Functionally Conformed Free Class I Heavy Chains Exist On The Surface Of β_2 Microglobulin Negative Cells

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Summary

Cytotoxic T lymphocytes (CTL) can recognize antigenic peptides bound in a groove formed by the $\alpha1$ and $\alpha2$ domains of the heterodimeric major histocompatibility complex class I molecule. Proper assembly, transport, and stability of functional class I molecules is thought to require β_2 microglobulin (β_2m), the light chain of the class I heterodimer. We show here that the requirement for β_2m is not absolute. β_2m$^-$ cells can be stained by the D^b $\alpha1$ domain-specific B22-249.1 monoclonal antibody, which detects a conformation-dependent epitope. Furthermore, β_2m$^-$ Con A blast target cells can be lysed by alloreactive CTL, even in serum-free conditions. Contrary to previous reports, the expression of low levels of conformed D^b heavy (H) chains is a property of both normal and transformed β_2m$^-$ cells. Finally, we present evidence that a subset of properly conformed H chains, free of β_2m, may have almost equal representation on β_2m$^+$ and $\beta2$m$^-$ cells.

MHC class I molecules are composed of a 45-kD polymorphic H chain noncovalently associated with β_2 microglobulin (β_2m)[1]. Previous studies have suggested that β_2m is critical for the normal function of class I molecules. In mutant cells that are unable to synthesize β_2m, most class I H chains accumulate intracellularly and are degraded (1, 2). Some class I H chains, such as D^b, reach the cell surface in β_2m-deficient cell lines such as the β_2m$^-$ R1E cell line (C58 origin, H-2^k), which had been transected with the D^b gene (R1E/D^b) (1). Cell surface expression of D^b in β_2m$^-$ tumor cells could be detected with the 28-14-8s mAb, that reacts with the $\alpha3$ domain of the D^b molecule. However, the levels of cell surface D^b expression were reduced in the β_2m$^-$ cells compared with the same cells supertransfected with β_2m (R1E/D^b/β_2m). More importantly, the peptide-binding domains of D^b molecules on the surface of R1E/D^b cells apparently assume an altered conformation, as shown by their failure to react with mAb specific for the $\alpha1$ (e.g., B22-249.1) or $\alpha2$ domains of D^b (1, 3). Furthermore, alloreactive or antigen-specific/class I-restricted cytotoxic T cells specific for D^b failed to react with R1E/D^b cells, suggesting an important role for β_2m in generating class I complexes visible to the TCR. These experiments suggested that β_2m plays an obligatory role in inducing and/or maintaining a functional conformation of class I molecules.

We have examined cell surface class I expression on cells from mice homozygous for a targeted mutation in their β_2m

gene. We previously reported (4) preliminary findings that cells from these mice fail to synthesize β_2m, and have essentially no serologically detectable levels of K^b or Qa2 class I molecules on their surface. However, low levels (about 1/20th the level found on β_2m$^+$ cells) of D^b molecules were detected on freshly isolated T cells from β_2m$^-$ mice by staining with D^b-specific mAbs 28-14-8s, B22-249.1, and 27-11-13s. Furthermore, β_2m$^-$ Con A blasts could be lysed, albeit inefficiently, by populations of alloreactive, H-2^b-specific CTL. Our findings that β_2m$^-$ cells can be stained by $\alpha1$-specific B22-249 mAb, and that β_2m$^-$ Con A blast target cells can be lysed by alloreactive CTL, contrast with previous reports that β_2m$^-$ R1E/D^b tumor cells are neither stained by B22-249.1 mAb, nor are lysed by alloreactive CTL.

Our earlier studies were performed in serum-containing medium. Serum contains free β_2m that could potentially bind to free class I H chains, resulting in a functional conformation of the molecules (5). Furthermore, β_2m shed from the CTL used in the functional experiments might have transferred onto the target cells. In the present paper, we provide direct evidence that functional, conformed free class I H chains exist on the surface of β_2m$^-$ cells in the absence of β_2m. The implications of these findings for the normal function of class I molecules are discussed.

