The Recognition of the Nonclassical Major Histocompatibility Complex (MHC) Class I Molecule, T10, by the γδ T Cell, G8

By Michael P. Crowley,* Ziv Reich,‡ Nasim Mavaddat,§ John D. Altman,§ and Yueh-hsiu Chien*§

*From the *Program in Immunology; ‡The Howard Hughes Medical Institute; and the §Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California 94305*

Summary

Recent studies have shown that many nonclassical major histocompatibility complex (MHC) (class Ib) molecules have distinct antigen-binding capabilities, including the binding of nonpeptide moieties and the binding of peptides that are different from those bound to classical MHC molecules. Here, we show that one of the H-2T region–encoded molecules, T10, when produced in *Escherichia coli*, can be folded in vitro with β_2-microglobulin (β_2m) to form a stable heterodimer in the absence of peptide or nonpeptide moieties. This heterodimer can be recognized by specific antibodies and is stimulatory to the γδ T cell clone, G8. Circular dichroism analysis indicates that T10/β_2m has structural features distinct from those of classical MHC class I molecules. These results suggest a new way for MHC-like molecules to adopt a peptide-free structure and to function in the immune system.

Classical MHC class I (class Ia) molecules possess a highly specialized groove occupied by short peptides that are acquired inside the cell during MHC heterodimer assembly. This peptide–MHC interaction not only contributes to the stability of the heterodimer on the cell surface, but forms the basis for its function, as complexes of intracellular pathogen derived peptides with MHC are the ligands for cytolytic αβ T cells. Recently, many nonclassical MHC class I molecules, such as those encoded in the Q, T, M, and CD1 regions, have been found to possess binding properties different from those of classical MHC molecules (as reviewed in references 1 and 2). These studies suggest that class Ib molecules have evolved for specific tasks that are distinct from those of classical class I MHC. For example, M3 and human CD1 have been proposed to play a special role in controlling microbial infection by binding and presenting *N*-formylated peptide and lipid antigens, respectively (3–7). Murine CD1 molecules have been shown to bind hydrophobic peptides and are thought to stimulate regulatory αβ T cells (8, 9). Some Qa molecules were found to bind mixtures of peptides with molecular properties different from those bound to classical MHC molecules (10, 11). Other Qa molecules have been suggested to have roles in the generation of regulatory T cells, as well as the elimination of bacterially infected cells (12, 13).

Although the majority of the cells that respond to class Ib ligands bear the αβ TCR, the H-2T–encoded T10 and the closely related T22 (94% identity) proteins were first identified as the ligands for two γδ T cells, KN6 and G8 (14–16). G8 was generated by immunizing BALB/c nude mice with B10.BR spleen cells (17), whereas KN6 was derived from a C57BL/6 double-negative thymocyte (18). Attempts to derive αβ T cells specific for these molecules, using either cells naturally expressing T10/T22 or transfected with these genes as immunogen, have been unsuccessful (reference 19; Schild, H., and Y.-h. Chien, unpublished data). Analysis of the recognition of T10/T22 by G8 shows it to be clearly different from MHC class I recognition by αβ T cells. In particular, G8 can respond to stimulator cells that lack functional peptide-loading mechanisms for either MHC class I or class II molecules (15, 16, 19). All variations in the ability of different stimulator cells to activate G8 can be attributed solely to the level of T10/T22 surface expression. In addition, G8 is able to respond to T10/T22 expressed on *Drosophila melanogaster* cells, which inherently lack peptide-loading machinery and therefore express MHC molecules that are devoid of peptide (20). Together, these experiments indicated that T10/T22 may not present peptide for its recognition by G8.

In this study, we evaluate directly whether components other than the T10/T22 heavy chain and β_2-microglobulin (β_2m) are necessary for its recognition and structural stability. We find that *Escherichia coli*–produced T10 and β_2m can be folded in vitro in the absence of peptide or nonpeptide moieties. This is in contrast with classical class I MHC molecules, whose folding of *E. coli*–produced heavy chain and β_2m can take place only in the presence of an appropriate peptide (21, 22). The reconstituted T10/β_2m heterodimer

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/04/1223/08 $2.00
Volume 185, Number 7, April 7, 1997 1223–1230

is biochemically homogeneous and can be recognized by specific antibodies and the G8 γδ T cell. The far-UV circular dichroic (CD)[1] spectrum of T10/β_2m is different from that of typical MHC class I molecules. These data suggest that T10 may have evolved to possess distinctive structural features capable of carrying out a specialized function in the immune system.

Materials and Methods

Construction of Expression Vectors. The expression cassettes for T10 and T10/L^d (T10/L^d, which has the α1 and α2 domains of T10 and α3 domain of the murine class I molecule L^d, can be recognized by the L^d α3-specific antibody 28.14.8S and G8. This hybrid gene was constructed previously to monitor cell surface expression of T10 in transfected cells in the absence of a T10/T22 heavy chain–specific antibody; reference 15) were constructed using PCR and the oligonucleotide primers GGAATTCCCATATGGGTTCACACTCGCTTAGG and GCGCAAGCTTTTACCATCTCAGGGTGAGGG containing the underlined EcoRI, NdeI, and HindIII restriction sites, respectively. The NdeI site in the EcoRI–NdeI oligonucleotide provides an ATG start codon and a stop codon TAA is included in the HindIII oligonucleotide to terminate the heavy chain following the α3 domain COOH-terminal tryptophan. The PCR was performed with the Ultma™ polymerase (Perkin Elmer, Norwalk, CT) and the recommended protocol. Amplified DNAs were ligated into pBluescriptKS(+) (Stratagene, La Jolla, CA) as EcoRI–HindIII fragments. Upon verification of the sequences, the heavy chain genes were ligated into pET24a^+ (Novagen, Madison, WI) as NdeI–HindIII fragments and expressed in *E. coli* BL21(DE3)pLysS. Clones containing inserts and producing protein upon induction with isopropyl β-d-thiogalactopyranoside (IPTG) were identified for large culture. The human HLA-A2 and human β_2m expression constructs are described in Garboczi et al. (22). The murine β_2m expression construct is described in Young et al. (23).

