Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7447–7451, October 1986
Immunology

β_2-Microglobulin is not required for cell surface expression of the murine class I histocompatibility antigen H-2D^b or of a truncated H-2D^b

(membrane biosynthesis/R1E cell line)

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Communicated by Phillip A. Sharp, June 19, 1986

ABSTRACT **β_2-Microglobulin (β_2m) has been thought essential for transport of all major histocompatibility complex class I antigens to the cell surface. Here, we show that the mouse class I antigen H-2D^b is expressed at the cell surface even when there is no β_2m present within the cell. This was established by transfecting the H-2D^b gene into the R1E cell line, which lacks β_2m. The conformation of the D^b antigen expressed by the R1E transfectant is very different from that of the native molecule. This D^b antigen is not recognized by D^b-allospecific and D^b-restricted cytotoxic T lymphocytes or by most monoclonal antibodies to the native D^b. We show further that a deletion construct of the D^b gene, which consists of exon 1 linked to exons 4–8, expresses a truncated D^b antigen lacking domains 1 and 2 [D^b−(1+2)] at the cell surface after transfection into the R1E line. Previous biochemical and crystallographic data have indicated that domain 3 is associated with β_2m; unexpectedly, D^b−(1+2) does not associate with β_2m when the mouse β_2m^b gene is transfected into the R1E transfectant expressing the truncated D^b. This suggests that interactions with domains 1 and 2 are important for the paired association of domain 3 and β_2m in the native D^b antigen.**

The major histocompatibility complex (MHC) class I antigens are cell surface glycoproteins comprising a heavy chain (≈45 kDa) associated noncovalently with β_2-microglobulin (β_2m, ≈12 kDa) (1, 2). The heavy chain has three external domains, each of about 90 amino acids, a short transmembrane segment, and a cytoplasmic peptide of about 35 amino acids. Biochemical and crystallographic data suggest that β_2m is associated with the third external domain of the heavy chain and support the overall structure of the class I antigen depicted in Fig. 1 (4, 5). β_2m has been thought essential for cell surface expression of MHC class I antigens, based on studies of the human Daudi and murine R1E cell lines (6–9). The Daudi cell line was established from a Burkitt lymphoma. R1E is a mutant cell line, derived from the C58 mouse thymoma R1.1 by chemical mutagenesis and *in vitro* selection for loss of TL expression. Both lines have mutant β_2m genes, fail to synthesize β_2m protein, and do not express class I antigens at the cell surface (10, 11). When β_2m protein is introduced into these lines by fusion with another cell, class I antigens derived from Daudi and R1E are expressed at the cell surface of the hybrids (6, 7, 9).

Analysis of HLA-A,B biosynthesis in the human B-lymphoblastoid cell line T5-1, which expresses HLA-A,B antigens normally, indicates that HLA-A,B heavy chains associate posttranslationally with β_2m in the endoplasmic reticulum (12). Association with β_2m is required for the rapid processing of the high-mannose oligosaccharides on the heavy chains that occurs in the Golgi apparatus (12, 13). HLA-A,B heavy chains in Daudi are glycosylated and inserted in the endoplasmic reticulum, where they are relatively stable (14). These observations suggest that association of HLA-A,B heavy chains with β_2m is required for transport from the endoplasmic reticulum to the Golgi apparatus and then on to the cell surface. Murine H-2 class I antigen biosynthesis is believed to be similar, but there is less direct evidence (15).

Recently Potter *et al.* (16, 17) have reported that the mouse class I antigen H-2D^b may not be associated with β_2m at the surface of the EL4/Mar line even though β_2m is present within the cell. Their conclusions have been questioned because the results they presented could also be explained by transport of D^b to the cell surface in association with the endogenous β_2m of EL4/Mar and then exchange of the murine β_2m with bovine β_2m from the cell culture medium (18, 19). To resolve the question of whether β_2m is required for expression of the D^b heavy chain, we have transfected the H-2D^b gene into R1E cells. We confirmed, by RNA blot hybridization analysis, that the subclone of the R1E line we used in these studies contains no β_2m RNA transcripts and, therefore, no β_2m protein (data not shown). We also transfected the mouse β_2m^b gene into the R1E transfectant expressing the D^b gene. This allowed us to compare the biosynthesis and function of D^b in the presence and absence of β_2m. In a similar fashion we also investigated the requirement for β_2m in biosynthesis of a truncated D^b antigen that lacks domains 1 and 2, D^b−(1+2). We establish first that the D^b heavy chain does not require β_2m for transport to the cell surface. Second, interactions with domains 1 and 2 are important for the paired association of domain 3 and β_2m in the D^b antigen. Third, comparison of antibody and cytotoxic-T-lymphocyte (CTL) recognition of D^b in the presence and in the absence of β_2m leads us to believe that the reports that the D^b antigen on EL4/Mar is free of β_2m are probably incorrect and that, instead, D^b may be associated with bovine β_2m from the cell culture medium.

