Classical and Nonclassical Class I Major Histocompatibility Complex Molecules Exhibit Subtle Conformational Differences That Affect Binding to CD8αα*

Received for publication, January 18, 2000

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The cell surface molecules CD4 and CD8 greatly enhance the sensitivity of T-cell antigen recognition, acting as “co-receptors” by binding to the same major histocompatibility complex (MHC) molecules as the T-cell receptor (TCR). Here we use surface plasmon resonance to study the binding of CD8αα to class I MHC molecules. CD8αα bound the classical MHC molecules HLA-A*0201, -A*1101, -B*3501, and -C*0702 with dissociation constants (K_d) of 90–220 μM, a range of affinities distinctly lower than that of TCR/peptide-MHC interaction. We suggest such affinities apply to most CD8αα/classical class I MHC interactions and may be optimal for T-cell recognition. In contrast, CD8αα bound both HLA-A*6801 and B*4801 with a significantly lower affinity (≥1 mM), consistent with the finding that interactions with these alleles are unable to mediate cell-cell adhesion. Interestingly, CD8αα bound normally to the nonclassical MHC molecule HLA-G (K_d ∼150 μM), but only weakly to the natural killer cell receptor ligand HLA-E (K_d ≥ 1 mM). Site-directed mutagenesis experiments revealed that variation in CD8αα binding affinity can be explained by amino acid differences within the α3 domain. Taken together with crystallographic studies, these results indicate that subtle conformational changes in the solvent exposed α3 domain loop (residues 223–229) can account for the differential ability of both classical and nonclassical class I MHC molecules to bind CD8.

Cytotoxic T lymphocytes (CTL)1 expressing the cell surface glycoprotein CD8 play an important role in immune protection against intracellular pathogens such as viruses. CD8 greatly enhances antigen recognition by CTL (1) and is referred to as a co-receptor, since it binds to the same peptide-major histocompatibility complex class I molecules as the T-cell receptor (TCR) (1). Generation of mature CTL also depends upon the presence of CD8 at their surface (2) and upon the interaction of CD8 with MHC (3, 4). CD8 exists at the cell surface as a homodimer of two α chains (CD8αα) and as a heterodimer or an α and a β chain (CD8αβ). Expression of the homodimeric form of CD8 is limited to the αβ T-cell population, whereas homodimeric CD8αα is present not only on αβ T-cells but also on subsets of γδ T cells and natural killer (NK) cells (5).

Direct binding of CD8αα to classical MHC alleles was initially demonstrated using cell-cell adhesion assays (6). Mutagenesis data suggested that CD8 and the TCR bind to separate sites on the MHC molecule (7). Recently, crystal structures of human and murine CD8αα-MHC complexes (8, 9) have shown that CD8αα binds to an extended site on classical MHC molecules, which includes nonpolymorphic residues from the α2 and α3 domain as well as β2-microglobulin. The two CD8α subunits contact the α3 domain in a manner resembling antibody-antigen interaction, with their complementarity determining region-like loops forming a pocket which accommodates an exposed loop (residues 223–229) linking the C and D strands. This mode of interaction prevents the simultaneous binding of a second MHC molecule to CD8αα. Furthermore, the crystal structure also indicates that the binding of CD8αα does not induce any significant changes in the TCR-binding platform of the MHC. We have recently studied the interaction of soluble forms of these molecules by surface plasmon resonance (10). Despite the large binding interface (total surface area buried upon binding ∼2000 Å²), CD8αα binds HLA-A2 with an extremely low affinity (K_d ∼200 μM). In agreement with the structural studies, binding of CD8αα to HLA-A2-peptide did not affect the binding of a TCR to the same peptide-MHC complex (10).

To investigate further the mechanism of co-receptor function, we have determined the extent to which the affinity of CD8αα/class I MHC interactions varies, by analyzing the binding of CD8αα to HLA-A, -B, and -C molecules. The genes that encode these classical MHC molecules are the most polymorphic human genes described (11). While CD8αα can bind to most classical MHC alleles, there is evidence that some alleles, such as HLA-A68 and -B48, possess polymorphisms in the α3 domain, which make them especially poor ligands for the co-
receptor (12, 13). Therefore CD8αα binding to a number of different alleles, including HLA-A68 and -B48, was tested. Also, since our understanding of the interaction of CD8 with nonclassical MHC molecules is limited, the binding of CD8αα to the nonclassical class I MHC molecules HLA-E and HLA-G was investigated. Site-directed mutagenesis was used to establish the molecular basis of the observed differences in CD8αα binding.