Protein Production and Purification. 1 L of Cells transformed with either heavy chain construct was grown to an OD_{600} of 0.3 and induced for 2 h with 1 mM IPTG. The harvested cells were resuspended in 10 ml of 25% sucrose, 50 mM Tris, pH 8.0, 1 mM EDTA, 1 mM PMSF, 1 mM DTT, and lysed at 37°C with 1% Triton X-100 and 1 mg/ml lysozyme (Sigma Chem. Co., St. Louis, MO) followed by freeze/thawing. The lysate was incubated for 30 min at 25°C with 30 mM $MgCl_2$ and 30 μg/ml DNase (DN-25; Sigma) followed by the addition of 50 mM EDTA. Inclusion bodies were collected by centrifugation and washed 4–6 times with 20 ml of wash buffer containing 0.5% Triton X-100, 50 mM Tris, pH 8.0, 100 mM NaCl, and 0.1% NaN_3. After two final washes in 20 ml of 50 mM Tris, pH 8.0, 100 mM NaCl, the inclusion bodies were solubilized in 6 M guanidine–HCl, 100 mM Tris, pH 8.0, 1 mM EDTA. HLA-A2, human β_2m (hβ_2m), and murine β_2m (mβ_2m) were expressed and inclusion bodies isolated as described (22, 23). Subunits for the T10/hβ_2m folding were size-purified on a Superdex 200 column (Pharmacia, Uppsala, Sweden) in the presence of 6 M urea. Before folding, 0.3 M DTT was added to all subunits.

Folding and Purification of the Heavy Chain–β_2m Complexes. Folding of the heterodimer was initiated by a 100-fold dilution of subunits into 1 L of nitrogen-saturated folding solution: 100 mM Tris, pH 8.0, 0.4 M l-arginine, 4 mM oxidized glutathione, 2 mM EDTA, 0.5 mM PMSF for T10/L^d/hβ_2m or T10/hβ_2m and 100 mM Tris, pH 8.2, 25% glycerol, 4 mM oxidized glutathione, 2 mM EDTA, 0.5 mM PMSF for T10/mβ_2m. Final protein concentrations were 1 μM T10 heavy chain and 2 μM β_2m. Folding reactions were incubated at room temperature for 48 h and concentrated to 30 ml in an Amicon stirred cell (10 kD cutoff) for fractionation on a Superdex 200 column (Pharmacia). Fractions containing associated T10/L^d heavy chain and hβ_2m were identified using a sandwich ELISA. The ELISA-positive fractions for T10/L^d and the corresponding sized fractions for T10/hβ_2m or T10/mβ_2m were each concentrated ~10 times and subjected to a Mono Q™ (Pharmacia) anion exchange column with a linear gradient of 0.1–0.3 M NaCl in 20 mM Tris (pH 7.5). The estimated yields of properly folded T10/hβ_2m and T10/mβ_2m are 2–4% and 1%, respectively.

The folding of HLA-A2 with HIV *pol* peptide RT309-317 (ILKEQVHGV) was carried out as described (22) with a folding yield routinely ~8%. No heterodimer could be detected when HLA-A2 and β_2m were folded in the absence of peptide.

ELISA. The sandwich ELISA for folded T10/L^d/hβ_2m heterodimer was performed using Immulon IV plates (Dynatech Laboratories, Inc., Chantilly, VA) coated overnight at 4°C with 10 μg/ml 28.14.8S antibody. After a 1-h incubation with analyte at room temperature, a rabbit anti–human β_2m polyclonal serum (Boehringer Mannheim, Indianapolis, IN) was added. The assay was developed with a goat anti–rabbit alkaline phosphatase conjugate (Jackson ImmunoResearch Labs., West Grove, PA).

G8 Stimulation Assay. G8 stimulation assays were performed in high binding polystyrene plates (Costar, Cambridge, MA) coated overnight at 4°C with purified T10/β_2m complex, moth cytochrome *c* peptide 88–103 loaded I-E^k, or using T10/L^d transfected CHO cells for stimulation of 10^5 G8 cells per well. Assays were also performed with T10/hβ_2m and T10/mβ_2m proteins that had been coated overnight at 4°C followed by a 10-h incubation with either PBS containing 2% BSA or RPMI containing 10% FCS at 22°C. The 24-h assay was carried out at 37 or 33°C for T10/hβ_2m and T10/mβ_2m, respectively. G8 cells express an alkaline phosphatase gene under control of the IL-2 gene *NFAT* promoter/enhancer (15). G8 stimulation is measured in fluorescence units, which represent measurements of *NFAT*-specific alkaline phosphatase activity, using the fluorescent substrate 4-methylumbelliferyl phosphate (Sigma). The dose–response curves are representative of at least three independent experiments.

Circular Dichroism Spectroscopy and Thermal Denaturation Studies. Far-UV CD spectra were recorded in a 0.1 path length cell on an AVIV 60 DS spectropolarimeter (Aviv Associates, Inc., Lakewood, NJ) equipped with a thermoelectric cuvette holder, using a step size of 0.25 nm, a bandwidth of 1 nm, and a time constant of 1 s. Spectra were recorded in sodium phosphate buffer (5 mM, pH 7.0). The spectra shown are representative of 3–5 independent measurements (each obtained from five repetitive scans) and were smoothed by the Savitzky–Golay algorithm using a sliding window of 9 (2.25 nm). Far-UV CD data is given as $[\Theta]_r$, the mean residue ellipticity. Thermal denaturation curves were obtained by following the CD signal at 223 nm as a function of the temperature. The temperature was increased in a step-wise mode (2°C intervals) with each temperature jump being followed by a 30-s equilibration time. Recording time was 100 s. Each point in the melting curves shown in the text represents the average of three independent experiments. Reversibility of the thermal transitions was determined by standard heating/cooling cycles. In each such

[1]*Abbreviations used in this paper:* CD, circular dichroic; FcRn, rat neonatal Fc receptor; IPTG, isopropyl β-d-thiogalactopyranoside.

