

EXPRESSION OF H-2D^b ON THE CELL SURFACE IN
THE ABSENCE OF DETECTABLE β 2 MICROGLOBULIN

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Class I molecules of the major histocompatibility complex (MHC) are expressed on the cell surface as a complex of two noncovalently associated units, a 40–45,000 dalton subunit called the heavy chain and an 11,000 dalton subunit called β 2 microglobulin (β 2m) (1). In the mouse this appears to be true for molecules encoded in the Qa-Tla region as well as for molecules encoded by the K, D, or L loci (2, 3). In contrast to the 45,000 dalton class I chain, β 2m is not integrated into the membrane. Similarly, it is believed that the heavy chains of class I molecules are not expressed in the absence of β 2m. Evidence for this comes primarily from the study of two cell lines. The Burkitt lymphoma line, Daudi, expresses neither class I HLA antigens nor β 2m on the cell surface (4). In this cell line the primary defect is the inability to synthesize β 2m, as shown by biochemical studies and by somatic cell hybridization experiments in which the expression of Daudi HLA antigens could be rescued either by mouse or human β 2m, provided by the normal partner in the hybrid (4). Essentially the same kind of data have been obtained by Hyman and his collaborators (5, 6) using a somatic cell variant of the C3H (H-2^k) thymoma, R1. These studies led to the conclusion that class I proteins have to be associated with β 2m for expression on the cell surface. We report here on a spontaneous variant of the B6 lymphoma EL4, which in contrast to the wild-type cell line, expresses neither H-2K^b nor β 2m but does express H-2D^b, which can be detected by serological reactivity as well as by cytotoxic T lymphocytes (CTL).

Materials and Methods

Antibodies. The monoclonal antibodies to H-2 specificities and to β 2m listed in Table I were obtained from various investigators. Rabbit anti-mouse β 2m was a kind gift from Dr. E. Appella.

Tumor Cell Lines. The original EL4 cell line was isolated by Dr. Peter Gorer (7) in 1945. We have examined two sublines: EL4/NY, which has been maintained in our laboratory for several years, and EL4/Mar, which is a variant that arose in the laboratory of Dr. P. Golstein. RDM-4 is an AKR/J thymoma cell line maintained in vitro and

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TABLE I
Reactivity of Monoclonal Anti-H-2 and Anti- β 2m Antibodies on EL4/NY and EL4/Mar

	Y-3 (K ^b)	EH-144 (K ^b)	28-14-8 (D ^b)	B22/249 (D ^b)	H141/51 (D ^b)	Lym-11 β 2m ^b	Clone 23 β 2m ^b
EL4/NY	+++	+++	+++	+++	+++	+++	+++
EL4/Mar	-	-	+++	+++	+++	-	-

Reactivity was assessed by microcytotoxicity using monoclonal antibodies at a concentration of 1:100 and rabbit complement at a final dilution of 1:9, as previously described (9). +++, 99% of cells dead after 60 min incubation; -, 5% of cells dead after 60 min incubation. Monoclonal antibodies were obtained as follows: B22/249 and H141/51, Dr. G. Hammerling; 28-14-8, Dr. T. Hansen; Y-3, Dr. S. Nathenson; Lym-11, Dr. U. Hammerling; clone 23, Dr. F. W. Shen.

expresses H-2^b-encoded products.

Cytotoxic T Cell Lines and Clones. The H-2K^b-specific polyclonal T cell line (KB5) of B10.BR origin and the cytotoxic T lymphocyte (CTL) clones derived from it (KB5-B1 and KB5-C20) were established and maintained as previously described (8). H-2D^b-specific CTL clones (K4R-E and K4R-G series) were derived in a similar manner using T cells from B10.BR mice immunized with B10.A (4R) spleen cells that were restimulated in vitro every 7 d with B10.A (4R) spleen cells and cloned by limiting dilution (8).

Immunoprecipitation and Electrophoresis of Radiolabeled Surface Molecules. Labeling of cell surface molecules with I¹²⁵ was performed by the lactoperoxidase-catalyzed reaction in the presence of hydrogen peroxide as described previously (9). After preclearing the membrane lysate, immunoprecipitates were prepared by adding monoclonal or xenon-antibodies to mouse β 2m, followed by a suspension of *Staphylococcus aureus*. The precipitates were subjected to electrophoresis on a 10-cm 15% acrylamide slab gel as described by Laemmli (10).

Results

Serological Analysis of H-2 and β 2m. The examination of the cytotoxicity of several monoclonal antibodies for the two EL4 sublines showed that these two cell lines differed in the expression of cell surface molecules (Table I). The anti-K^b (Y-3, EH-144), anti-D^b (28-14-8, H-141/51), and the two anti- β 2m (Lym-11, clone 23) monoclonal antibodies were all cytotoxic for EL4/NY, whereas only the anti-D^b antibodies were cytotoxic for EL4/Mar. The inability of anti-K^b and anti- β 2m antibodies to kill EL4/Mar was also observed at dilutions of the antibodies lower than those presented in Table I.

The reactivity of anti-H-2 and anti- β 2m antibodies was also examined by immunofluorescence on the fluorescence-activated cell sorter (FACS). The reactivity observed was essentially the same as that observed in microcytotoxicity testing. EL4/NY reacted with anti-K^b (EH-144) (Fig. 1) anti-D^b (28-14-8, H-141/51) (Fig. 1) and anti-Lym-11 (Fig. 2) antibodies; on the other hand, only the anti-D^b antibodies (28-14-8, H-141/51) (Fig. 1) but not anti-K^b (Fig. 1) or anti-Lym-11 (Fig. 2) antibodies showed any reactivity with EL4/Mar. In addition, it was apparent that the level of reactivity of the D^b antibodies with EL4/Mar was essentially the same as that seen with EL4/NY. Thus, by both serological criteria, microcytotoxicity and immunofluorescence, EL4/NY was K^{b+} D^{b+} Lym-11 (β 2m)⁺ whereas EL4/Mar was K^{b-} D^{b+} Lym-11⁻.

