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*J Immunol* 2003; 170:2030-2036; doi: 10.4049/jimmunol.170.4.2030

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Modulation of HLA-A*0201-Restricted T Cell Responses by Natural Polymorphism in the IE1_{315–324} Epitope of Human Cytomegalovirus

Virginie Prod’homme,* Christelle Retière,* Berthe-Marie Imbert-Marcille, † Marc Bonneville,* and Marie-Martine Hallet‡ *

Cytotoxic T lymphocytes play a central role in the control of persistent human CMV (HCMV) infection and reactivation. In healthy virus carriers, the specific CD8⁺ T cell response is almost entirely directed against the virion tegument protein pp65 and/or the 72-kDa major immediate early protein, IE1. Studies that included a large panel of HCMV⁺ donors suggested that immunorelevance of pp65 and IE1 was directly related with individual HLA haplotype difference. Nevertheless, there are no data on the incidence of HCMV natural polymorphism on virus-specific CTL responses. To assess the impact of IE1 polymorphism on CTL response, we have sequenced in 103 clinical isolates the DNA region corresponding to IE1_{315–324}, an immunodominant epitope presented by HLA-A*0201 molecules. Seven peptidic variants were found with extensive difference in their frequencies. The response of four HLA-A*0201-restricted anti-IE1 T lymphocyte clones, which were previously generated from one donor against autologous B lymphoblastoid cells expressing a recombinant clinical variant of IE1, was then evaluated using target cells loaded with mutant synthetic peptides or expressing rIE1 variants. One of four clones, which have been sorted 19 times among 22 clones targeted against IE1_{315–324}, recognized six of the seven tested variant epitopes. All three other clones showed distinct reactivity patterns to target cells loaded with the different mutant peptides or expressing IE1 variants. Therefore, in the HLA-A2 context, clonal expansions of anti-IE1 memory CTLs may confer a protection against HCMV successive infections and reactivations by killing cells presenting most of the naturally occurring IE1_{315–324} epitope variants. The Journal of Immunology, 2003, 170: 2030–2036.

After a primary infection often asymptomatic, CMVs establish with healthy hosts a stable relationship in which they persist under the control of the immune system. CD8⁺ T lymphocytes play a key role in the clearance of CMV productive infection as well as in the maintenance of the latent state (1–4). In the murine model, the 89-kDa IE1 protein, an immediate-early viral trans regulator, is considered as an immunodominant Ag for the CD8⁺ CTL response (5). In humans, an early report has shown that a high frequency of CTLs was specifically targeted to 72-kDa IE1 in CMV healthy carriers (6), but further data obtained from an in vitro fibroblast infection model have put into question the strong immunogenicity of this protein (7, 8). This explains in part why, for several years, the virion tegument protein pp65 has been regarded as the only immunodominant human CMV (HCMV) Ag. More recently, two reports have reassessed the immunogenicity of 72-kDa IE1 through analysis of memory T cell responses against CMV-derived peptic epitopes or whole HCMV proteins expressed by recombinant vaccinia viruses (9, 10). Both studies showed that in some donors, the anti-IE1 CTL response could be stronger than the anti-pp65 one and suggested that immunodominance of pp65 and IE1 should depend on individual polymorphism (for a review, see Ref. 11).

To date, the CD8⁺ T cell repertoire against HCMV has almost always been investigated using the AD169 laboratory strain, but little is known yet about the natural polymorphism of viral proteins and its impact on CTL response. Therefore, current assessments of the immunogenicity of HCMV proteins might be biased by peptide variation in antigenic gene products of viral strain(s) carried by donors. Available data suggest that CD8⁺ T cell response against pp65 targets conserved epitopes, at least in the HLA-A*0201 context. Infrequent natural mutations, which had only a faint effect on cytolytic targeting, have been described in the HLA-A*0201-restricted pp65_{495–503} immunodominant epitope (12), and no mutation was found in pp65_{14–22} and pp65_{120–126}. Two HLA-A*0201-restricted subdominant epitopes (13). Unlike pp65, the IE1-exon 4 sequence exhibits significant polymorphism that has been partially described by several authors (14–16). Nevertheless, there are only limited data (reviewed in Ref. 11) on sequence variations occurring within MHC class I-restricted IE1 epitopes and no information about its consequence on CTL responses.