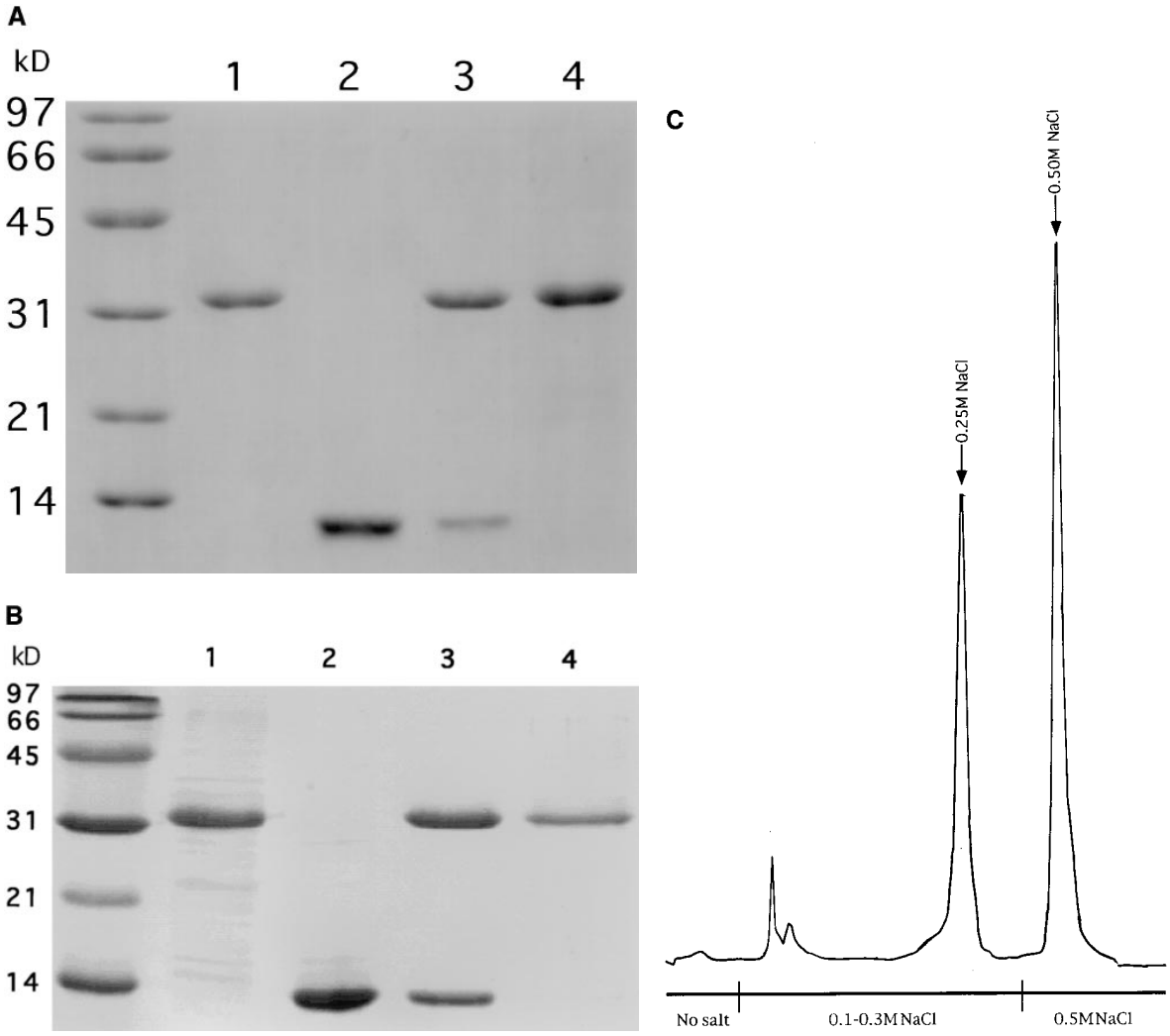

Figure 1. Purification of reconstituted T10/β_2m heterodimer by ion exchange chromatography. (*A*) SDS-PAGE analysis of T10 heavy chain (lane *1*) and hβ_2m (lane *2*) in urea, a 0.25 M NaCl ion exchange column peak fraction from the T10/hβ_2m purification (lane *3*), and a 0.5 M NaCl high salt wash ion exchange column fraction (lane *4*). Subunits in lanes *1* and *2* have been size purified in 6 M urea after solubilization in guanidine–HCl. The gel was stained with Coomassie blue. (*B*) SDS-PAGE analysis of T10 heavy chain (lane *1*) and mβ_2m (lane *2*) solubilized in guanidine–HCl, a 0.27 M NaCl ion exchange column peak fraction from the T10/mβ_2m purification (lane *3*), and a 0.5 M NaCl high salt wash ion exchange column fraction (lane *4*). The gel was stained with Coomassie blue. (*C*) Ion exchange chromatography profile from the T10/hβ_2m heterodimer purification. Two major peaks, one at 0.25 M NaCl in the 0.1–0.3 M NaCl gradient and a second peak eluting at 0.5 M NaCl in the high salt wash, are observed.

cycle, spectra were initially recorded at 25°C and the samples were heated to temperatures above the T_m of the protein complex analyzed and immediately cooled to 25°C. Posttransition spectra were recorded after an equilibration period of 1 h. The CD spectra at high temperatures were recorded separately to avoid the formation of kinetically driven, irreversibly unfolded species due to long incubation times at high temperatures.

Thermodynamic parameters were derived from the CD data presented in the text, assuming a two-state unfolding model. Derivation of the free energy change, ΔG, at physiological temperature (37°C), which lies below the transition region of the unfolding curves where the equilibrium constant, K, cannot be directly derived, was made using the following form of the Gibbs–Helmholtz equation: $\Delta G(T) = \Delta H_m(1 - T / T_m) - \Delta C_p([T_m - T] + T \ln [T / T_m])$, where T is the Kelvin temperature, T_m is the midpoint temperature of the thermal unfolding transition, ΔH_m is the enthalpy change for unfolding measured at T_m, and ΔC_p is the difference in heat capacity between the folded and unfolded conformations. T_m and ΔH_m were obtained from the van't Hoff equation: $\Delta H = RT^2(\delta \ln K)/(\delta T)$, in which R is the gas constant. ΔH_m values used to derive the free energy of the two T10 forms were 96 and 85 kcal/mol for the human–mouse and mouse–mouse combinations, respectively. ΔC_p values were assumed to be independent of temperature (24) and were estimated from the following: $\Delta C_p = (\delta \Delta H)/(\delta T)_p$. An average value of 0.8 kcal/mol · deg was used in all calculations. Values of $K(T)$ inside the transition region of the unfolding curves were derived from the following relation: $K(T) = (\Theta_N - \Theta_T) / (\Theta_T - \Theta_U)$, where (Θ_N) and (Θ_U) are the limiting ellipticity values representing the native and unfolded states, respectively, and (Θ_T) is the observed ellipticity at T. Baseline corrections of the row ellipticity values were made only for data below the transition zone. The end product of the main unfolding transition was represented by a single molar ellipticity value.