MATERIALS AND METHODS

Plasmid Construction. The Mo/D^b gene was constructed in three steps. First, the 2400-bp *Bam*HI fragment, containing exons 4–8, was inserted in the *Bam*HI site of the expression vector pTCF, which contains the ampicillin-resistance gene and the aminoglycoside 3′-phosphotransferase gene (20). Second, this subclone was partially digested with *Bam*HI, the linearized vector singly cut with *Bam*HI was purified by gel

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Abbreviations: β_2m, β_2-microglobulin; CTL, cytotoxic T lymphocyte; LTR, long terminal repeat; mAb, monoclonal antibody; MHC, major histocompatibility complex; Mo-MuLV, Moloney murine leukemia virus; bp, base pair(s); kb, kilobase(s).

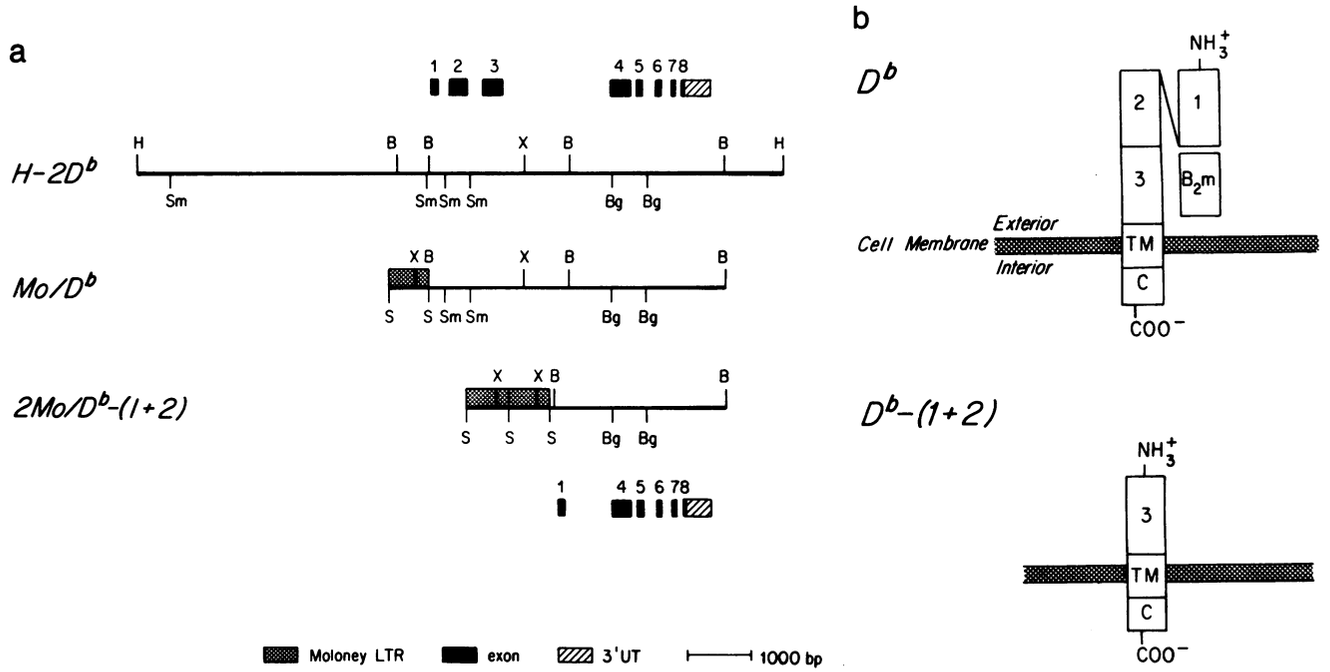

FIG. 1. (*a*) Restriction maps of H-2D^b, Mo/D^b, and 2Mo/D^b−(1+2) genes. Restriction sites (B, *Bam*HI; Bg, *Bgl* II; H, *Hin*dIII; S, *Sal* I; Sm, *Sma* I; X, *Xba* I) shown are those used to construct and confirm structure of Mo/D^b and 2Mo/D^b−(1+2) genes. The locations of the exons of the H-2D^b, Mo/D^b, and 2Mo/D^b−(1+2) genes are shown (3). LTR, long terminal repeat 3'UT, 3' untranslated region; bp, base pairs. (*b*) Domain structure of D^b and D^b−(1+2) antigens. 1, 2, and 3 are external domains; TM, transmembrane segment; C, cytoplasmic peptide.