In a previous study, we generated from one donor four different HLA-A*0201-restricted anti-IE1 CD8⁺ T cell clones against autologous B lymphoblastoid cells expressing a recombinant clinical variant of IE1. One of these four clones was immunodominant and corresponded to 19 of 22 anti-IE1 HLA-A*0201-restricted T cell clones generated from this donor. All four clones released TNF in an autopresentation assay in which they were loaded with a 9-mer IE1_{315–323} (YILEETSVM) minimal peptide (17).

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Received for publication September 23, 2002. Accepted for publication December 10, 2002.

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‡ Abbreviations used in this paper: HCMV, human CMV; BLCL, B lymphoblastoid cell line; PMNL, polymorphonuclear leukocyte.
In this study, the DNA region corresponding to this epitope was PCR amplified and sequenced in 103 clinical isolates from different origins, which led to the identification of seven IE1315–324 peptide variants with extensive differences in their frequency. The impact of these mutations on T cell clone responses was then evaluated using HLA-A*0201 target cells loaded with mutant synthetic peptides or expressing rIE1 variants. Six of the seven tested variant epitopes efficiently stimulated HLA-A*0201-restricted lytic activity of the immunodominant clone. All three other clones reacted to fewer IE1315–324 variant epitopes. Our data suggest an in vivo clonal amplification of CTLs that are able to cross-react with multiple HCMV IE1 variants.

Materials and Methods

Cells

CD8 T lymphocyte clones C1, C2, C3, and C4 were previously generated from PBMCs of an HCMV+ healthy donor C (HLA class I haplotype: A*0201A*B501, B*1801B*4402, Cw*0501Cw*1203) stimulated with an autologous B lymphoblastoid cell line (BLCL) expressing a recombinant clinical variant of IE1 (17). All these four clones released TNF when stimulated with the minimal HLA-A*0201-restricted 9-mer peptide IE1315-323 (YILEETSVML). C2 is a B220+1.B6 immunodominant clone that was found 19 times among 22 anti-IE1 HLA-A*0201-restricted clones that were sorted from PBMCs of donor C. The 3 other anti-IE1 HLA-A*0201-restricted clones, C1, C3, and C4, respectively exhibited Vp21S3J21.1, Vp41S1J12, and Vp1S1J13 segments (17). T cell clones were maintained in RPMI 1640/10% human serum supplemented with rIL-2 (60 IU/ml) and restimulated every 3 wk under polyclonal stimulation, as described (18).

The BLCL derived from donor C (17) and U373 MG cells, which express the HLA-A*0201 allele, were used as target cells for Ag presentation to T lymphocytes. BLCL/IE1-1, previously used as stimulator to generate C1, C2, C3, and C4, stably express a clinical variant of IE1 (17). U373 MG/IE1-1, U373 MG/IE1-2, and U373 MG/IE1-3 were generated by stable transfection with vectors coding for three IE1 variants.

Sequence strategy

DNA was rapidly extracted from frozen polymorphonuclear leukocytes (PMNLs), obtained at the time of active CMV infection. Cell pellets were suspended in 10:1 Tris EDTA, heated 10 min at 95°C, and centrifuged (10 min at 13,000 × g). A first round of amplification (35 cycles: denaturation for 1 min at 94°C; annealing for 1 min at 50°C; extension for 2 min at 72°C) was conducted from 10 μl of each sample with primers IE1A (5′-GGGGAAGATGGTGAGGGC-3′) and IE1B (5′-CTCCTACCTCTGTCGTCATCTG-3′). A second round (35 cycles: denaturation for 1 min at 94°C; annealing for 1 min at 55°C; extension for 2 min at 72°C) was performed on 5 μl of the first PCR samples to amplify a 310-bp DNA fragment with primers IE1A (5′-AGGGTCTCAGCGACATTGTGC-3′) and IE1B (5′-AGGAGAAGACTCTCCAGGAAG-3′). ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA) using Factura software was used to sequence amplified DNA fragments. Labeling reactions were performed with the primer IE1A (5′-TGATATCTTCCAATCTGTTGGG-3′).