Results

E. coli–produced T10 and β_2m Subunits Can Be Folded into a Stable Heterodimer in the Absence of Peptide. It was shown previously that T10/T22 protein can be expressed stably on cells lacking a functional peptide-loading mechanism (15, 16, 19). In addition, incubation of T10/L^d-expressing cells with peptide libraries of 8 amino acids in length or shorter does not increase the level of surface T10/L^d expression (Schild, H., M. Jackson, and Y.-h. Chien, unpublished data). These results suggest that T10/T22 may not require peptide binding for stable expression on the cell surface at physiological temperature. The fact that T10/T22 expressed on these peptide loading–deficient cells can stimulate G8 as well as those molecules expressed on normal cells further suggests that a peptide-free form of these molecules is functional. To evaluate definitively whether T10 and β_2m without peptide are sufficient for maintaining the structural stability and function of the complex, we expressed both components separately in *E. coli*, purified and denatured each component, and folded them together in vitro.

To detect properly folded material in the absence of an anti-T10/T22 antibody or a suitable mouse β_2m antibody, we first perfomed folding experiments with a soluble form of the chimeric T10/L^d heavy chain molecule and human β_2m. T10/L^d, which has the α1 and α2 domains of T10 and α3 domain of the murine class I molecule L^d, can stimulate G8 and can be recognized by the L^d α3-specific antibody 28.14.8S (15). Human β_2m can be recognized by an anti-hβ_2m polyclonal serum.

Soluble forms of the T10/L^d and T10 heavy chain proteins were produced by truncating the extracellular domains just before the transmembrane region. All proteins (T10, T10/L^d, hβ_2m, and mβ_2m) were produced as inclusion bodies and, thus, they could be isolated to a high level of purity by standard washing procedures (Fig. 1, *A* and *B*). For the folding of T10/hβ_2m, subunits were subjected to gel filtration in the presence of 6 M urea to further purify heavy chain and hβ_2m away from residual bacterial components (Fig. 1 *A*). The folding of subunits (T10/L^d with hβ_2m, T10 with hβ_2m, and T10 with mβ_2m) was initiated by dilution of the denatured subunits according to modified published procedures (22, 25). To isolate heterodimer, the folding reactions were concentrated and fractionated by gel filtration chromatography. Fractions containing the renatured T10/L^d/hβ_2m were detected by a sandwich ELISA. Corresponding fractions from the T10/hβ_2m or T10/mβ_2m foldings were combined and the heterodimers were further purified by ion exchange chromatography. In a gradient of 0.1–0.3M NaCl, a major peak eluting at 0.25 M NaCl or at 0.27 M NaCl was observed for the T10/hβ_2m and T10/mβ_2m, respectively, while the rest of the protein eluted in the 0.5 M NaCl high salt wash (Fig. 1 *C*; data not shown). In each case, SDS-PAGE indicated that the major peak within the gradient contained both the heavy chain and β_2m, whereas fractions from the high salt wash contained heavy chain alone (Fig. 1, *A* and *B*).

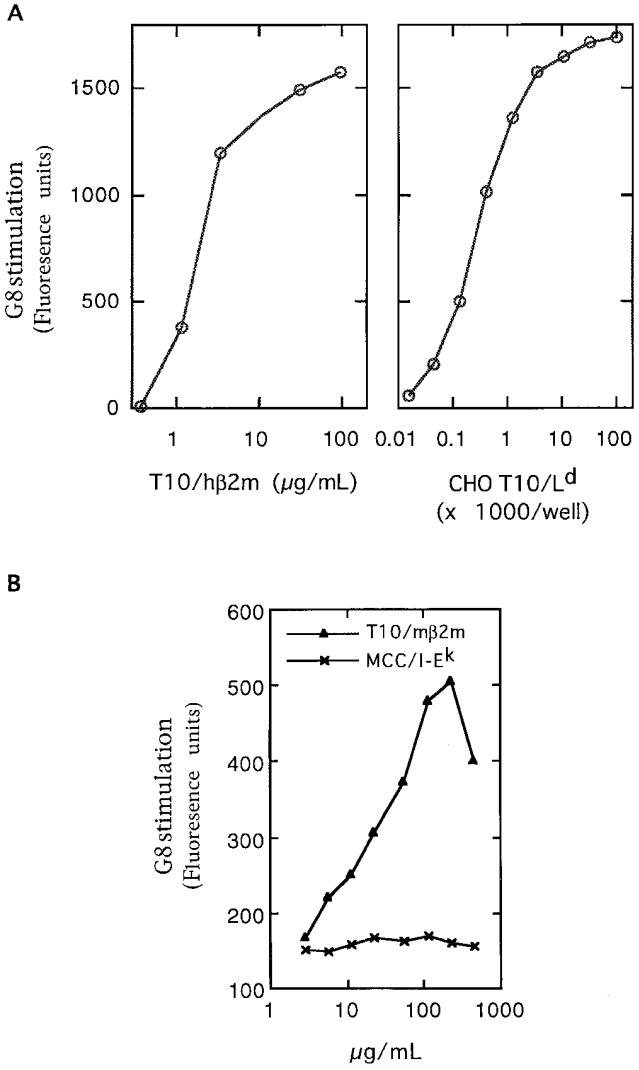

Figure 2. (*A*) Stimulation of G8 γδ T cells by purified T10/hβ_2m heterodimer and by CHO T10/L^d cells. (*B*) Stimulation of G8 γδ T cells by purified T10/mβ_2m heterodimer and by the moth cytochrome c 88–103 peptide-loaded MHC class II molecule, I-E^k.