electrophoresis, and the 2200-bp *Bam*HI fragment, containing exons 1–3, was inserted. Clones with the 2200-bp *Bam*HI fragment in the correct site and orientation were isolated. Third, the Moloney murine leukemia virus (Mo-MuLV) LTR (provided by R. Grosschedl, University of California, San Francisco) was inserted as a 650-bp *Sal* I fragment in the *Sal* I site of the pTCF vector, adjacent to exon 1 of D^b. The Mo/D^b−(1+2) gene was similarly constructed. First, the subclone of the 2400-bp *Bam*HI fragment (exons 4–8) in pTCF was linearized by partial digestion with *Bam*HI (see above), the recessed 3' termini were repaired by DNA synthesis with the Klenow fragment of DNA polymerase I, and the 240-bp *Sma* I fragment, containing D^b exon 1, was inserted. Second, two Mo-MuLV LTRs were inserted in the *Sal* I site of the pTCF vector adjacent to exon 1 of D^b. These constructs were made by standard methods (3, 21).

Transfection and Cell Culture. R1E cells were transfected with the Mo/D^b, 2Mo/D^b−(1+2) and β_2m^b genes by electric field-mediated transfection as described by Potter *et al.* (22). Transfectants R1E/D^b and R1E/D^b−(1+2) were selected and then grown in the presence of the neomycin analog G418 (2 mg/ml); R1E/β_2m in the presence of mycophenolic acid (0.25 μg/ml), hypoxanthine (15 μg/ml), and xanthine (250 μg/ml); and R1E/D^b+β_2m and R1E/D^b−(1+2)+β_2m in the presence of G418, mycophenolic acid, hypoxanthine, and xanthine. Medium was RPMI 1640 with glutamine, penicillin, streptomycin, and 10% fetal bovine serum.

RNA Analysis. Total RNA was isolated from cell lines by the guanidinium isothiocyanate/CsCl method (21). RNAs were separated by electrophoresis in formaldehyde-containing 1% agarose gels (10 μg of RNA loaded per lane) and transferred to GeneScreen filters (21, 23). Filters were hybridized and washed essentially as described by Cate *et al.* (24).

Immunoprecipitation. Cells were surface labeled with ^{125}I as described (25). Immunoprecipitations were carried out essentially as described by Krangel *et al.* (26).

CTL Assay. D^b-allospecific CTLs were generated by stimulating 5 × 10^6 AKR/J spleen cells with 5 × 10^6 γ-irradiated B10.A(2R) spleen cells. Mo-MuLV-specific, D^b-restricted CTLs were generated by stimulating 5 × 10^6 C57BL/6 Mo-MuLV-immune spleen cells with 3 × 10^5 γ-irradiated MBL-2 cells (27). Target cell lines were labeled with ^{111}In by incubating 2.5 × 10^6 cells in 0.25 ml of RPMI 1640/10% fetal bovine serum with 25 μCi (1 Ci = 37 GBq) of ^{111}In (as indium oxide) for 15 min. The spontaneous release of ^{111}In from labeled cells ranged from 1.9% to 11.9%.

RESULTS AND DISCUSSION

Construction and Transfection of Mo/D^b and 2Mo/D^b−(1+2) Genes. We replaced the 5' flanking sequences of the D^b and truncated D^b genes with the Mo-MuLV LTR (Fig. 1*a*) because of our earlier observation that the level of K^b mRNA was very low in R1E cells stably transfected with the complete K^b gene (data not shown). The D^b gene construct (Mo/D^b) contains the Mo-MuLV LTR linked to exons 1–8 of the D^b gene and encodes a complete D^b heavy chain. The truncated D^b gene construct [2Mo/D^b−(1+2)] has two Mo-MuLV LTRs linked to exons 1, 4, 5, 6, 7, and 8 of D^b and encodes a truncated D^b heavy chain consisting of the third external domain, transmembrane segment, and cytoplasmic peptide [D^b−(1+2)] (Fig. 1*b*). R1E cells were transfected with the Mo/D^b and 2Mo/D^b−(1+2) genes linked to the selectable marker aminoglycoside phosphotransferase, by electric field-mediated transfection (22). Clones resistant to G418 were screened for antibody binding of the monoclonal antibody (mAb) 28-14-8, which recognizes a determinant located on domain 3 of D^b (3). Clones R1E/D^b and R1E/D^b−(1+2), from transfections with the Mo/D^b and 2Mo/D^b−(1+2) genes respectively, gave a high level of binding of 28-14-8 and were selected for further study. The R1E/D^b, R1E/D^b−(1+2), and R1E lines were then transfected with the mouse β_2m^b gene (11), linked to the selectable marker guanine phosphoribosyltransferase, to compare the biosynthesis and function of D^b and D^b−(1+2) antigens in the presence and absence of β_2m. Clones resistant to both G418 and mycophenolic acid were screened with the K^k- and

D^k-specific mAb 15-1-5 for cell surface expression of the endogenous K^k and D^k antigens of the R1E line (H-2^k) (28). Three clones, R1E/D^b+β_2m, R1E/D^b−(1+2)+β_2m, and R1E/β_2m, from transfection of R1E/D^b, R1E/D^b−(1+2) and R1E cells respectively, expressed reasonable levels of K^k and D^k antigens and were retained for further study.