Materials and Methods

Mice. All β_2m$^-$ strains (4) were bred at the Life Science Addition Animal Facility (University of California, Berkeley) or the

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[1] *Abbreviation used in this paper:* β_2m, β_2 microglobulin.

Center for Cancer Research at the Massachusetts Institute of Technology (Cambridge, MA). All other strains were purchased from The Jackson Laboratory (Bar Harbor, ME).

Tumor Cells. R1E/D^b and R1E/D^b/β_2m (6) were a generous gift from M. Zuñiga (University of California, Santa Cruz).

Cell Culture Media. Media containing bovine β_2m: RPMI 1640, 5% FCS, 5 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin, 20 mM Hepes, and 50 μM 2-ME (RP-5). β_2m free media: AIM-V (Gibco Laboratories, Grand Island, NY), and 50 μM 2-ME.

Antibodies. Allophycocyanin-conjugated streptavidin was obtained from Molecular Probes (Eugene, OR). Fluorescein-conjugated goat anti–mouse Ig (cat. no. 1010-02) was obtained from Southern Biotechnology Associates (Birmingham, AL). Sheep anti-fluorescein-conjugated magnetic beads (cat. no. 4310D) were obtained from Advanced Magnetics (Cambridge, MA). Anti-CD8 (AD4[15]), anti-CD4 (RL174), anti-NK1.1 (PK136), and anti-K^k (16-1-2n) can be obtained from the American Type Culture Collection (Rockville, MD). Anti-D^b α1 (B22-249.1) was a gift from P. Walden (Max Planck Institute, Tübingen, Germany) and was biotinylated using standard methods. Anti-Thy-1.2 (J1J) was a gift from J. Sprent (The Scripps Research Institute, La Jolla, CA).

Radiation Bone Marrow Chimeras. Bone marrow was prepared from female β_2m$^-$ H-2^k homozygous mice (5 mo-old) resulting from the intercross of the 4th backcross generation of B10.BR $\times$ (B6 $\times$ 129)F_1, and was depleted of T cells by treatment with anti-Thy1.2 (J1J) plus complement. Ten million T-depleted marrow cells were injected into female B10.BR mice (3–5 mo-old) irradiated (980 rad from a ^{137}Cs source) $\sim$2 h earlier. To prevent rejection of the class I-deficient marrow grafts by NK cells, all recipients were depleted of NK1.1$^+$ cells by intraperitoneal injection with 200 μg PK136 1 d earlier (7). Spleen cells were harvested for analysis 12 wk later.

Immunofluorescent Staining Analysis. Lymph node suspensions from individual mice or tumor cells were stained at 10^6 cells/25 μl for 20 min at 4°C in either PBS, 5% FCS, 0.2% NaN$_3$ (medium containing β_2m), or PBS, 14 μg/ml β-galactosidase (Sigma Chemical Co., St. Louis, MO), and 0.2% NaN$_3$ (β_2m-free staining media). Between stains, cells were washed two times. After the final wash, cells were fixed in 1% paraformaldehyde and stored, foil wrapped, at 4°C until analysis. Analysis was performed on a FACStar$^+$® flow cytometer. Typically, 20,000 cells per sample were analyzed.

Cytolysis Analysis. Primary MLC were established essentially as described (8). Briefly, 25 $\times$ 10^6 responder splenocytes and 25 $\times$ 10^6 stimulator splenocytes (receiving 3,000 rad from a ^{137}Cs source) were cultured together in 20 ml of RP-5 in upright T-25 flasks (Corning Glass Works, Corning, NY) for 5 d and then harvested for cytolysis assay. Cells harvested on day 9 or 10 from 1° MLC were reestablished in 2° MLC (2–3 $\times$ 10^6 responder cells/culture) with fresh stimulator cells (25 $\times$ 10^6 cells/culture) and cultured for 4 d before harvesting for assay. The cytolysis assay was performed essentially as described (8) either in RP-5 or AIM-V, as indicated. For target cell preparation, splenocytes were cultured for 2 or 3 d in 24-well plates (Falcon Plastics, Lincoln Park, NJ) at 2 $\times$ 10^6 cells/ml with 2 μg/ml Con A (when culturing in RP-5), and 0.33 μg/ml Con A (when culturing in AIM-V).