Peptides

Synthetic IE1 mutant peptides were purchased from Genosys (Pampsisford, U.K.). Their purity was greater than 70% by HPLC analysis. Peptides were dissolved in aqueous solution containing 10% DMSO and 0.1% acetic acid, and then frozen in small aliquots at −80°C. Before use, peptide concentration was estimated using HPLC 1640 supplemented with 10% FCS. Bioinformatics and molecular analysis section HLA peptide-binding predictions were used to estimate t1/2 dissociation of IE1 mutant peptides from HLA-A*0201 (19).

Expression vectors

IE1-1 full-length coding sequence was previously amplified by RT-PCR and cloned into MRC-5 fibroblasts infected with a CMV clinical strain, IE1315-324 amino acid sequence (YILEETSVML) encoded by the IE1-1 cDNA was identical with that of the Towne strain (17). IE1 DNA fragments of 1.1 kb, which included a BsmI unique site at position 381 and the stop codon at position 1469–1471 (i.e., corresponding to the 3′ major part of exon 4 coding region), were cloned by PCR from the DNA of MRC-5 fibroblasts infected with the laboratory strain AD169 (fragment IE1-2 coding for the IE1315-324 YILEETSVML sequence) or with another clinical strain (fragment IE1-3 coding for the IE1315-324 YILEETSVML sequence). Sequences were controlled on three independently amplified PCR products. IE1-2 and IE1-3 fragments were then fused with the ATG-BsmI 5′ part of IE1-1 in place of the BsmI-stop nucleotide sequence of IE1-1, to generate full-length cDNAs coding for IE1-2 and IE1-3 mutant proteins. For expression in U373 MG cells, the three IE1 full-length cDNAs were subcloned in a pcDNA3 vector (Invitrogen, San Diego, CA), deleted from two PvuII fragments that carry neomycin resistance gene. A DNA fragment containing an internal ribosome entry site and the aminoglycoside phosphotransferase coding sequence that confers resistance to neomycin was inserted between the IE1 coding sequence and the bovine growth hormone poly(A). Then the three constructed vectors allow together IE1 and selection marker expressions under the control of CMV promoter.

Cytotoxicity assays

The cytotoxic activity of T cell clones against target cells stably transfected with IE1 natural variants or loaded with mutant synthetic peptides was estimated by a standard 4-h 51Cr release assay. Briefly, target cells were incubated for 1 h at 37°C with Na2H235CrO4 (NEN-PerkinElmer Life Sciences, Paris, France) and washed twice. Nontransfected BLCL and U373 MG cells were then pulsed with various concentrations of peptide (1 h at 37°C in RPMI 1640/10% FCS) and washed three times. Target cells (3 × 103 cells/well in 96-well round-bottom culture plate) were incubated with T cells at various E:T ratios in 150 μl RPMI 1640/10% FCS. After 4 h of incubation at 37°C, the culture supernatant was harvested and counted for released radioactivity. Each test was performed in triplicate. Percent specific lysis was calculated as previously described (20).

Proliferation assays

The proliferative activity of T cells, taken >3 wk after the late stimulation, was estimated by a 6-h pulse with 1H[3H]thymidine after a 6-h coculture with autologous irradiated (35 Gy) BLCL loaded with 108 nM peptides at 2.5:1 E:T ratio. Cells were then harvested and counted for 1H incorporation. Results were expressed as the mean of triplicates.

Cytokine expression assays

BLCL was pulsed for 1 h at 37°C with 104 nM of various mutant peptides in RPMI 1640 medium/10% FCS and washed twice. T lymphocyte clones (103 cells) were stimulated by peptide-pulsed BLCL (2 × 105 cells) in 2 ml of RPMI 1640 medium/10% FCS in the presence of 10 μg/ml of brefeldin A (Brefeldin A; Sigma-Aldrich, Saint Quentin Fallavier, France) for 5 h at 37°C. For intracytoplasmic staining, cells were washed and fixed 10 min at room temperature in a solution of PBS at 4% paraformaldehyde and permeabilized with PBS containing 0.1% BSA (A9647; Sigma-Aldrich) and 0.1% saponin (S-2149; Sigma-Aldrich). Fixed stimulated T cells were then stained for cytokines using the method described by Jung et al. (21), with 250 ng of PE-conjugated anti-human IL-2 (MQ1-17H12; BD Pharmingen, Pont de Claix, France) or 125 ng of FITC-conjugated anti-human IFN-γ mAbs (4S.B3; BD Pharmingen), for 30 min at room temperature. Reagent dilutions and washes were done with PBS containing 0.1% BSA and 0.1% saponin. After labeling, cells were resuspended in PBS and analyzed on a FACSscan flow cytometer using CellQuest software (BD Biosciences, Pont de Claix, France).