The D^b and D^b−(1+2) mRNAs in the transfectants were analyzed by hybridization to an oligonucleotide specific for exon 4 of D^b (Fig. 2). The level of D^b mRNA in R1E/D^b is similar to that in EL4, an H-2^b murine lymphoma, and the level of D^b mRNA in R1E/D^b+β_2m is approximately 4 times greater. The level of D^b−(1+2) mRNA in R1E/D^b−(1+2) is approximately one-fourth, and in R1E/D^b−(1+2)+β_2m, one-half, that of D^b mRNA in EL4.

Expression of D^b and D^b−(1+2) Antigens. The five R1E transfectants and control R1E, EL4, and R1.1 lines were screened for binding of mAbs specific for H-2D^b, -K^b, -K^k and -D^k and β_2m (Table 1). The binding of mAb 28-14-8 suggests that D^b and D^b−(1+2) antigens are expressed at a high level on the cell surface both in the presence and in the total absence of β_2m in the cells. The three β_2m gene transfectants [R1E/β_2m, R1E/D^b+β_2m, and R1E/D^b−(1+2)+β_2m] all reexpress the K^k and D^k antigens, albeit at a level one-fifth that of R1.1. Sixteen other clones from two transfections of the R1E line with the β_2m^b gene all had the same low level of K^k and D^k expression (data not shown). Transfectant R1E/D^b+β_2m shows a high level of binding of β_2m^b-specific mAb Lym11 (29), whereas R1E/D^b−(1+2)+β_2m and R1E/β_2m show similar low levels of binding of Lym11, which we attribute in both cases to β_2m^b associated with D^k and K^k. This indicates that D^b is associated with β_2m when present within the cell (R1E/D^b+β_2m) but D^b−(1+2) is not. The high level of binding of Lym11 to R1E/D^b+β_2m cells also suggests that the low expression of

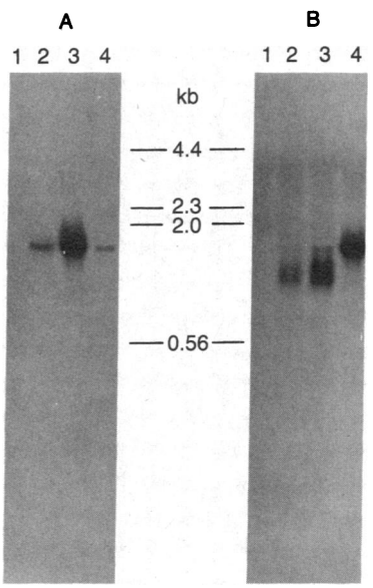

FIG. 2. Blot hybridization analysis of D^b and D^b−(1+2) mRNAs. (*A*) Lanes 1–4: RNA from cell lines R1E, R1E/D^b, R1E/D^b+β_2m, and EL4, respectively. Exposure time was 2 hr. (*B*) Lanes 1–4: RNA from R1E, R1E/D^b−(1+2), R1E/D^b−(1+2)+β_2m, and EL4. Exposure time was 16 hr. The D^b-specific oligonucleotide probe is complementary to nucleotides 25–47 of exon 4. The D^b−(1+2) mRNA is smaller than the D^b mRNA by the length expected due to deletion of exons 2 and 3 (270 and 276 nucleotides long, respectively). In both R1E/D^b−(1+2) and R1E/D^b−(1+2)+β_2m there is a low level of a larger RNA. This may be either a partially spliced precursor of D^b−(1+2) mRNA or a mature form of D^b−(1+2) mRNA that is initiated from the promoter in the first rather than the second of the Mo-MuLV LTRs in the 2Mo/D^b−(1+2) gene. kb, Kilobases.

Table 1. H-2 class I- and β_2m-specific mAb binding to D^b and D^b−(1+2) antigens

Cell line	Antibody binding, ^{125}I cpm			
	28-14-8 (D^b)	K10-56.1 (K^b)	Lym11 (β_2m^b)	15-1-5 (K^k/D^k)
EL4	3618	2651	2203	137
R1E/D^b	2969	105	105	125
R1E/D^b+β_2m	6717	109	1576	343
R1E/D^b−(1+2)	3685	95	104	123
R1E/D^b−(1+2)+β_2m	5516	97	407	337
R1E/β_2m	114	107	421	292
R1E	142	103	107	105
R1.1	88	82	88	1532

The specificities of the mAbs (shown in parentheses) have been characterized (3, 28, 29). Binding assays were performed essentially as described (3). Results are expressed as cpm of ^{125}I bound, each result is the average from triplicate samples, and the variation in all cases was not >10%.