Immunomagnetic Depletion of B Cells. β_2m$^-$ spleen cells were stained with affinity-purified, fluorescein-conjugated, goat anti–mouse Ig antibody in β_2m-free staining media for 20 min at 4°C, washed two times, then treated with sheep anti-fluorescein-conjugated magnetic beads (Advanced Magnetics) for 30 min at 4°C with gentle rocking. Ig$^+$ (and hence, magnetic) cells were

removed by exposing the cell suspension for 15 min at room temperature to a strong magnetic field using a Biomag device (Advanced Magnetics). Nonimmobilized cells were recovered and passed a second time through the Biomag device to remove any remaining Ig$^+$ cells. Recovered cells were washed into AIM-V medium before the cytolysis assay.

Antibody Plus Complement Depletion. CTL populations were resuspended at 20 $\times$ 10^6 cells/ml in RP-5, and the optimal dilution of mAb and a mixture of rabbit and guinea pig complement. After incubation at 37°C for 40 min, viable cells were recovered by passage over a ficoll gradient and washed into the appropriate media.

Results

Polyclonal CTL populations specific for H-2^b were generated in MLC, in which BALB/c (H-2^d) or B10.BR (H-2^k) spleen cells were stimulated with B6 (H-2^b) stimulator spleen cells. In most cases, the CTL were restimulated with B6 spleen cells before assaying their activity. The CTL were tested for their capacity to lyse Con A blast targets from H-2^b-type β_2m$^-$ or β_2m$^+$ mice (Fig. 1). Corroborating our earlier study, β_2m$^-$ target cells were lysed by the CTL, although substantially less efficiently than were β_2m$^+$ target cells. To achieve equivalent lysis of β_2m$^-$ target cells, 50 times more CTL from primary MLC were usually required than for lysis of β_2m$^+$ target cells (Fig. 1 A). CTL from secondary MLC appeared capable of more efficient relative lysis of β_2m$^-$ target cells, since only 10–20 times more CTL from secondary MLC were typically required for lysis of β_2m$^-$ target cells than for lysis of β_2m$^+$ target cells (Fig. 1 B). There was no significant lysis of syngeneic (BALB/c or B10.BR) β_2m$^+$ target cells by any of these CTL, demonstrating their specificity.

Previous studies have shown that bovine β_2m, present in FCS, can associate with class I H chains on cells cultured in serum-containing medium (5). It was therefore possible that lysis of β_2m$^-$ cells by CTL depended on the binding of serum β_2m to free class I H chains on the surface of β_2m$^-$ cells. To test this possibility, we compared experiments performed in FCS-containing medium with those performed in AIM-V serum-free medium. The two media were used for generating target cell blasts and for the cytolysis assay. Because AIM-V medium failed to support the generation of high levels of CTL activity in MLC, the CTL were generated in FCS-containing medium, and were washed extensively in AIM-V medium before the cytolysis assay. As shown in Fig. 1, A and B, a similar extent of lysis of β_2m$^-$ target cells was observed in FCS-containing medium as in AIM-V serum-free medium, indicating that serum β_2m is not necessary for lysis of β_2m$^-$ cells by CTL.