EXPERIMENTAL PROCEDURES

Preparation of Soluble CD8αα—The extracellular fragment of soluble CD8αα (residues 1–120) was expressed in Escherichia coli, refolded, and purified as described previously (14). The majority of the protein (>90%) was correctly folded, as determined by mAb binding (10).

Expression of HLA Heavy Chains—The extracellular portion (residues 1–276) of both classical and nonclassical HLA class I molecules were expressed in E. coli BL21pLysS(DE3) strain using the T7 promoter based vectors pET23d (Novagen) or pGMT7 (15). Expression of HLA-A*02, -B*35, and -C*07 and HLA-E heavy chains has been reported previously (16–18). HLA-A*11, -A*6801, and -B*4801 heavy chains were cloned into pET23d using the unique restriction sites NcoI/HindIII. HLA-G heavy chains were cloned into pGMT7 with the unique restriction sites NdeI/HindIII. HLA-G heavy chains were purified from inclusion bodies and solubilized in denaturants as described previously (16).

Preparation of Biotinylated HLA Class I Complexes—Biotinylated soluble HLA class I complexes were prepared as described previously (10, 19, 20). Briefly, β2-microglobulin was refolded and then chemically biotinylated using hydroxysuccinimido-biotin (Sigma, Gillingham, UK). HLA complexes were then prepared by refolding the relevant HLA heavy chain and peptide with the chemically biotinylated β2-microglobulin in a standard manner (16). HLA-peptide complexes were subsequently purified by size exclusion using a Superdex 75 column (Amerham Pharmacia Biotech, Uppsala, Sweden). The elutionvolume of each complex was similar to that of the 43 kDa molecular mass standard ovalbumin.

The class I-binding peptides used in this study were synthesized by Genosys Biotechnologies (Cambridge, UK) and are described below. For HLA-A*0201, ILKPEFHGV (derived from human immunodeficiency virus, type 1 polymerase); for HLA-A*1101, AIFQSSMTK (human immunodeficiency virus, type 1 polymerase); for HLA-A*6801, KTG-

FIG. 1. The affinity of CD8αα binding to HLA-A11, B35, and C7. A, top panel, CD8αα was injected at increasing concentrations (from 7 to 930 μM) over flow cells with either HLA-A*1101 (~2100 RU, solid trace) or the control protein OX68 (~2800 RU, dotted trace) immobilized. Binding to HLA-A*1101 at each concentration was calculated as the difference between the responses at equilibrium in the HLA-A*1101 and control flow cells and is plotted in the lower panel. Lower panel, the solid line represents a nonlinear fit of the Langmuir binding isotherm to the specific binding responses (solid squares). Inset, a Scatchard transformation of the same data; the Kd was obtained from the slope by linear regression (Kd = 1/slope). B and C, similar experiments were performed using flow cells with HLA-B*2501 (~3800 RU, solid trace) and HLA-C*0702 (~3500 RU, solid trace) immobilized, respectively. In each case immobilized OX68 (~4400 RU, dotted traces) was used as a control protein.
Characterization of CD8αα binding affinity to HLA-A, B, and C alleles.

**RESULTS AND DISCUSSION**

Classical HLA-A, -B, and -C Alleles Define a Standard CD8αα Binding Affinity—We used SPR to directly measure the affinity of CD8αα binding to HLA-A*1101, HLA-B*3501, and HLA-C*0702 alleles. Equilibrium affinity measurements (see Fig. 1) were carried out by injecting soluble recombinant CD8αα (10, 14) over sensor chip surfaces coated with particular peptide-MHC complexes, or a control protein, as described under "Experimental Procedures." The difference in the responses obtained in the immobilized peptide-MHC and control surfaces represented specific binding to the peptide-MHC. The equilibrium dissociation constant ($K_d$) was measured by repeating such injections over a range of CD8αα concentrations. As shown in Fig. 1, CD8αα bound to HLA-A*1101, HLA-B*3501 and HLA-C*0702 with affinities ranging from 90 to 220 μM. These compare with an affinity of 140 μM, which was previously determined for the CD8αα/HLA-A*0201 interaction.