Results

Cytolytic responses of CTL clones against IE1315–324 and IE1316–324 synthetic peptides

The cytotoxic activities of HLA-A*0201-restricted CTL clones generated from donor C against the decapetide IE1315–324 (YILEETSVML) and its two internal nonapeptides, IE1315–323 and IE1316–324, were evaluated (Fig. 1). All CTL clones reacted against all three peptides, but responded better to the decamer than to the two internal nonamers. Furthermore, IE1315–324 was always a less efficient stimulator than IE1316–324. Then we chose to use IE1315–324 mutant synthetic decapetides to carry on a study on the impact of the IE1 polymorphism on the HLA-A*0201-restricted CTL responses.
analyzed clinical isolates, were the two most frequent sequences PA2, which corresponded respectively to 39 and 31% of the 103 infected individuals. Sequence PA1 matched sequence PA2, except for an I/V substitution at position 2 (Table I). PA1 and PA2, and PA3 peptidic sequences at IE1315–324 position. BLCL/IE1-1, previously used as a target to generate the IE1-specific CTL clones, was included in experiments as a positive control. As shown in Fig. 2, all four clones recognized better BLCL loaded with 10^4 nM of SPA1, SPA2, or SPA3 synthetic peptides, and IE1-2, or the IE1-3 variant. Unlike the C2 clone, C1 and C3 clones efficiently killed target cells loaded with 10^3 nM of SPA4 and SPA6, but were differently reactive against lower concentrations of these synthetic peptides. Taken together, these data show that the immunodominant clone C2 was stimulated by six of the seven mutant peptides. Minimal peptide concentrations required to stimulate cytolyis by clone C2

Response of CTL clones against PA1, PA2, and PA3 mutant epitopes

A first round of experiments was performed to measure the impact of natural sequence variation in PA1, PA2, and PA3 epitopes on the biological responses of C1, C2, C3, and C4 T cell clones against IE1 variants. Autologous BLCL and U373 MG target cells, which express HLA-A*0201, were first loaded with various concentrations of the three synthetic peptides, and their ability to stimulate cytolyis by C1, C2, C3, and C4 clones was measured. As shown in Fig. 2, all four clones recognized better BLCL loaded with the SPA1 10-mer synthetic peptide corresponding to the PA1 original epitope as compared with SPA2 and SPA3 synthetic peptides that carried, respectively, PA2 and PA3 sequences. All clones recognized SPA1 and SPA2 at high concentrations (≥10^5 nM), but the C2 immunodominant clone was the only one that was able to efficiently kill target cells loaded with the SPA3 peptide. Similar results were obtained with U373 MG target cells (data not shown).

Consistent results were obtained when proliferation and intracytoplasmic expression of IL-2 and IFN-γ of the four clones against target cells loaded with 10^4 nM of SPA1, SPA2, or SPA3 were measured (Fig. 3). C2 was the only clone that was similarly activated by the three peptides. C1 and C3 were poorly stimulated by SPA3 in both cytokine production and cell proliferation assays. Despite an intracellular IL-2 production that was as high as that of the three other clones (Fig. 3B), clone C4 proliferated poorly against the three mutant peptides (Fig. 3A). Nevertheless, this clone yielded strong IFN-γ responses when incubated with 10^4 nM of SPA1, SPA2, or SPA3 peptides (Fig. 3C), which is consistent with its significant cytolytic activity toward target cells loaded with 10^3 nM of SPA3 (Fig. 2).