The lysis of β_2m$^-$ target cells is not mediated by MHC class II (MHC-II)-specific CTL, because T-blast target cells, which do not express MHC-II in the mouse, were employed for all of these experiments. To rule out the possibility that contaminating B cell blasts in the Con A blast preparation are responsible for the observed lysis, we employed as target cells β_2m$^-$ Con A blasts that had been depleted of class II$^+$ B cells before culture, by immunomagnetic separation with
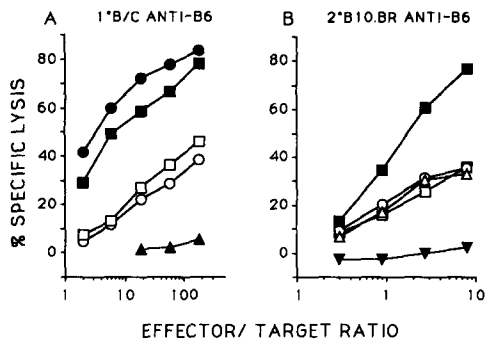
Figure 1. Lysis of β_2m^- target cells by allospecific CTL in serum-free medium. (*A*) Anti-H-2^b effector cells from a primary BALB/c anti-B6 MLC tested on Con A blast target cells. (*B*) Anti-H-2^b effector cells from a secondary B10.BR anti-B6 MLC tested on Con A blast target cells. Target cells were prepared by culture on medium containing FCS or in serum-free AIM-V medium. Assays were performed in AIM-V medium. Target cells were: (■) H-2^b β_2m^+, FCS; (●) H-2^b β_2m^+, AIM-V; (□) H-2^b β_2m^-, FCS; (O) H-2^b β_2m^-, AIM-V; (△) H-2^b β_2m^-, AIM-V, B cell-depleted; (▲) H-2^d β_2m^+ FCS; (▼) H-2^k β_2m^+, FCS. The H-2^b β_2m^+ and β_2m^- targets were from (B6 × 129)F2-4 animals, the H-2^d targets were from BALB/c mice, and the H-2^k targets were from B10.BR mice.

anti-Ig coated magnetic beads. These target cells were lysed as efficiently as target cells from which B cells had not been specifically depleted (Fig. 1 *B*).

The effector cells that lyse H-2^b, β_2m^- target cells were typed for expression of cell surface markers by treating the effector cell population with specific mAb and complement before the cytolysis assay. Treatment of the population with anti-CD8 mAb plus complement strongly reduced lysis of β_2m^- cells, whereas treatment with anti-CD4 plus complement or anti-NK1.1 plus complement had no effect. Therefore, lysis of β_2m^- cells by these cytolytic cells is mediated by CTL of the conventional CD8$^+$CD4$^-$NK1.1$^-$ phenotype (Fig. 2). Although our previous results indicate that NK1.1$^+$CD8$^-$ NK cells can also lyse β_2m^- target cells (9),
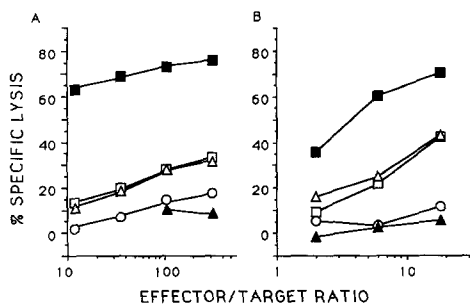

Figure 2. Lysis of β_2m^- targets is mediated by CD8$^+$CD4$^-$NK1.1$^-$ cells. Anti-H-2^b effector cells from B10.BR anti-B6 MLC were treated with antibody and complement, or complement alone, before assay on β_2m^- or β_2m^+ targets, as indicated. (■, □, ▲) C alone; (O) anti-CD8 plus C; (△) anti-NK1.1 in panel *A*, anti-CD4 plus C in panel *B*. (□, O, △) H-2^b β_2m^- targets; (■) H-2^b β_2m^+ targets; and (▲) H-2^k β_2m^+ targets. The H-2^b β_2m^+ and β_2m^- targets were from (B6 × 129)F2-4 animals, and the H-2^k targets were from B10.BR mice.
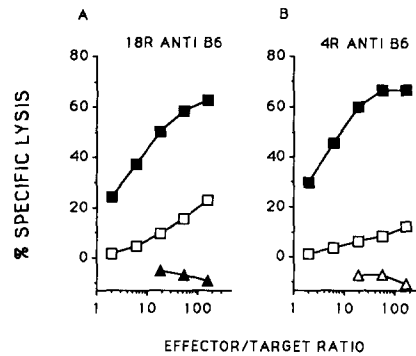