Fractions from the ion exchange column were assayed for their ability to stimulate G8 γδ T cells. For all heterodimer purifications, only the material eluting within the major peak in the gradient was active in these assays. The folded T10/hβ_2m complex was found to stimulate G8 to the same degree as T10/L^d transfected CHO cells (Fig. 2 *A*), which stimulate G8 to a higher level than the naturally expressing EL4 or PCC3 cells (15). The T10/mβ_2m complex was also stimulatory (Fig. 2 *B*), but at an ~10-fold lower level.

The lower stimulatory activity of T10/mβ_2m in these experiments is most likely due to its lower thermal stability compared with that of the T10/hβ_2m form (as discussed below). This could cause T10/mβ_2m to be more sensitive to the denaturing effects of the coating process (26). We have preincubated T10/hβ_2m and T10/mβ_2m with media

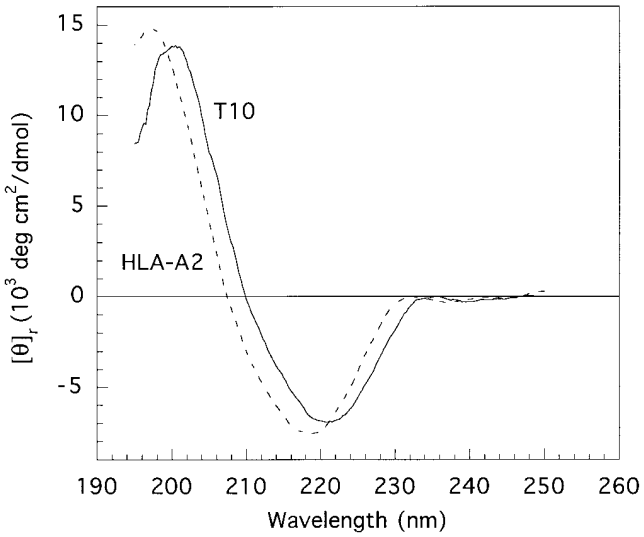

Figure 3. Far-UV CD spectra of T10/hβ_2m (0.15 mg/mL; *solid line*) and HLA-A2 (0.25 mg/mL; *dotted line*) heterodimers in 5 mM sodium phosphate buffer (pH 7.0). Spectra were recorded using 0.1 cm cells at 25°C.

at 22°C for 10 h before T cell stimulation assays. This treatment does not change the dose–response curves for either complex compared with those without preincubation. Together, these results clearly indicate that the complex has been correctly folded and can be recognized by G8, without the addition of peptide or nonpeptide components and, likely, without the contribution of a media or serum component.

T10/β_2m Is Structurally Different from Classical MHC Class I Molecules. Far-UV CD spectroscopy has been used to analyze the structure and thermal stability of classical MHC class I molecules and the rat neonatal Fc receptor (FcRn), an MHC-like molecule that functions as an IgG transporter (27–31). To obtain similar parameters for the reconstituted T10/β_2m heterodimer, we subjected both T10/hβ_2m and T10/mβ_2m to CD analysis (Fig. 3, data not shown). Interestingly, the spectra of both T10/hβ_2m and T10/mβ_2m are red-shifted compared with those reported for classical MHC class I molecules and FcRn (27–31). This difference was further verified by comparing the CD spectrum of T10 with that of *E. coli*–expressed and folded HLA-A2 molecules complexed with HIV *pol* peptide (Fig. 3). These data suggest that although these molecules are likely to have similar folds, T10 has structural properties distinct from classical class I MHC molecules (32).

The thermal denaturation profile of the T10/hβ_2m complex is shown in Fig. 4 *A*. At neutral pH , the melting curve reveals two transitions. The first is characterized by a transition temperature midpoint (T_m) of 49°C and reflects the simultaneous dissociation and unfolding of the T10 heavy chain. The second transition, with a T_m at 63–64°C, is characterized by a sign reversal of the CD signal and closely parallels the unfolding profile of free β_2m ($T_m \approx 64$°C, data not shown; references 29, 30), implying that its denaturation is largely independent of the heavy chain. Consistent with its

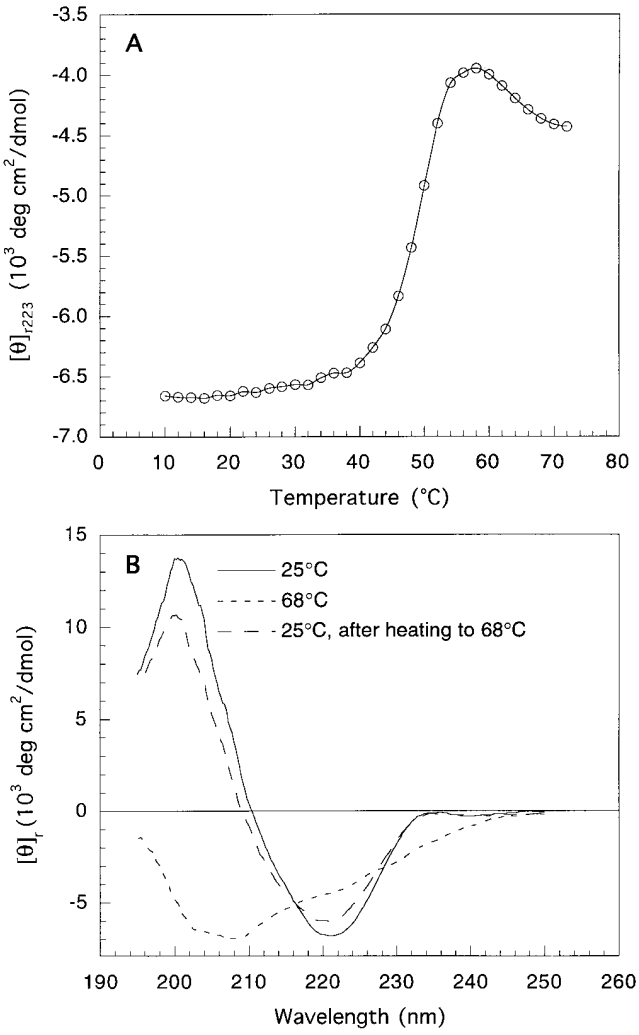

Figure 4. (*A*) Thermal denaturation of the T10/hβ_2m heterodimer. Protein concentration was 0.15 mg/mL. (*B*) CD scans of native, unfolded, and renatured T10/hβ_2m.

lower activity in G8 stimulation assays, the T10/mβ_2m complex is less stable than the mouse–human combination, with a T_m = 43°C (Fig. 5). Mouse β_2m was observed to have a $T_m \approx 62$°C (data not shown). For each heterodimer, the thermal transition is largely reversible (see Fig. 4 *B*; data not shown).