The immunogenicity features of IE1 epitope variants were then assessed after natural processing by A*0201 target cells. To this end, U373 MG line was stably transfected with vectors encoding the IE1-1, IE1-2, or IE1-3 variants that carried, respectively, PA1, PA2, and PA3 peptideic sequences at IE1315–324 position. BLCL/IE1-1, previously used as a target to generate the IE1-specific CTL clones, was included in experiments as a positive control. As shown in Fig. 4, cytotoxic responses of C1, C2, and C3 clones against BLCL/IE1-1, U373 MG/IE1-1, U373 MG/IE1-2, and U373 MG/IE1-3 at 5:1, 10:1, 20:1, and 40:1 E:T ratios were consistent with the results obtained in peptide-loading experiments (Fig. 2). The C2 clone lytic activity was unaffected by the polymorphism of the IE1 proteins expressed by U373 MG cells. Unlike the C2 clone, C1 and C3 clones efficiently killed target cells expressing the IE1-1 and IE1-2, but not the IE1-3 variant.

Cytolytic activities of CTL clones against the four other mutant peptides

The cytolytic activity of C1, C2, and C3 T cell clones was measured against autologous BLCL and U373 MG target cells loaded with various concentrations of synthetic peptides SPA4, SPA5, SPA6, and SPA7, which corresponded to all the other variants found for the IE1315–324 epitope. The SPA1 peptide control was for the three clones the best stimulator at 0.1 nM concentration (Fig. 5). The SPA5 peptide, which carries E→D substitution at position 5, did not stimulate any of the three T cell clones. SPA7 was not recognized by clone C3, but stimulated C1 and C2 at a concentration of 10^3 nM. All three clones were cytotoxic against cells loaded with 10^5 nM of SPA4 and SPA6, but were differently reactive against lower concentrations of these synthetic peptides.

Table I. Natural mutations in the IE1315–324 epitope

<table>
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<th>Amino Acid Sequence</th>
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<th>BIMAS Score</th>
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</tr>
<tr>
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</tr>
<tr>
<td>YVLETSSL</td>
<td>PA7</td>
<td>130</td>
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* Target residues for natural polymorphism are indicated in boldface.
* Estimate of k_d of dissociation (min) from the HLA-A*0201 allele (19).
* Original clinical strain and Towne strain sequences.
* AD169 strain sequence.

FIGURE 1. Cytotoxic responses of C1, C2, and C3 HLA-A*0201-restricted anti-IE1 T cell clones against autologous BLCL pulsed or not with IE1315–324 (YLEETSVM), IE1316–324 (YILEETSVM), or IE1316–324 (ILEETSVM) synthetic peptides at 10^4 nM. Their relative antigenic activity was measured by a standard 5^1Cr release assay at a 20:1 E:T ratio.
were always lower as compared with the other clones. Similar results were obtained when U373 MG were used as target cells (data not shown).

**Discussion**

The four CTL clones used in this study corresponded to the whole HLA-A*0201-restricted anti-IE1 repertoire that was sorted out from PBMCs of a healthy HCMV+ individual, and one of them was immunodominant (17). We first compared the specific cytolytic activity of these CTL clones against the IE1_315–324 (YILEETSVML) decapeptide and its two internal IE1_315–323 and IE1_316–324 nonapeptides. The three synthetic peptides were designed on the sequence of a recombinant clinical variant of IE1 that has been previously used as a target to generate the four clones (17). All clones were found cross-reactive against the three peptides in standard cytotoxic assays (i.e., using HLA-A*0201 target

![Figure 2](image-url) **FIGURE 2.** Cytotoxic responses of C1, C2, C3, and C4 HLA-A*0201-restricted anti-IE1 T cell clones against autologous BLCL pulsed with various concentrations of SPA1 (YILEETSVML), SPA2 (YVLEETSVML), or SPA3 (YILEETSVLM) synthetic peptides. Relative antigenic activities of target cells were measured by a standard 51Cr release assay at a 20:1 E:T ratio.

![Figure 3](image-url) **FIGURE 3.** Biologic responses of C1, C2, C3, and C4 HLA-A*0201-restricted anti-IE1 T cell clones to autologous BLCL loaded with 10^4 nM of SPA1 (YILEETSVML), SPA2 (YVLEETSVML), or SPA3 (YILEETSVLM) synthetic peptides or of an irrelevant peptide IP. A, Proliferation response measured by [3H]thymidine labeling assay; B, intracellular expression of IL-2; and C, intracellular expression of IFN-γ. Intracytoplasmic IL-2 and IFN-γ were stained, respectively, with PE-conjugated anti-human IL-2 and FITC-conjugated anti-human IFN-γ mAbs after treatment of T cells with brefeldin A and permeabilization with 0.1% saponin. Cytokine production results are expressed as percentages of labeled cells.