K^k and D^k on R1E/D^b+β_2m, R1E/D^b−(1+2)+β_2m, and R1E/β_2m is not due to a lack of β_2m protein in these transfectants.

The conformation of the D^b antigen expressed on R1E/D^b was investigated with a panel of six mAbs specific for determinants on domains 1 and 2 of the native D^b (Table 2). None of these mAbs bind to R1E/D^b, but all six give the same pattern of binding on R1E/D^b+β_2m and EL4. This establishes that the conformation of domains 1 and 2 of D^b on the surface of R1E/D^b is very different from that of the native molecule. In contrast, the binding of 28-14-8 to R1E/D^b indicates that the determinant recognized by this mAb is retained on domain 3 of D^b even in the absence of β_2m. The conformation of domain 3 of D^b on R1E/D^b thus appears to be less disrupted than that of domains 1 and 2, which is perhaps unexpected in view of the paired association of domain 3 and β_2m in the native D^b antigen.

Cell surface expression of D^b, D^b−(1+2), D^k, and K^k antigens was analyzed by immunoprecipitation of lysates from surface-radioiodinated cells with a rabbit anti-H2 antigen serum (anti-H-2, ref. 30) and mAb 28-14-8 (Fig. 3*a*). The D^b heavy chain is specifically precipitated from R1E/D^b by both anti-H2 and 28-14-8 and is detected as a band at ≈46 kDa. This establishes that the D^b heavy chain is expressed at a high level at the cell surface of R1E/D^b even though there is no β_2m protein within the cell. The variation in electrophoretic mobility of the D^b heavy chain is due to heterogeneity in carbohydrate chains, since endoglycosidase F digestion generates deglycosylated heavy chains of 30 kDa from both R1E/D^b and R1E/D^b+β_2m (data not shown). With longer autoradiographic exposure, the precipitation with 28-14-8 shows an additional band at 12 kDa (Fig. 3*b*), which we believe is bovine β_2m taken up from the cell culture medium (19). In contrast, the D^b heavy chains precipitated by

Table 2. D^b-specific mAb binding to D^b antigens expressed with and without β_2m

mAb	Location of allodeterminant	Antibody binding, ^{125}I cpm		
		R1E/D^b	R1E/D^b+β_2m	EL4
27-11-13	D^b domain 1	153	5669	4601
B22-249.1	D^b domain 1	166	3762	2487
28-11-5	D^b domain 2	144	2021	1469
H141-31	D^b domain 2	72	1674	1260
K15-25	D^b domain 1 and/or 2	137	2653	1510
28-8-6	D^b/K^b domain 1 and/or 2	103	3076	2008
28-14-8	D^b domain 3	1956	5926	2763
K10-56.1	K^b domain 1 and/or 2	66	51	1893