Assuming a standard two-state unfolding model, the free energy change at a particular temperature, $\Delta G(T)$, can be estimated from the melting curves shown here. At physiological temperature (37°C), we calculate free energy changes of ~3.3 and 1.5 kcal/mol for the T10/hβ_2m and T10/mβ_2m heterodimers, respectively. These values for T10/hβ_2m and T10/mβ_2m can be translated into expected ratios of folded to unfolded species of 200:1 and 11:1, respectively, at 37°C. The structural basis for the different thermal stabilities of these heterodimers is presently under investigation.

By comparison, both forms of T10 are less stable than classical class I molecules complexed with an appropriate

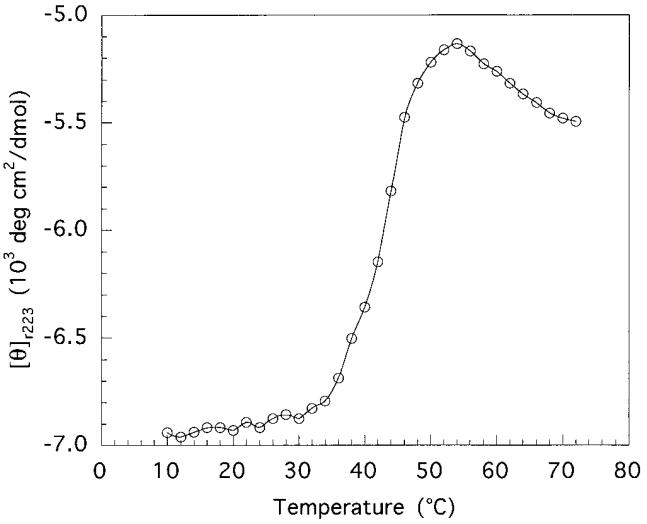

Figure 5. Thermal denaturation of the T10/mβ_2m heterodimer. Protein concentration was 0.15 mg/mL.

peptide, for which free energies >5kcal/mol and T_m of 65–72°C have been reported (29, 30). However, with the exception of the K^d molecule, MHC class I molecules are critically unstable in the absence of peptide and can not be assembled. On the other hand, the stability of FcRn, which does not bind peptide for either stability or function, lies in between these extremes, with T_m of 62°C and 51°C at pH 6.0 and pH 8.0, respectively, the latter being very similar to the the T_m of the T10/hβ_2m heterodimer (31).

Discussion

We set out to evaluate directly whether a component other than the T10 heavy chain and β_2m is necessary for its recognition by G8 and for the structural stability of the molecule by producing the two subunits separately in *E. coli* and folding them together in vitro. We find that the complex of T10 with murine β_2m can be assembled in the absence of any additional factors and that the heterodimer is stimulatory to G8. However, T10/mβ_2m has a rather low thermal stability, similar to that of the empty K^d molecule. The ability of plate bound T10/mβ_2m to stimulate G8 is also lower than cells expressing T10, by ~10-fold.

Based on these observations, one possibility is that the heterodimer expressed on the cell surface is further stabilized by a factor(s) other than the primary amino acid sequences of T10 and mβ_2m. This stabilizing factor for T10/mβ_2m in vivo may be the carbohydrate moieties that are covalently linked to the T10 heavy chain. Although T10 and T22 have three potential N-linked glycosylation sites in the α1 and α2 domains, two more than classical MHC class I molecules, the *E. coli*–produced subunits are not glycosylated. Recently, it was shown that the elimination of a single N-glycan site in the adhesion domain of human CD2, a protein belonging to the immunoglobulin superfamily, severely reduces the stability of the protein (33). It is possible that one or more of the carbohydrates of T10/T22 plays a critical role in stabilizing the structure of the heterodimer. Alternatively, T10/mβ_2m might also be stabilized by association with a molecule not covalently linked to the complex. However, regardless of the nature of such a stabilizing factor, by substituting the mouse β_2m with human β_2m in our in vitro system, we were able to increase the stability of the T10 complex. T10/hβ_2m can stimulate G8 at levels similar to that of the best stimulator cells. These results indicate that, while an additional factor may be necessary for the stable expression of T10 on the cell surface, it does not confer specificity. Thus, the only essential feature required for G8 recognition is a properly folded and stable T10 heavy chain and β_2m heterodimer.

This scenario is reminiscent of the recognition of murine class II I-E^k by the $\gamma\delta$ T cell LBK5. While I-E^k requires peptide for stable expression on the cell surface, the bound peptide does not confer specificity for its recognition by LBK5. As shown previously, LBK5 can recognize I-E^k stabilized by a variety of different peptides (15). Thus, these two examples of $\gamma\delta$ T cell recognition differ fundamentally from the recognition by $\alpha\beta$ T cells of classical or nonclassical MHC. In the latter case, the peptide or nonpeptide moieties being presented contributes significantly to the specificity of the recognition. However, unlike I-E^k, there is no evidence that T10 binds peptide. This assertion is based on the observation that no peptide(s) other than those derived from the antibodies used for immunoprecipitation can be eluted from T10/L^d molecules isolated from cells (19), as well as our result presented here that the folding of heterodimer does not require peptide. These observations are consistent with the primary amino acid sequences of T10/T22, which suggest that these molecules may lack the necessary structural features to bind peptide. Most notably, T10/T22 possess a 3–amino acid deletion within the α1 domain and a 12–amino acid deletion within the α2 domain (14). Thus, assuming that T10 and T22 molecules fold similarly to classical MHC molecules, the α2 α-helical region, the conserved COOH-terminal peptide-binding pocket (pocket F), as well as the outermost β strand of the α1 domain and the outermost β strand of the α2 domain, would all be significantly altered (14).