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<th>Origin</th>
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<td>9 (9%)</td>
<td>4 (4%)</td>
<td>1 (1%)</td>
<td>14 (13.5%)</td>
<td>3 (3%)</td>
</tr>
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</table>

Table II. Frequencies of IE1_{315–324} mutant epitopes in 103 HCMV clinical isolates.
As their responses were always stronger against IE1315–324, we chose to use the decamer for additional experiments. These results are not entirely consistent with recently published data showing very low levels of cross-reactivity against the two nonamers, IE1315–323 and IE1316–324, in ELISPOT responses and unsuccessful attempts to stain IE1315–323-specific clones with IE1316–324-HLA peptide tetramers (22). As we have observed that our clones did not kill efficiently target cells loaded with the IE1315–323 nonamer at concentrations lower than 10^4 nM (data not shown), this discrepancy was probably a consequence of differences in methodologies. Another possible explanation is that peptides used in our study carried an I3V substitution at residue IE1316.

Seven IE1315–324 natural variants were characterized by sequencing the DNA region corresponding to this epitope in 103 clinical isolates from different origins. Natural mutations at P2 (I or V), P9 (M or L), and P10 (L or M) of the decapeptide did not abrogate activity of the C2 immunodominant clone. All three other clones were differently sensitive to several combinations of mutations targeted to these three positions. The modulation of their cytotoxic response against target cells expressing recombinant IE1-1, IE1-2, and IE1-3 variants was consistent with those induced by cells loaded with the corresponding SPA1, SPA2, and SPA3 peptides. Taken together, our data show that these natural residue exchanges, which were targeted at P2 and P10 anchor positions in the IE1315–324 decapeptide, did not alter intracellular processing and/or presentation of the Ag. The E→D substitution at P5 was observed in only 1 of our 103 clinical isolates. No clone was found cytotoxic against the SPA5 peptide that carried a D at P5, whereas all clones were stimulated by 10^4 nM of SPA6, which carried an E at P5, but was identical with SPA5 at all other positions. Although loss of MHC binding of SPA5 cannot be excluded, our data strongly suggest that the E→D substitution affected a key TCR-contacting residue. This observation is consistent with crystallographic data showing that in an HLA-A2 molecule, the P5 residue of a viral peptide is bound in a deep pocket at the center of the TCR in which the CDR3 loops converge (23).

Highly focused memory CTL responses associated with a very limited repertoire recognizing the same MHC/peptide complex have been observed by several authors in CMV infection. Data obtained from mice in the H-2^d haplotype showed that the memory T lymphocyte response to the virus is highly focused against dominant antigenic peptides from genes m123 (encoding IE1, pp89) and m164, with very minor contribution of CD8+/H11001 CTLs specific for peptides encoded by m04, m18, M83, and M84. In contrast, a broader immune response to acute CMV infection was observed in draining lymph nodes and in the lung (24–26). In humans, a clonal or an oligoclonal repertoire of CD8+/H11001 CTLs recognizing the same MHC/peptide complex has been described in healthy virus carriers as well as for pp65 (27) and IE1 (17, 22). Oligoclonal amplifications of CMV-specific CTLs can represent up to one-quarter of the

![FIGURE 4](image_url) Cytotoxic responses of C1, C2, and C3 HLA-A*0201-restricted anti-IE1 T cell clones against autologous BLCL stably expressing the recombinant protein IE1-1 (BLCL/IE1-1) and against U373 MG cells stably expressing the recombinant proteins IE1-1 (U373 MG/IE1-1), IE1-2 (U373 MG/IE1-2), or IE1-3 (U373 MG/IE1-3), which carried, respectively, YILEETSVML, YVLEETSVML, and YILEETSVLM peptide sequences at position 315–324. Relative antigenic activities of target cells were measured by a standard 51Cr release assay. Assays were performed with various E:T ratios.