Figure 3. Lysis of β_2m^- target cells by polyclonal allospecific CTL specific for D^b region molecules, or K^b A^b region molecules. CTL specific for D^b region molecules were raised in secondary B10.A(18R) anti-B6 MLC (*A*). CTL specific for K^b and A^b region molecules were raised in secondary B10.A(4R) anti-B6 MLC. The CTL were tested for lysis of the indicated target cells. (■) H-2^b β_2m^+ targets; (□) H-2^b β_2m^- targets; (▲) B10.A(18R) targets; and (△) B10.A(4R) targets.

the conditions of the MLC apparently do not support the growth or activation of NK cells.

To identify the MHC molecule(s) responsible for lysis of β_2m^- target cells, we used spleen cells from MHC-recombinant congenic mice as responder cells to generate polyclonal CTL specific for K^b or D^b. By stimulating B10.A(18R) (K^{b}A^{b}E^{b}D^d) spleen cells with B6 cells, CTL specific for D^b were obtained. B10.A(4R) (K^{k}A^{k}E^{null}D^b) responder spleen cells stimulated with B6 cells resulted in a population containing K^b-specific CTL. Fig. 3 shows that both populations lysed β_2m^+, H-2^b Con A blast target cells, and with less efficiency, β_2m^-, H-2^b target cells. In this and other experiments, B10.A(18R) anti-B6 CTL achieved better and more reproducible lysis of β_2m^- target cells than did the B10.A(4R) anti-B6 CTL. These results suggest that anti-D^b CTL are more efficient than anti-K^b CTL at lysing β_2m^- target cells.

To directly test whether the B10.A(18R) anti-B6 CTL recognize D^b on β_2m^- target cells, antibody blocking experiments were performed. These experiments employed the mAb B22-249.1, which recognizes the $\alpha1$ domain of D^b. Binding of B22-249.1 mAb has been used as evidence that the $\alpha1$ domain of D^b is in a properly folded conformation (1, 3, 10). To minimize any sources of soluble β_2m in the experiment, the target cells were generated by culture in AIM-V medium, the assay was performed in AIM-V medium, and purified B22-249.1 IgG was employed. Nonsaturating effector/target ratios were used, based on a preliminary titration experiment (Fig. 4 *A*). As shown in Fig. 4 *B*, B22-249.1 IgG, in a dose-dependent manner, inhibited lysis of both β_2m^+ and β_2m^- target cells by the B10.A(18R) anti-B6 CTL. The antibody had no effect on lysis of H-2^k targets by B10.A(18R) anti-B10.BR CTL, demonstrating the specificity of the effect. These results demonstrate directly that allospecific CTL can recognize D^b molecule on β_2m^- cells. The results further suggest that the D^b molecules recognized are in a folded conformation detected by the B22-249.1 mAb.
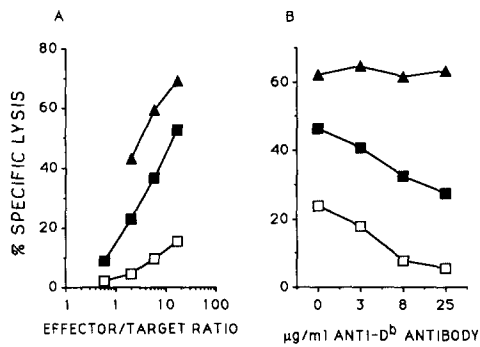
Although the previous experiments were performed under

Figure 4. Inhibition of D^b region-specific CTL by D^b-specific mAb. In A, D^b-specific CTL were titrated on H-2^b β_2m$^+$ targets (■) or H-2^b β_2m$^-$ targets (□); and control H-2^k-specific CTL were titrated on H-2^k targets (▲). In B, purified anti-Db antibodies (B22-249.1 mAb) were titrated into each CTL-target reaction at a constant E/T ratio of 17:1.