**TABLE I**

Summary of CD8αα/HLA affinity measurements

| HLA   | $K_d$ μM |
|-------|----------|
| A*0201 | 130 ± 30 (n = 7) |
| A*1101 | 100 ± 10 (n = 3) |
| B*3501 | 130 ± 10 (n = 2) |
| C*0702 | 220 ± 20 (n = 2) |
| A*6801 | 1000 ± 500 (n = 3) |
| B*4801 | 1000 ± 500 (n = 3) |
| G     | 160 ± 3 (n = 2) |
| E     | ≥1000 (n = 2) |
| α3 mutants |         |
| A2-245AV | 500 (n = 1) |
| A69-245VA | 110 (n = 1) |
| A2-254AT | 470 (n = 1) |
| B48-245TA | 120 (n = 1) |
| EloopA | 160 ± 6 (n = 2) |
| AloopE | ≥1000 (n = 1) |

* Mean ± S.D. or, for n = 2, mean ± range of n independent determinations.

**FIG. 2.** The affinity of CD8αα binding to HLA-A68, A68-V245A, HLA-B48, and B48-T245A. A, main panel, CD8αα was injected at the concentrations indicated, over flow cells with either HLA-A*6801 (~3500 RU, solid trace) or the control protein OX68 (~4900 RU, dotted trace) immobilized. Specific binding responses were calculated as in Fig. 1 and are plotted in the inset. Inset, the solid line represents a nonlinear fit of the Langmuir binding isotherm to the data. B–D, similar experiments were performed using flow cells with A68-V245A (~3900 RU, solid trace), HLA-B48 (~2600 RU, solid trace), or B48-T245A (~4000 RU, solid trace) immobilized, respectively. In each experiment, immobilized OX68 (~4600 RU for B and D, ~3200 RU for C, dotted traces) was used as a control protein.
Collectively, the exact α3 domain sequences of these molecules are representative of a large number of HLA alleles, and it is, therefore, likely that CD8αα will bind to the great majority of HLA alleles with affinities in this range ($K_d, 90-220 \mu M$ at 25 °C).

**HLA-A*6801 and HLA-B*4801 Bind CD8αα with a Severely Reduced Affinity**—As shown in Fig. 2, A and C, CD8αα bound to HLA-A*6801 and HLA-B*4801 with a significantly lower affinity than the alleles described above. Despite the injection of CD8αα at high concentrations ($\approx 1800 \mu M$), binding did not reach saturation, making precise estimates of affinities difficult. However, the affinities of the HLA-A*6801 and HLA-B*4801 interactions were both $\approx 1 \text{mM}$ (see Table I). Previously reported cell binding experiments and mutational studies have indicated that for both alleles; the polymorphism at position 245 accounts for these reduced affinities (12, 13). We sought to confirm this by mutating 245 to the consensus Ala and testing whether affinity was increased. As can be seen in Fig. 2, B and D, CD8αα bound to the two mutant proteins A245-T245 and B245-T245 with affinities comparable with HLA-A*0201. This is strong evidence confirming that polymorphism at residue 245 is indeed responsible for the low affinity of CD8αα binding to HLA-A*6801 and HLA-B*4801. In support of this, introduction of reciprocal mutations to Val or Thr at position 245 of both molecules confirmed that this lower affinity is due to polymorphism at residue 245 (see Table I). Previously reported cell binding experiments and mutational studies have indicated that for both alleles; the polymorphism at position 245 accounts for these reduced affinities (12, 13). We sought to confirm this by mutating 245 to the consensus Ala and testing whether affinity was increased. As can be seen in Fig. 2, B and D, CD8αα bound to the two mutant proteins A245-T245 and B245-T245 with affinities comparable with HLA-A*0201. This is strong evidence confirming that polymorphism at residue 245 is indeed responsible for the low affinity of CD8αα binding to HLA-A*6801 and HLA-B*4801. In support of this, introduction of reciprocal mutations to Val or Thr at position 245 of both molecules confirmed that this lower affinity is due to polymorphism at residue 245 (see Table I). Previously reported cell binding experiments and mutational studies have indicated that for both alleles; the polymorphism at position 245 accounts for these reduced affinities (12, 13). We sought to confirm this by mutating 245 to the consensus Ala and testing whether affinity was increased. As can be seen in Fig. 2, B and D, CD8αα bound to the two mutant proteins A245-T245 and B245-T245 with affinities comparable with HLA-A*0201. This is strong evidence confirming that polymorphism at residue 245 is indeed responsible for the low affinity of CD8αα binding to HLA-A*6801 and HLA-B*4801. In support of this, introduction of reciprocal mutations to Val or Thr at position 245 of both molecules confirmed that this lower affinity is due to polymorphism at residue 245 (see Table I). Previously reported cell binding experiments and mutational studies have indicated that for both alleles; the polymorphism at position 245 accounts for these reduced affinities (12, 13). We sought to confirm this by mutating 245 to the consensus Ala and testing whether affinity was increased. As can be seen in Fig. 2, B and D, CD8αα bound to the two mutant proteins A245-T245 and B245-T245 with affinities comparable with HLA-A*0201. This is strong evidence confirming that polymorphism at residue 245 is indeed responsible for the low affinity of CD8αα binding to HLA-A*6801 and HLA-B*4801. In support of this, introduction of reciprocal mutations to Val or Thr at position 245 of both molecules confirmed that this lower affinity is due to polymorphism at residue 245 (see Table I). Previously reported cell binding experiments and mutational studies have indicated that for both alleles; the polymorphism at position 245 accounts for these reduced affinities (12, 13). We sought to confirm this by mutating 245 to the consensus Ala and testing whether affinity was increased. As can be seen in Fig. 2, B and D, CD8αα bound to the two mutant proteins A245-T245 and B245-T245 with affinities comparable with HLA-A*0201. This is strong evidence confirming that polymorphism at residue 245 is indeed responsible for the low affinity of CD8αα binding to HLA-A*6801 and HLA-B*4801. In support of this, introduction of reciprocal mutations to Val or Thr at position 245 of both molecules confirmed that this lower affinity is due to polymorphism at residue 245 (see Table I). Previously reported cell binding experiments and mutational studies have indicated that for both alleles; the polymorphism at position 245 accounts for these reduced affinities (12, 13). 