![FIGURE 5](image_url) Cytotoxic responses of C1, C2, and C3 HLA-A*0201-restricted anti-IE1 T cell clones against autologous BLCL loaded with various concentrations of SPA1 (YILEETSVML), SPA4 (YILEETSVM), SPA5 (YILEDTSVLL), SPA6 (YILEETSVLL), and SPA7 (YVLEETS VLL) mutant peptides. Their relative antigenic activity was measured by a standard 51Cr release assay at a 20:1 E:T ratio.
total CD8+ T cell population in healthy elderly individuals (28). Conversely, the case of a patient with primary HIV-1, EBV, and CMV coinfections was reported in which the immune system mounted CTL responses to multiple viral Ags simultaneously, albeit with different strengths. Memory CTLs for IE1 Ag were detected by limiting dilution analysis during the first week of anti-viral therapy, and after a delay of 1-2 wk, CTLs directed against other structural components (gB, pp65, pp150) reached peak levels (29). Such a narrowing of the memory T cell repertoire is generally observed in successive infections with homologous or heterologous viruses as well as in persistent infections associated with sporadic reactivations of a latent virus (for a review, see Ref. 30). There are data suggesting that HCMV could present a considerable and recurrent burden to the immune system (31), which is periodically boosted by a low level of HCMV replication (32). This virus is now considered as a major factor in driving oligoclonal expansions in elderly individuals (28).

Healthy people are frequently concomitantly infected with multiple HCMV strains (33), and consequently, their immune system must ensure a protection against homologous viruses carrying polymorphism in their antigenic proteins. In persistent infection, the T cell response against a single epitope could theoretically be protective against mutations or re-infection by variant strains if a control is exercised by polyclonal T lymphocyte response with strict specificity for each peptidic variant, or by amplification of a cross-reactive oligoclonal or clonal population. Our results strongly suggest that an efficient control of >99% of HCMV variants could be exerted by broadly cross-reactive immunodominant T cell clones specific of the IE1315-324 epitope in the HLA-A*0201 context. These results are consistent with observations made with other viral infections. In individuals with persistent hepatitis B virus infection, the control of emergence of viral mutations in a given epitope was not found exerted by specific CTLs with heterogeneous TCR, but by preferentially amplified cross-reactive T cells (34). Similarly, in a mouse model of successive infections with a series of natural variants of influenza A, the polyclonal T cell response against variants was completely dominated by infrequent cross-reactive T cells that expanded from an original memory population (35). During a primary infection of lymphocytic choriomeningitis virus, the polyclonal memory T cell repertoire depends on prior Ag encounters, and can provide protection against a range of antigenic variants. Viruses bearing epitope variants re-stimulate the cross-reactive memory repertoire rather than a T cell population specific for new variants in a subsequent infection (36).

Mutations that were found in the IE1315-324 peptide sequence of the clinical isolates did not correspond to synonymous substitutions, but to a variety of replacements in which residues at positions 316, 323, and 324 of IE1 were very significantly associated (p < 0.5% using Fisher’s exact test). These data strongly suggest a viral selection process that may be due to differences in the functional characteristics and/or immunogenicity of IE1 variants. Several regulatory activities have been described for IE1, which depends a kinase activity residing in the exon 4 encoded region (37) and interacts with viral and cellular factors to regulate expression of HCMV and host genes (for a review, see Ref. 38). Therefore, the selection of replacements that were observed in IE1315-324 amino acid sequence should be driven by functional constraints. Although our results showing that anti-IE1 HLA-A*0201-restricted CTLs from donor C were able to kill target cells presenting most of the IE1315-324 variant epitopes do not argue for immunological selection, such a pressure may exist at the population level. This study is the first to investigate the impact of IE1 polymorphism on CTL response, and we cannot exclude that mutations in IE1315-324 provide selective HCMV escape from CTL-mediated killing in immunosuppressed patients via alteration of overlapping epitope recognition in different HLA contexts.

In a recent study that used mathematical modeling approach, authors calculated that HCMV could be eradicated by the preventive immunization of 66--75% of the human population with a putative vaccine (39). Until now, all candidate vaccines have been developed on the basis of antigenic properties of HCMV laboratory strains (40). The existence of a natural polymorphism in HCMV antigenic proteins raises questions about its consequences on the establishment of a protective immunity against natural strains. Theoretically, a good peptide candidate for inclusion in an antiviral vaccine strategy should be located in conserved protein regions. However, our data suggest that the IE1315-324 epitope, despite its polymorphism, can be a good candidate Ag to induce protective responses in HLA-A*0201 individuals against ~99% of the natural HCMV isolates.

References