Figure 5. Lysis of β_2m$^-$ targets by β_2m$^-$ CTL. Anti-H-2^b CTL were prepared in secondary MLC against H-2^b (B6) stimulator cells. Responding CTL were from normal B10.BR mice (A), or from bone marrow chimeras of the type (H-2^k β_2m$^-$ → B10.BR) (B). They were tested for lysis of the indicated target cells: H-2^b β_2m$^+$ targets (■, ●); H-2^b β_2m$^-$ targets (□, ○); and H-2^k β_2m$^+$ targets (▲). Before the assay, the CTL were depleted of class I$^+$ cells (●, ○), or not (■, □, ▲), and washed into serum-free (AIM-V) medium.

conditions minimizing sources of soluble β_2m, it remained possible that β_2m from the CTL themselves was transferred to the target cells during the cytolysis assay. To address this possibility, we sought to employ a population of CTL that were β_2m$^-$. β_2m$^-$ mice are deficient in CD8$^+$ CTL. However, differentiation of β_2m$^-$ hematopoietic stem cells in irradiated β_2m$^+$ mice leads to the efficient differentiation of CD8$^+$ CTL precursors under the influence of the host thymus (Bix and Raulet, manuscript submitted for publication). Therefore, to generate β_2m$^-$, H-2^k CTL precursors, we produced radiation chimeras by inoculating irradiated B10.BR mice with bone marrow cells from H-2^k, β_2m$^-$ donors. 13 wk later, spleen cells from the reconstituted chimeras and control B10.BR mice were isolated and stimulated twice consecutively in MLC with B6 spleen cells to generate anti-H-2^b CTL. To deplete the minor fraction of radioresistant hematopoietic cells of host (class I$^+$) origin often found in radiation chimeras, the chimeric CTL population was depleted of residual radioresistant β_2m$^+$, H-2^k cells by treatment with anti-H-2^k mAb (16-1-2n) plus complement on the day of the cytolysis assay. As before, the cytolysis assay and target cell cultures were performed in AIM-V medium. Thus, essentially no β_2m was present in this experiment.

As shown in Fig. 5 B, the β_2m$^-$, anti-H-2^k CTL lysed β_2m$^-$, H-2^b target cells. Comparing dose–response curves, the lysis of β_2m$^-$ versus that of β_2m$^+$ target cells by the β_2m$^-$ CTL was similar to that mediated by the β_2m$^+$ (B10.BR) CTL, although the latter population was somewhat more potent (compare Fig. 5, A and B). The depletion of residual H-2^{k+} cells from the chimeric CTL population made no difference in the dose–response curves. Depletion of H-2^k cells from the control B10.BR CTL population eliminated CTL activity, demonstrating the effectiveness of the antibody plus complement treatment. These results suggest that lysis of β_2m$^-$ H-2^b target cells by allospecific CTL does not depend upon β_2m.

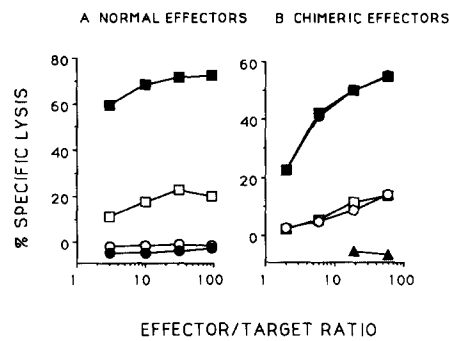The data in the previous experiments do not distinguish whether most anti-H-2^b CTL in the populations lyse β_2m$^-$,