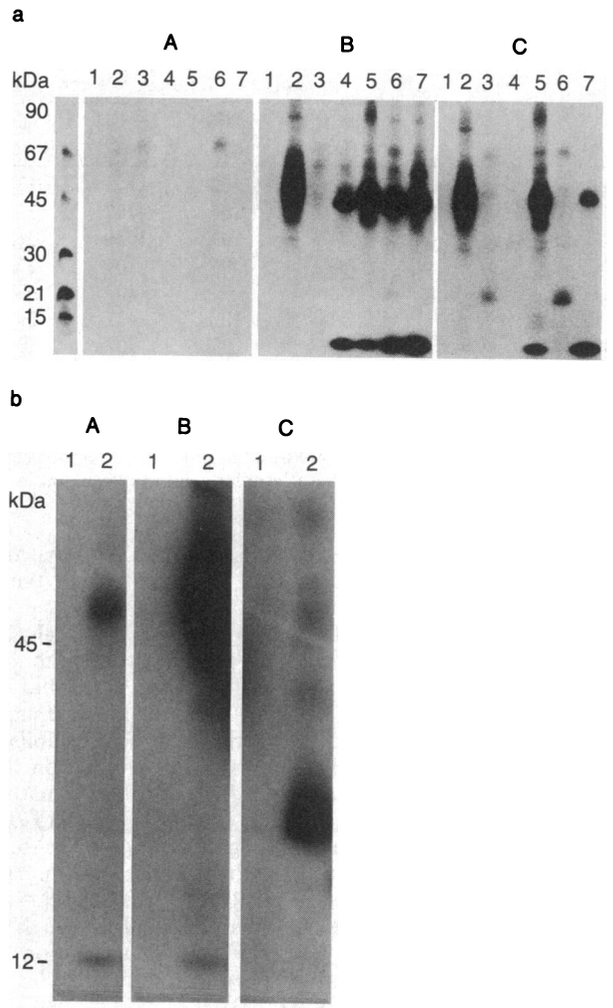

FIG. 3. Immunoprecipitation of D^b and $D^b-(1+2)$ antigens from surface-radioiodinated cells. (*a*) Results of precipitation with nonimmune rabbit serum (*A*), rabbit anti-H2 antigen serum (anti-H-2) (*B*), and with mAb 28-14-8 (*C*). Lanes: 1, R1E; 2, R1E/D^b; 3, R1E/$D^b-(1+2)$; 4, R1E/β_2m; 5, R1E/$D^b+\beta_2$m; 6, R1E/$D^b-(1+2)+\beta_2$m; 7, EL4. Precipitation was achieved with 2 μl of nonimmune rabbit serum, anti-H-2, or 28-14-8. Relative cpm of ^{125}I-labeled lysates used for precipitation were as follows: R1E, 2×; R1E/D^b, 4×; R1E/$D^b-(1+2)$, 4×; R1E/β_2m, 3×; R1E/$D^b+\beta_2$m, 1×; R1E/$D^b-(1+2)+\beta_2$m, 4×; EL4, 1×. Gels were exposed to film for 2 days at −70°C. Standards are shown in lane at far left. (*b*) Precipitation of lysates of surface-labeled R1E/$D^b+\beta_2$m (*A*), R1E/D^b (*B*), and R1E/$D^b-(1+2)+\beta_2$m (*C*) with control P3X63Ag8 culture supernatant (31) (lanes 1) or mAb 28-14-8 (lanes 2). Precipitation was achieved with 50 μl of P3X63Ag8 or 28-14-8 culture supernatants. The relative cpm of ^{125}I were as follows: R1E/$D^b+\beta_2$m, 1×; R1E/D^b, 4×; R1E/$D^b-(1+2)+\beta_2$m, 8×. For *A*, autoradiographic exposure was overnight; for *B* and *C*, exposure was for 7 days.

28-14-8 from both R1E/$D^b+\beta_2$m and EL4 are strongly associated with β_2m. Isoelectric focusing indicates that in this case the β_2m is a mixture of murine and bovine molecules (data not shown). $D^b-(1+2)$ is specifically precipitated from R1E/$D^b-(1+2)$ and R1E/$D^b-(1+2)+\beta_2$m by mAb 28-14-8 as a species of 24 kDa (Fig. 3*a*). The level of expression of $D^b-(1+2)$ is higher on R1E/$D^b-(1+2)+\beta_2$m, which we believe results from the higher level of $D^b-(1+2)$ mRNA in this transfectant. Removal of carbohydrate with endoglycosidase F reduces the apparent molecular mass of $D^b-(1+2)$ to 18.5 kDa, which is in agreement with the size predicted from the primary structure (data not shown). $D^b-(1+2)$ is not stably associated with β_2m on the cell surface even when β_2m protein is present within the cell (R1E/$D^b-(1+2)+\beta_2$m) (Fig. 3*b*). The K^k and D^k heavy chains, together with β_2m, are precipitated by anti-H-2 from R1E/$D^b+\beta_2$m, R1E/$D^b-(1+2)+\beta_2$m, and R1E/β_2m (Fig. 3*a*).

CTL Recognition of D^b and $D^b-(1+2)$ Antigens. Recognition of the D^b and $D^b-(1+2)$ antigens by CTLs was investigated with both D^b-allospecific CTLs and D^b-restricted Mo-MuLV-specific CTLs (Tables 3 and 4). The D^b antigen expressed on R1E/D^b is not recognized by the D^b-specific CTLs. This result is in agreement with our mAb binding data (Table 2), which indicate that the conformation of the D^b on R1E/D^b is very different from that of the native molecule. The $D^b-(1+2)$ antigen is also not recognized by the D^b-specific CTLs; this is expected, as CTL recognition sites on D^b, like those on other H-2 class I antigens, map to domains 1 and 2 (3). McCluskey *et al.* (32) have obtained similar results with the equivalent L^d and D^d truncated antigens [$L^d-(1+2)$ and $D^d-(1+2)$] expressed in L cells but were able to generate CTLs that recognized these antigens by *in vivo* priming and *in vitro* restimulation with the L cells expressing them.