**The Nonclassical MHC Molecules HLA-E and HLA-G Have Different Affinities for CD8αα—**HLA-E and HLA-G are similar to classical MHC molecules in terms of homology, secondary structure, and association with β2-microglobulin, but exhibit only very limited polymorphism (21). Within the α3 domain, both molecules contain differences from the consensus sequence of classical HLA molecules. As can be seen in Fig. 4, these occur at positions 219, 223, 224 in the HLA-E sequence and 214 and 228 in the HLA-G sequence. All of these changes are located extremely close to the HLA/CD8αα interface in or around the 223–229 loop (see Figs. 4 and 5) and could potentially affect binding to CD8αα.

Despite the presence of such amino acid differences, CD8αα bound HLA-G (see Fig. 3A) with an affinity ($K_d, \approx 150 \mu M$) comparable with that of CD8αα/HLA-A*0201 interaction. In contrast, HLA-E bound poorly to CD8αα (Fig. 3B), and even at high concentrations ($> 800 \mu M$) binding responses were small and did not reach saturation. The affinity of CD8αα for HLA-E was estimated to be $\approx 1 \text{mM}$ (see Table I), a figure comparable with interaction with HLA-A*6801 and -B*4801.

**Conformational Changes in the α3 Domain 223–229 Loop Can Account for Variation in CD8αα Binding—**Direct interaction of CD8αα with the 223–229 loop of the HLA α3 domain was predicted from mutagenesis studies (7). Similar studies also indicated that polymorphism at position 245 in the α3 domain of HLA-A*68 resulted in a loss of CD8αα-mediated cell-cell adhesion (12). The crystal structure of an HLA-A*02/CD8αα complex confirmed that this loop lies at the center of the interface, making direct contacts to both CD8αα subunits (8). Comparison of HLA-A*6801 and HLA-A*0201 structures showed that despite the similarity in the overall fold of the molecules, the introduction of a bulky Val side chain at position 245 in HLA-A*68 causes a steric conflict with Thr228, triggering a small but significant distortion of the 223–229 loop in the α3 domain (8) (see Fig. 6). This distortion is sufficient to reduce binding to CD8αα severely.