Such structural features in T10 are not shared by FcRn, a class Ib molecule that does not bind peptide. Crystallographic studies revealed that FcRn is structurally similar to MHC molecules in its conservation of the α1 and α2 domain topology of two helices that span a single β sheet, but the presence of a proline at position 162 introduces a break in the α2 helix of the molecule, causing a shift of its α helices and resulting in a peptide-binding groove filled with side chains (34). Consistent with the crystallographic analysis, it was shown that the CD spectrum of FcRn and classical MHC are very similar (35). However, both spectra are blue-shifted when compared with that of T10/β_2m. This observation indicates that T10 is likely to possess structural properties not found in either MHC class I molecules or in FcRn. Thus, these results suggest a new way for an MHC class I–like molecule to adopt a peptide-free structure.

An alternative to the hypothesis that T10/T22 needs an

additional factor for stability is the possibility that rapid turnover is a useful property in a ligand for γδ T cell recognition. There is strong evidence that at least one role for γδ T cells in the immune system is the surveillance for cells that have become stressed or damaged (36–39). T10 molecules expressed on the cell surface are likely to have a shorter half-life than classical MHC molecules. Consistent with this, preliminary antibody stainings indicate low T10 cell surface expression on primary lymphoid cells and EL4 cells (Crowley, M., and Y.-h. Chien, unpublished data). It is arguable that a rapid turnover may better enable T10 to act as a transient target of sensory immune cells.

We thank Drs. D. Garboczi and D. Wiley for providing the human HLA-A2 and hβ_2m constructs, Drs. J. Sacchettini and S. Nathenson for providing the mβ_2m construct, and Dr. M. Davis for critically reading the manuscript.

M.P. Crowley was supported by a fellowship from the National Science Foundation. Z. Reich was supported by The Rothchild Foundation, Israel, and is currently supported by The Howard Hughes Medical Institute. N. Mavaddat is supported by the National Health and Medical Research Council of Australia. We also thank the National Institutes of Health for grant support.

Address correspondence to Dr. Y.-h. Chien, D333 Fairchild Building, Department of Microbiology and Immunology, Stanford University, School of Medicine, Stanford, California 94305. The current address of N. Mavaddat is the Walter and Eliza Hall Institute of Medical Research, The Royal Melbourne Hospital, Victoria 3050, Australia.

Received for publication 10 April 1996 and in revised form 27 January 1997.

References

1. Shawar, S.M., J.M. Vyas, J.R. Rodgers, and R.R. Rich. 1994. Antigen presentation by major histocompatibility complex class I-B molecules. *Annu. Rev. Immunol.* 12:839–880.
2. Stroynowski, I., and K.F. Lindahl. 1994. Antigen presentation by nonclassical class I molecules. *Curr. Opin. Immunol.* 6:38–44.
3. Kurlander, R.J., S.M. Shawar, M.L. Brown, and R.R. Rich. 1992. Specialized role for a murine class 1-b MHC molecule in prokaryotic host defenses. *Science (Wash. DC).* 257:678–679.
4. Shawar, S.M., R.G. Cook, J.R. Rodgers, and R.R. Rich. 1990. Specialized functions of MHC class I molecules. I. An N-formyl peptide receptor is required for construction of the class I antigen *Mta*. *J. Exp. Med.* 17:872–912.
5. Pamer, E.G., C.R. Wang, L. Flaherty, K.F. Lindahl, and M.J. Bevan. 1992. H-2M3 presents a Listeria monocytogenes peptide to cytotoxic T lymphocytes. *Cell.* 70:215–223.
6. Beckman, E.M., S.A. Porcelli, C.T. Morita, S.M. Behar, S.T. Furlong, and M.B. Brenner. 1994. Recognition of a lipid antigen by CD1-restricted alpha beta+ T cells. *Nature (Lond.).* 372:691–694.
7. Sieling, P.A., D. Chatterjee, S.A. Porcelli, T.I. Prigozy, R.J. Mazzaccaro, T. Soriano, B.R. Bloom, M.B. Brenner, M. Kronenberg, P.J. Brennan et al. 1995. CD1-restricted T cell recognition of microbial lipoglycan antigens. *Science (Wash. DC).* 269:227–230.
8. Castano, A.R., S. Tangri, J.E. Miller, H.R. Holcombe, M.R. Jackson, W.D. Huse, M. Kronenberg, and P.A. Peterson. 1995. Peptide binding and presentation by mouse CD1. *Science (Wash. DC).* 269:223-226.
9. Bendelac, A., O. Lantz, M.E. Quimby, J.W. Yewdell, J.R. Bennink, and R.R. Brutkiewicz. 1995. CD1 recognition by mouse NK1+ T lymphocytes. *Science (Wash. DC).* 268:863–865.
10. Rotzschke, O., K. Falk, S. Stevanovic, B. Grahovac, M.J. Soloski, G. Jung, and H.G. Rammensee. 1993. Qa-2 molecules are peptide receptors of higher stringency than ordinary class I molecules. *Nature (Lond.).* 361:642–644.
11. Joyce, S., P. Tabaczewski, R.H. Angeletti, S.G. Nathenson, and I. Stroynowski. 1994. A nonpolymorphic major histocompatibility complex class Ib molecule binds a large array of diverse self-peptides. *J. Exp. Med.* 179:579–588.
12. Jiang, H., R. Ware, A. Stall, L. Flaherty, L. Chess, and B. Pernis. 1995. Murine CD8+ T cells that specifically delete autologous CD4+ T cells expressing V beta 8 TCR: a role of the Qa-1 molecule. *Immunity.* 2:185–194.
13. Imani, F., and M.J. Soloski. 1991. Heat shock proteins can regulate expression of the Tla region-encoded class Ib molecule. *Qa-1. Proc. Natl. Acad. Sci. USA.* 88:10475–10479.
14. Ito, K., K.L. Van, M. Bonneville, S. Hsu, D.B. Murphy, and S. Tonegawa. 1990. Recognition of the product of a novel MHC TL region gene (27b) by a mouse γδ T cell receptor. *Cell.* 62:549–561.
15. Schild, H., N. Mavaddat, C. Litzenberger, E.W. Ehrich, M.M. Davis, J.A. Bluestone, L. Matis, R.K. Draper, and Y.H. Chien. 1994. The nature of major histocompatibility complex recognition by γδ T cells. *Cell.* 76:29–37.
16. Weintraub, B.C., M.R. Jackson, and S.M. Hedrick. 1994. Gamma delta T cells can recognize nonclassical MHC in the absence of conventional antigenic peptides. *J. Immunol.* 153: 3051–3058.
17. Bluestone, J.A., R.Q. Cron, M. Cotterman, B.A. Houlden, and L.A. Matis. 1988. Structure and specificity of T cell receptor gamma/delta on major histocompatibility complex antigen-specific CD3+, CD4−, CD8− T lymphocytes. *J. Exp. Med.* 168:1899–1916.
18. Bonneville, M., K. Ito, E.G. Krecko, S. Itohara, D. Kappes, I. Ishida, O. Kanagawa, C.A. Janeway, D.B. Murphy, and S. Tonegawa. 1989. Recognition of a self major histocompatibility complex TL region product by gamma delta T-cell receptors. *Proc. Natl. Acad. Sci. USA.* 86:5928–5932.
19. Kaliyaperumal, A., R. Falchetto, A. Cox, R. Dick, J. Shabanowitz, Y.H. Chien, L. Matis, D.F. Hunt, and J.A. Bluestone. 1995. Functional expression and recognition of nonclassical