Structure and Biosynthesis of MHC Class I Antigens. We have shown that the D^b class I heavy chain is expressed at the cell surface even when there is no β_2m present within the cell. Potter *et al.* (16, 17) have analyzed the expression of the D^b antigen on EL4/Mar, which is a mutant line derived from the H-2^b thymoma EL4. They reported that the EL4/Mar D^b may not be associated with β_2m at the cell surface even though β_2m is present within the cell. In contrast, our results show that the native D^b is associated with β_2m on the cell surface of both R1E/$D^b+\beta_2$m and EL4 (Table 1 and Fig. 3). Potter *et al.* (16) further reported that the EL4/Mar D^b is recognized by mAb B22-249.1 and by D^b-allospecific CTLs. We have shown in this paper that the D^b antigen expressed on R1E/D^b is not recognized, whereas the native D^b expressed on R1E/$D^b+\beta_2$m and EL4 is recognized by B22-249.1 and by D^b-specific CTLs (Tables 2–4). This comparison suggests that the EL4/Mar D^b is not free of β_2m and instead may be associated with bovine β_2m from the culture medium (18, 19).

Earlier studies (12, 33) have suggested that the conformation of HLA-A,B heavy chains is markedly altered when they are not associated with β_2m. Here, the lack of recognition of the D^b antigen on R1E/D^b by both D^b-specific CTLs and most D^b-specific mAbs clearly establishes that this antigen has a very different conformation from that of the native antigen. Thus the D^b heavy chain does not appear to retain a more native conformation in the absence of β_2m than do other MHC class I heavy chains.

The low expression of K^k and D^k on the three β_2m gene transfectants [R1E/β_2m, R1E/$D^b+\beta_2$m, and R1E/$D^b-(1+2)+\beta_2$m] suggests that there may be some additional defect in the R1E line which affects these antigens. Thus it is not clear that the failure of D^k and K^k to be expressed on the surface of R1E cells results only from the absence of β_2m.

Table 3. Allospecific CTL recognition of D^b and $D^b-(1+2)$ antigens

Cell line	% specific ^{111}In release			
	E/T 3:1	E/T 6:1	E/T 12:1	E/T 25:1
MBL-2	25.7	34.5	41.6	47.8
R1E/D^b	−2.4	−1.8	−0.9	0.3
R1E/$D^b+\beta_2$m	16.0	17.4	24.4	31.3
R1E/$D^b-(1+2)$	2.1	2.9	4.3	8.2
R1E/$D^b-(1+2)+\beta_2$m	1.0	2.3	4.5	8.4
R1E/β_2m	2.3	4.0	6.2	6.7
R1E	0.9	2.2	2.5	4.5

E/T, effector/target cell ratio.

Table 4. Recognition of D^b and $D^b-(1+2)$ antigens by Mo-MuLV-specific CTLs

Cell line	% specific ^{111}In release			
	E/T 3:1	E/T 6:1	E/T 12:1	E/T 25:1
MBL-2	25.7	34.5	41.6	47.8
R1E/D^b	0.2	0.0	0.7	0.4
R1E/D^b+β_2m	3.1	12.4	16.0	22.4
R1E/$D^b-(1+2)$	1.6	1.2	0.6	1.2
R1E/$D^b-(1+2)$+β_2m	−0.3	−0.8	−0.4	3.2
R1E/β_2m	−2.2	−0.3	2.0	2.8
R1E	0.9	−0.3	0.6	0.5

MBL-2 is an H-2^b Mo-MuLV-induced lymphoma. The five R1E transfectants and the R1E line were infected with Mo-MuLV as described (27). The ^{111}In release from uninfected target cells was <4% for all lines.

The question of whether D^b is unusual in its ability to be expressed in the absence of β_2m, compared to other MHC class I antigens, also remains to be determined.

The conformation of domains 1 and 2 of K^b and D^b class I antigens is influenced by "lateral" interaction between the two domains (3). Here, analysis of the expression of $D^b-(1+2)$ indicates that "vertical" interaction of domains 1 and 2 with domain 3 and β_2m is important for association of β_2m with the D^b heavy chain. This suggests that, in general, the conformation of MHC class I antigens may be sensitive to alteration, since conformational change in any of the external domains or β_2m may be transmitted to the other domains via both "lateral" and "vertical" interactions. Conformational lability of class I antigens might be important in their ability to direct recognition of viral antigens by CTLs.

We thank Jane Parnes for the β_2m^b gene; Rudi Grosschedl for the Mo-MuLV LTR and advice on electric field-mediated transfection; Sune Kvist, Gunter Hämmerling, Richard Maziarz, and Derry Roopenian for antibodies; and Kevin Conlon for electrode construction. This work was supported by Biogen N.V. and by National Institutes of Health Grant AI15669. J.F. had a Fogerty International fellowship and a Cancer Research Institute fellowship during this work.