H-2^b targets poorly, or alternatively, that only a subset of anti-H-2^b CTL lyse β_2m$^-$ targets. As one means of addressing this question, we generated CTL by stimulating B10.BR responder spleen cells twice consecutively with either β_2m$^-$ or β_2m$^+$ H-2^b stimulator spleen cells in MLC. Although significant CTL activity was not induced after only one round of stimulation with β_2m$^-$ cells (4), significant activity was usually evident after two or more rounds of stimulation (Fig. 6). These effector cells lysed β_2m$^+$ and β_2m$^-$ H-2^b targets with nearly equal efficiency, but did not lyse B10.BR target cells (Fig. 6 B). As in previous experiments, the CTL raised against β_2m$^+$, H-2^b stimulator cells lysed β_2m$^+$ target cells much better than they lysed β_2m$^-$ target cells. These data suggest that a subset of anti-H-2^b CTL lyse β_2m$^+$ and β_2m$^-$ target cells with nearly equal efficiency.

To examine the expression of serologically detected class I epitopes on the β_2m$^-$ cells, we stained lymph node cells with a conformation-dependent anti-class I antibody (B22-249.1), and examined them by flow cytometry. To ensure that no β_2m was present in the experiment, we employed purified antibody preparations, harvested the cells in serum-free medium, and stained the cells in medium that contained
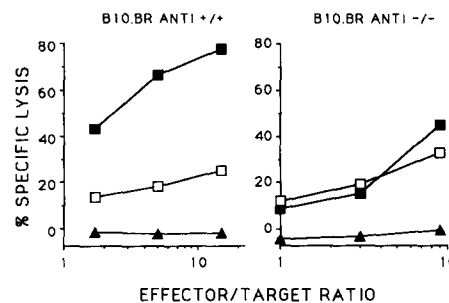

Figure 6. Stimulation with β_2m$^-$ cells enriches CTL that react equally well with β_2m$^-$ and β_2m$^+$ target cells. CTL were raised by stimulating B10.BR spleen cells with H-2^b β_2m$^+$ (A) or H-2^b β_2m$^-$ (B) spleen cells. CTL from secondary MLC were tested against H-2^b β_2m$^+$ (■), H-2^b β_2m$^-$ (□), or H-2^k β_2m$^+$ (B10.BR) (▲) target cells.
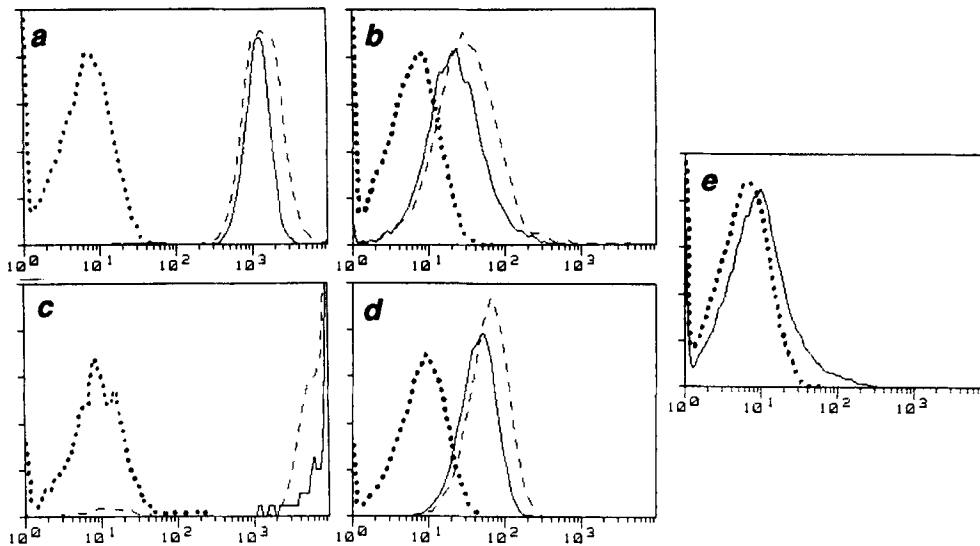
β-galactosidase as a source of protein, instead of serum. We compared the staining of β_2m$^-$ lymph node cells to the staining of the β_2m$^-$ R1E/D^b tumor cells. As shown in Fig. 7, β_2m$^-$ lymph node cells stained with B22-249.1 mAb, although the intensity of staining was about 1/20th that of β_2m$^+$ cells. This level of staining is similar to that observed in the presence of serum, which provides a source of β_2m. The specificity of the staining is shown by the failure of the antibody to significantly stain B10.BR (H-2^k cells).