Our data show that CD8αα also binds HLA-B*4801 with an affinity similar to that of the CD8αα/HLA-A*6801 interaction and confirms that this lower affinity is due to polymorphism at residue 245 (see Fig. 2). The two amino acids implicated, Val (HLA-A*6801) and Thr (B*4801), have similar-sized side chains, and this would suggest that in HLA-B*4801, steric conflict between Thr224 and Thr228 is likely to induce structural perturbation of the 223–229 loop via a similar mechanism to that operating in HLA-A*6801. It has been suggested that B*8101, which like B*4801 has a Thr at position 245, will also have a reduced affinity for CD8αα (13).
HLA-E bound CD8α with an affinity far lower than the majority of classical HLA alleles (>1 mM, see Fig. 3). While the overall conformation of HLA-E is similar to HLA-A*0201 (22), detailed comparisons of the HLA α3 domains indicate that the 223–229 loop adopts a conformation distinct from that of HLA-A2 and similar to that of HLA-A68 (Fig. 6). A probable cause of this is subtle adjustments in the packing of the 219 and 224 side chains against conserved core residues of the α3 domain centered on Tyr257. In contrast, the Thr to Val change at 228 in the HLA-G sequence lies directly within the loop (see Fig. 5). However, in HLA-A2 this side chain does not interact directly with CD8. Also, both Thr and Val are of a similar size, and the substitution of a γ oxygen for a β carbon atom would appear unlikely to perturb the structure of the α3 domain.

**CD8αα Interactions with Classical MHC Molecules**—One critical feature of CD8+ function in both thymic selection and peripheral activation of T-cells is a requirement that CD8 and TCR bind to the same MHC molecule (4, 23–25). This raises the question of how CD8/MHC interaction is able to increase the sensitivity of antigen recognition, and contribute to thymic selection events, without compromising specificity. To resolve this question it is important to know the physical properties of CD8/MHC interaction and how they relate to TCR/MHC binding.

Our results suggest that most HLA-A, -B, and -C alleles are likely to interact similarly with CD8αα. These interactions are characterized by a fairly narrow range of affinities (90–220 μM), lower than typical adhesion molecule interactions (e.g. $K_d$).
CD8αα Interactions with Class I MHC Molecules

Fig. 5. Amino acid differences in the α3 domain of HLA-E and HLA-G. Main panel, the α3 domain of the HLA-A*0201 class I heavy chain is shaded blue, with the 223–229 loop highlighted in yellow. The amino acid differences in HLA-E and HLA-G documented in Fig. 4 are shown as red and green spheres, respectively. Both molecules have changes at positions 268 (to Glu) and 275 (to Lys), which are shown in magenta. Inset, the structure of CD8αα complexed with HLA-A2 (adapted from Gao et al. (8)). HLA-A2 heavy chain is shown in blue and β2-microglobulin and antigenic peptide in gray. CD8αα is shown in cyan. The pink box outlines the α3 domain shown in the main panel. The figure was prepared using the programs BOBSCRIPT (35) and RASER3D (36).

Fig. 6. Conformational differences in the 223–229 loop region of class I HLA molecules. Superimpositions of the α3 domains of HLA-B*3501 (green), A*6801 (red), and E (yellow) onto that of HLA-A*0201 (blue) in complex with CD8αα (cyan). Most Cα atoms superimpose closely, but there are conformational differences in the 223–229 loop. Whereas the HLA-B*35 loop adopts a conformation similar to that of HLA-A*0201, that of both HLA-A*6801 (8) and HLA-E adopt distinct conformations. Superimpositions were carried out using SHP (37). The figure was drawn using BOBSCRIPT (35) and RASER3D (36).

CD2–CD48, 30–50 μM at 25 °C. The standard CD8αα/HLA interaction is also considerably weaker than TCR/peptide-MHC interaction (1–50 μM at 25 °C). Although this study has been focused upon the affinity of CD8αα/HLA interactions, it is highly likely that the extremely fast dissociation kinetics measured for HLA-A*02/CD8αα engagement (10) are typical of CD8αα interactions with other class I MHC molecules. Consequently, when both TCR and CD8αα, which may be physically associated, engage the same peptide-MHC complex during antigen recognition, the binding is likely to be dominated by the TCR, which imparts antigen specificity. In comparison, CD8αα/MHC interaction is both weaker, and remarkably short-lived, with CD8αα-MHC complexes up to 100-fold less stable than TCR-MHC complexes (10). Furthermore, the low solution affinity of CD8/class I MHC interaction suggests that in general, significant engagement only occurs following recruitment of CD8 into the TCR-CD3 complex, as suggested previously (10).