1. Nathenson, S. G., Uehara, H., Ewenstein, B. M., Kindt, T. J. & Coligan, J. E. (1981) *Annu. Rev. Biochem.* **50,** 1025–1052.
2. Ploegh, H. L., Orr, H. T. & Strominger, J. L. (1981) *Cell* **24,** 287–299.
3. Allen, H., Wraith, D., Pala, P., Askonas, B. & Flavell, R. A. (1984) *Nature (London)* **309,** 279–281.
4. Yokoyama, K. & Nathenson, S. G. (1983) *J. Immunol.* **130,** 1419–1425.
5. Bjorkman, P., Strominger, J. L. & Wiley, D. C. (1985) *J. Mol. Biol.* **186,** 205–210.
6. Arce-Gomez, B., Jones, E. A., Barnstable, C. J., Solomon, E. & Bodmer, W. (1977) *Tissue Antigens* **11,** 96–112.
7. Fellous, M., Kamoun, M., Wiels, J., Dausset, J., Clements, G., Zeuthen, J. & Klein, G. (1977) *Immunogenetics (N.Y.)* **5,** 423–436.
8. Hyman, R. & Stallings, V. (1976) *Immunogenetics (N.Y.)* **3,** 75–84.
9. Hyman, R. & Stallings, V. (1977) *Immunogenetics (N.Y.)* **4,** 171–181.
10. Rosa, F., Berissi, H., Weissenbach, J., Maroteaux, L., Fellous, M. & Revel, M. (1983) *EMBO J.* **2,** 239–243.
11. Parnes, J. R. & Seidman, J. G. (1982) *Cell* **29,** 661–669.
12. Krangel, M. S., Orr, H. T. & Strominger, J. L. (1979) *Cell* **18,** 979–991.
13. Kornfeld, R. & Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54,** 631–664.
14. Ploegh, H. L., Cannon, L. E. & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2273–2277.
15. Dobberstein, B., Garoff, H., Warren, G. & Robinson, P. J. (1979) *Cell* **17,** 759–769.
16. Potter, T. A., Boyer, C., Schmitt-Verhulst, A. M., Golstein, P. & Rajan, T. V. (1984) *J. Exp. Med.* **160,** 317–322.
17. Potter, T. A., Zeff, R. A., Schmitt-Verhulst, A. M. & Rajan, T. V. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2950–2954.
18. Maloy, W. L. & Coligan, J. E. (1985) *Immunol. Today* **6,** 263–264.
19. Bernabeu, C., Van de Rijn, M., Lerch, P. G. & Terhorst, C. P. (1985) *Nature (London)* **308,** 642–645.
20. Grosveld, F. G., Lund, T., Murray, E. J., Mellor, A. L., Dahl, H. H. M. & Flavell, R. A. (1982) *Nucleic Acids Res.* **10,** 6715–6732.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
22. Potter, H., Weir, L. & Leder, P. (1985) *Proc. Natl. Acad. Sci. USA* **81,** 7161–7165.
23. Church, G. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
24. Cate, R. L., Mattaliano, R. J., Hession, C., Tizard, R., Farber, N. M., Cheung, A., Ninfa, E., Frey, A. Z., Gash, D. J., Chow, E. P., Fisher, R. A., Bertonis, J. M., Torres, G., Wallner, B. P., Ramachandran, K. L., Ragin, R. C., Manganaro, T. F., MacLaughlin, D. T. & Donahoe, P. K. (1986) *Cell* **45,** 685–698.
25. Brenner, M. B., Trowbridge, I. S. & Strominger, J. L. (1985) *Cell* **40,** 183–190.
26. Krangel, M. S., Taketani, S., Biddison, W. E., Strong, D. M. & Strominger, J. L. (1982) *Biochemistry* **21,** 6313–6321.
27. Flyer, D. C., Burakoff, S. J. & Faller, D. V. (1985) *J. Immunol.* **135,** 2287–2292.
28. Ozato, K., Mayer, N. & Sachs, D. H. (1980) *J. Immunol.* **124,** 533–540.
29. Tada, N., Kimwa, S., Hatzfeld, A. & Hammerling, U. (1980) *Immunogenetics (N.Y.)* **11,** 441–444.
30. Kvist, S., Ostberg, L. & Peterson, P. A. (1978) *Scand. J. Immunol.* **7,** 265–276.
31. Kohler, G. & Milstein, C. (1975) *Nature (London)* **256,** 495–497.
32. McCluskey, J., Bluestone, J. A., Coligan, J. E., Maloy, W. L. & Margulies, D. H. (1986) *J. Immunol.* **136,** 1472–1481.
33. Lancet, D., Parham, P. & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3844–3848.