sequence for peptide gH16 seem to be a rather selective event, since residue 285 (position 2 in gH16) is the only position within gH16 where mutation of more than one nucleotide is necessary to bring about the observed sequence diversity. In addition, an HCMV strain recently isolated from an AIDS patient, which differs in only four amino acids from AD169, includes an alanine-to-threonine change within the sequence of gH16 [47].

A further key feature of immunodominance of T cell determinants is promiscuous binding to different MHC class II alleles [48–50]. Data on the HLA class II determinants that are associated with presentation of gH16 supported this assumption. In our inhibition experiments, the peptide was presented in association with HLA-DR. DR52 is the only DR allele that was shared by the 3 donors with reactivity for gH16. However, DR52 was also present in donors whose samples did not respond to gH16. It seems likely, therefore, that gH16 (aa 284–302) associates with different HLA molecules since no other DR allele was common to all 3 donors. Furthermore, evidence...
supporting the importance of recognition of gH16 comes from results obtained with line J1. Although this line reacted with gH16, it did not show a differential response to the full-length protein, since the dominant response was directed against the amino-terminal peptide API (aa 34–51), which did not show strain-specific stimulation of T cells.

Although the T cell–reactive domain on peptide API (aa 34–51), which was recognized by line J1, is located in a part of gH that shows a high degree of sequence variability among strains, its reactivity was not influenced by this amino acid variability. The line responded equally well to peptides representing either the AD169 or Towne strains. This result suggests that the variable part of gH between aa 36 and 37 was not part of the epitope. The deletion of a proline with the additional change of a histidine to lysine in strain Towne could be expected to result in loss of reactivity if these residues were part of the MHC class II binding peptide. This epitope, however, might contribute to the observed strain-specific humoral immune response against gH since the neighboring B cell epitope, located between aa 34 and 43 (AD86), is strain specific [22].

The proximity of T and B cell epitopes has been suggested as a mechanism for the induction of an efficient immune response [51–54]. The dominance of AD86 as a B cell–reactive epitope is consistent with this prediction.

The domains of gH recognized by the T cell lines from donors J3 and J4 were completely different. This was unsuspected since both donors had an identical HLA haplotype. J4 responded to a single domain in the area between aa 110 and 141, whereas J3 showed reaction with aa 284–302, 324–342, 474–492, and 484–502.

Antigen processing and subsequent presentation to T cells is a critical step in determining the nature of the generated immune response. Besides macrophages, several other cells that constitutively express MHC class II can function as antigen-presenting cells, including B lymphocytes [54, 55]. In contrast to other antigen-presenting cells, B cells express individual membrane immunoglobulin as specific receptors for a particular antigen. It has recently been shown that antibodies that associate with different parts of antigens at different affinity constants can have a significant influence on the outcome of antigen processing, possibly by altering the nature of the substrate for processing [52, 54, 56, 57]. All 5 donors had antibody levels against gH that were comparable to gB (data not shown). Since gH induces predominantly conformation-dependent antibodies, it was not possible to analyze the fine specificity of the B cell response of our donors. However, from similar investigations of gB of HCMV and other viral glycoproteins, it can be assumed that a variety of individual B cell specificities have evolved during the persistent HCMV infection in each donor [20, 21]. Thus, B cells could be involved in the presentation of gH in immune individuals, which might explain the observed differences in T cell–reactive domains between HLA-identical donors.

In summary, we have identified a limited number of T cell–reactive determinants on gH of HCMV. The pattern of recognition is unique for each donor, and no cross-reactive epitope could be identified that stimulated PBMC from all 5 donors. In addition to the previously established strain-specific epitope of humoral immune response against gH, we identified a dominant strain-specific T cell epitope in three T cell lines. Considering a homology between gH molecules from different isolates that is >95%, this is not trivial [58, 59]. For the clinical situation, this might have two consequences: First, in situations in which immune functions are impaired, existing immunity might not be sufficient to protect from reinfecting HCMV strains, and second, vaccines consisting of antigen from 1 strain might not induce sufficient protection. Recent studies have demonstrated that in addition to evasion of an existing immune response, virus variants that involve T cell determinants can exert antagonistic effects on the development of an effective immune response [60–62].

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