MHC class I $T10^b$ is not peptide-dependent. *J. Immunol.* 155: 2379–2386.
20. Jackson, M.R., E.S. Song, Y. Yang, and P.A. Peterson. 1992. Empty and peptide-containing conformers of class I major histocompatibility complex molecules expressed in Drosophila melanogaster cells. *Proc. Natl. Acad. Sci. USA*. 89:12117–12121.
21. Silver, M.L., K.C. Parker, and D.C. Wiley. 1991. Reconstitution by MHC-restricted peptides of HLA-A2 heavy chain with beta 2-microglobulin, in vitro. *Nature (Lond.)*. 350:619–622.
22. Garboczi, D.N., D.T. Hung, and D.C. Wiley. 1992. HLA-A2–peptide complexes: refolding and crystallization of molecules expressed in Escherichia coli and complexed with single antigenic peptides. *Proc. Natl. Acad. Sci. USA*. 89:3429–3433.
23. Young, A.C.M., W. Zhang, J.C. Sachettini, and S.G. Nathenson. 1994. The three-dimensional structure of H-2Db at 2.4Å resolution: implications for antigen-determinant selection. *Cell*. 76:39–50.
24. Privalov, P.L., and S.J. Gill. 1988. Stability of protein structure and hydrophobic interaction. *Adv. Prot. Chem.* 39:191–234.
25. Buchner, J., and R. Rudolph. 1991. Renaturation, purification and characterization of recombinant Fab-fragments produced in *Escherichia coli*. *Bio/technology*. 9:157–162.
26. Butler, J.E. 1991. Perspectives, configurations, and principles. *In* Immunochemistry of Solid-phase Immunoassay. J.E. Butler, editor. CRC Press, Inc., Boca Raton, FL. 3–26.
27. Lancet, D., P. Parham, and J.L. Strominger. 1979. Heavy chain of HLA-A and HLA-B antigens is conformationally labile: a possible role for beta 2-microglobulin. *Proc. Natl. Acad. Sci. USA*. 76:3844–3848.
28. Gorga, J.C., A. Dong, M.C. Manning, R.W. Woddy, W.S. Caughey, and J.L. Strominger. 1989. Comparison of the secondary structures of human class I and class II major histocompatibility complex antigens by Fourier transform infrared and circular dichroism spectroscopy. *Proc. Natl. Acad. Sci. USA*. 86:2321–2325.
29. Fahnestock, M.L., I. Tamir, L. Narhi, and P.J. Bjorkman. 1992. Thermal stability comparison of purified empty and peptide-filled forms of a class I MHC molecule. *Science (Wash. DC)*. 258:1658–1662.
30. Bouvier, M., and D.C. Wiley. 1994. Importance of peptide amino and carboxyl termini to the stability of MHC class I molecules. *Science (Wash. DC)*. 265:398–402.
31. Raghavan, M., L.N. Gastinel, and P.J. Bjorkman. 1993. The class I major histocompatibility complex related Fc receptor shows pH-dependent stability differences correlating with immunoglobulin binding and release. *Biochemistry*. 32:8654–8660.
32. Greenfield, N., and G.D. Fasman. 1969. Computed circular dichroism spectra for the evolution of protein conformation. *Biochemistry*. 8:4108–4116.
33. Wyss, D.F., J.S. Choi, J. Li, M.H. Knoppers, K.J. Willis, A.R. Arulanandam, A. Smolyar, E.L. Reinherz, and G. Wagner. 1995. Conformation and function of the N-linked glycan in the adhesion domain of human CD2. *Science (Wash. DC)*. 269:1273–1278.
34. Burmeister, W.P., L.N. Gastinel, N.E. Simister, M.L. Blum, and P.J. Bjorkman. 1994. Crystal structure at 2.2 Å resolution of the MHC-related neonatal Fc receptor. *Nature (Lond.)*. 372:336–343.
35. Gastinel, L.N., N.E. Simister, and P.J. Bjorkman. 1992. Expression and crystallization of a soluble and functional form of an Fc receptor related to class I histocompatibility molecules. *Proc. Natl. Acad. Sci. USA*. 89:638–642.
36. Carding S., W. Allan, S. Kyes, A. Hayday, and P. Doherty. 1990. Late dominance of the inflammatory process in murine influenza by γ-δ^+ T cells. *J. Exp. Med.* 172:1225–1231.
37. Hara, T., Y. Mizuno, K. Takaki, H. Takada, H. Akeda, T. Aoki, M. Nagata, K. Ueda, G. Matsuzaki, Y. Yoshikai et al. 1992. Predominant activation and expansion of Vγ9-bearing $\gamma\delta$ T cells in vivo as well as in vitro in salmonella infection. *J. Clin. Invest.* 90:204–210.
38. Hou, S., M. Kats, P.C. Doherty, and S.R. Carding. 1992. Extent of $\gamma\delta$ T cells involvement in the pneumonia caused by Sendai virus. *Cell. Immunol.* 143:183–193.
39. Hiromatsu, K., Y. Yoshikai, G. Matsuzaki, S. Ohga, K. Muramori, K. Matsumoto, J. Bluestone, and K. Nomoto. 1992. A protective role of γ-δ T cells in primary infection with Listeria monocytogenes in mice. *J. Exp. Med.* 175:49–56.