To our surprise, β_2m$^-$ R1E/D^b cells also stained specifically with B22-249.1 mAb. The absolute intensity of staining was similar to that of β_2m$^-$ lymph node cells. By comparison, the β_2m$^+$ variant of these cells, R1E/D^b/β_2m, stained more intensely than β_2m$^+$ lymph node cells. Considering that the tumor cells are larger than lymph node cells, it appears that the β_2m$^-$ tumor cells may express a lower density of D^b on their surface than the β_2m$^-$ lymph node cells.

Discussion

Our results suggest that the D^b molecule can assume a functional conformation in the absence of β_2m. This conclusion is based on the findings that β_2m$^-$ cells were stained by the conformation-dependent B22-249.1 mAb in serum-free medium, and were lysed by allospecific β_2m$^-$ CTL in serum-free medium. Furthermore, the D^b-specific lysis of β_2m$^-$ cells could be blocked with the conformation-dependent B22-249.1 mAb.

Our results with the R1E/D^b cell line contrast with previous results suggesting that this cell line fails to present properly conformed H chain at the cell surface (1). The different results are not due to the use of different reagents, because the earlier studies, like ours, employed the B22-249.1 mAb. It is likely that previous studies with R1E/D^b were not sufficiently sensitive to detect correctly conformed D^b on the cells, since we find that successful detection requires a highly sensitive staining protocol. Although R1E/D^b

stains relatively weakly compared with the staining of R1E/D^b/β_2m, the results indicate that the expression of low levels of conformed D^b H chains is a property of both normal and transformed β_2m$^-$ cells.

We further find that a subset of allospecific CTL, generated by multiple stimulations with β_2m$^-$ cells, lyse β_2m$^-$ and β_2m$^+$ cells equally well. This observation, taken together with other findings, is most simply explained by assuming that these effectors recognize a set of properly conformed H chains, free of β_2m, that are roughly equally represented on β_2m$^+$ and β_2m$^-$ cells. Whether these free H chains are associated with peptides is not known. Evidence for CTL recognition of peptide-free, β_2m-containing class I molecules has been reported (11). However, it seems unlikely that H chains free of both peptides and β_2m would maintain a functional conformation at the cell surface.

We favor an alternative possibility, that is suggested by recent findings that in the absence of β_2m, free D^b H chains incubated with physiologically relevant, nonamer peptides assume a conformation reactive with B22-249.1 mAb (3). High (micromolar) concentrations of peptide were required to observe this effect. Nonetheless, it is possible that some cellular peptides bind to free D^b H chains in β_2m$^-$ cells tightly enough to stabilize the properly folded conformation for transport to the cell surface. Such complexes might be expected to have a short half-life in the absence of β_2m. Alternatively, a subset of peptides may bind free H chains very tightly, resulting in stable complexes at the cell surface.

A great deal of recent effort has been devoted to attempts to understand the assembly of class I H chains with antigenic peptides, and β_2m in vivo (3, 11–14). An important question is whether peptides associate with H chain before or after β_2m, and the relative contribution of peptide binding and β_2m binding to the stability of the trimolecular complex. Our results raise the possibility that, at least in some instances, peptides may bind H chains in the absence of β_2m with sufficient affinity to establish a functionally conformed molecule on the cell surface.

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