This study establishes that HLA-A*6801 and HLA-B*4801 are able to bind CD8αα, albeit with affinities that are markedly lower (≥1 mM) than the majority of other classical alleles. This is consistent with cell binding experiments, which have shown that interaction of CD8αα with either allele is insufficient to mediate cell-cell adhesion (12, 13). Furthermore, our measurements confirmed the conclusion of these studies that polymorphism at position 245 is responsible for the lower affinities. Our data are also consistent with the finding that killing of target cells by HLA-A*6801-restricted CTL is functionally CD8-independent (26). One question that this observation prompted was how such CTL are positively selected in the thymus in the absence of CD8/HLA-A*6801 engagement. Our measurements suggest a possible explanation, that the relatively lower affinity of HLA-A68 for CD8αα, despite being too weak to enhance killing by mature CTL, may be sufficient to affect positive selection of such CD8+ cells in the thymus. One idea, which is currently being investigated, is whether such cells compensate for suboptimal CD8/HLA-A68 interactions by selecting TCRs that bind with a proportionally higher affinity to HLA-A68.

Unlike CTL recognition of HLA-A*6801, alloreactive recognition of HLA-B*4801 by CTL has been shown to be CD8-dependent (13). This is surprising since CD8αα binds each of these alleles with an affinity ≥1 mM. However, because of the difficulties associated with measuring such low affinity interactions, it is possible that CD8αα binds significantly stronger to HLA-B*4801 than to HLA-A*6801. A different explanation (13) of this discrepancy is that alloreactive CTL recognition differs from conventional CTL recognition. Alternatively, it could be that HLA-B*4801-restricted CTL killing is enhanced by CD8αβ, but not CD8αα, and HLA-A*6801-restricted killing enhanced by neither. This might imply that HLA-B*4801 binds preferentially to CD8αβ, as suggested previously (13), and that HLA-A*6801 binds significantly to neither. If so, this would predict that preferential binding to CD8αβ could be mediated by small shifts in the α3 domain loop and that the shifts induced by the polymorphism at residue 245 in HLA-B*4801 and HLA-A*6801 are subtly, but significantly, different.

CD8αα Interactions with HLA-E and HLA-G—Complexes of HLA-E bound to the conserved leader peptides of a range of class I MHC molecules (including that of HLA-G) are known to mediate protection from lysis by NK cells, by interacting directly with inhibitory CD94/NKG2A or B receptors on their cell surface (27). Most NK cells do not express CD8 at their cell surface. Consequently, our finding that HLA-E binds CD8αα extremely weakly due to local conformational differences in the α3 domain presumably reflects the fact that HLA-E recognition by CD56+ CD8− NK cells does not require CD8.

HLA-G is mainly expressed in the placenta, but also in a number of other tissues, including the thymus. Its tissue distribution within the placenta is consistent with a role in the interface with the maternal immune system (21). It is expressed at a time when classical class I molecules are largely absent from the placenta. The expression of HLA-G has been
shown to play a role in inhibiting lysis of target cells by NK cells derived from placenta (28, 29), including clones that bear CD94/NKG2 receptors at their cell surface (30).

Since studies have shown that HLA-G is competent to present a range of peptides of intracellular origin in a manner similar to classical class I molecules (31, 32), HLA-G may therefore have the potential to play a role in protecting the fetus from viral infection by presenting viral peptides on infected placental tissue to maternal CTL during a period when classical HLA molecules are not expressed on the placenta. Consistent with this hypothesis is the fact that HLA-G is expressed in thymic epithelial cells and could therefore induce tolerance to the maternal repertoire. Furthermore, since HLA-G has a limited polymorphism, alloreactive maternal T-cell responses directed toward placental HLA-G would be unlikely.

Our results show that HLA-G is able to bind CD8αα with an affinity (150 μM), which is in the middle of the range of classical HLA/CD8αα affinities we have measured (90–220 μM). These measurements are consistent with experiments that indicate that HLA-G is able to mediate cell-cell adhesion by interacting with CD8αα (33). Therefore it is possible that HLA-G/CD8 interaction may mediate the positive and negative selection of class I-restricted CTL and facilitate HLA-G-restricted antigen recognition by such cells, in a manner similar to conventional classical class I-restricted CTL (3, 4, 34).

Acknowledgments—We thank Linda Barber, David Allen, Liz Davies, Gavin Wright, Marion Brown, Pokrath Hansausta, David Bainbridge, Shirley Ellis, Graham Ogg, Veronique Braud, and Andrew McMichael for generously providing materials and valuable advice.

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J. Biol. Chem. 2000, 275:15232-15238.
doi: 10.1074/jbc.275.20.15232

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