Immunoprecipitation of β 2m from Cell Surfaces. The failure of EL4/Mar to react with the anti-Lym-11 antibody may have been due to a mutation resulting in the loss of allotypic specificity. To test this possibility, we immunoprecipitated β 2m from lysates of cells labeled with I¹²⁵, using xenogeneic as well as allogeneic antibodies. Precipitation with either the allotypic antibodies, Lym-11 or clone 23 (Fig. 3), or the xenogeneic antibody (rabbit α - β 2m) (Fig. 3) to mouse β 2m,

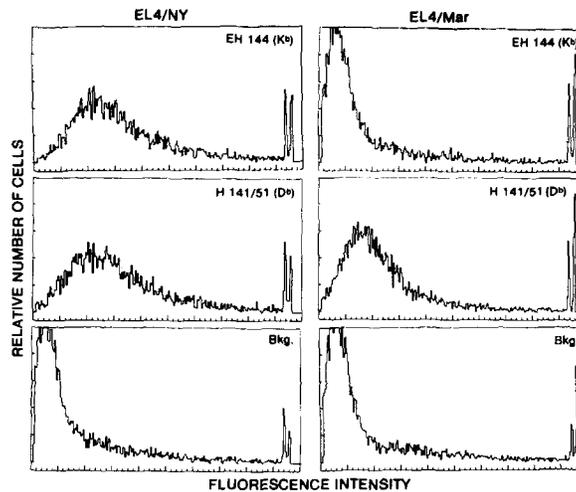


FIGURE 1. Reactivity of anti-H-2 antibodies with EL4/NY and EL4/Mar measured on the FACS. The binding of the monoclonal alloantibodies was detected with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin antibody (FGAMIg), as previously described (9). Bkg, reactivity of FGAMIg alone.

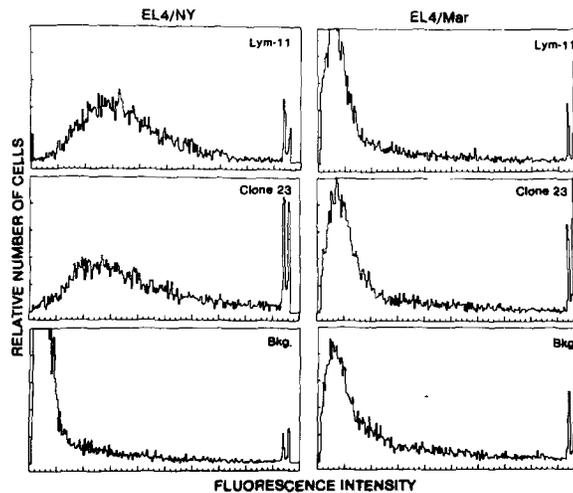


FIGURE 2. Reactivity of anti- β 2m antibodies with EL4/NY and EL4/Mar measured on the FACS. The binding of the monoclonal alloantibodies was detected with FGAMIg. Bkg, reactivity of FGAMIg alone.

brought down β 2m from EL4/NY but not from EL4/Mar lysates (Fig. 3). Since the xenogeneic antibody recognizes determinants distinct from the allotypic specificity on β 2m (e.g., this antibody can precipitate β 2m from both C57BL/6 and BALB/c cell lines), the failure to precipitate β 2m from EL4/Mar using the xenoantiserum is strong evidence that this cell line does not express β 2m on the cell surface.

Cell-mediated Cytotoxicity. The reactivity of previously characterized (8) anti- K^b - and anti- D^b -specific alloreactive CTL was also examined on the EL4 sublines. The anti- K^b -specific polyclonal (line KB5) or monoclonal (clones KB5.B1 and C20) B10.BR CTL lysed EL4/NY but not EL4/Mar (Table II). By contrast,

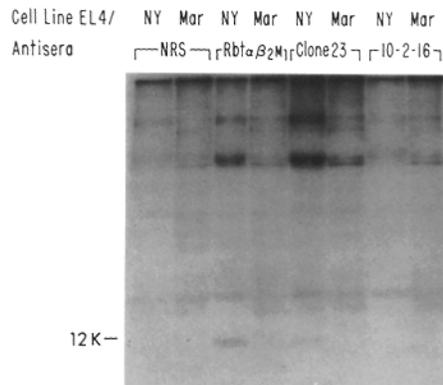


FIGURE 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of molecules precipitated from EL4/NY and EL4/Mar. Membrane preparations labeled with I^{125} were precipitated with anti- $\beta 2m$ antibodies, rabbit α - $\beta 2m$ or clone 23 (monoclonal anti- $\beta 2m$); 10-2-16 is a monoclonal anti-Ia reagent used as a negative control. $\beta 2m$ runs slightly faster than the 12,000 dalton marker and can be identified in the third and fifth lanes only. NRS, normal rabbit serum.

TABLE II

H-2D^b-specific, But Not H-2K^b-specific CTL Clones Lyse the EL4/Mar Tumor Target Cells

A. Anti-K ^b effector cells					
Effector cells*	Percent specific ^{51}Cr release on target cells				
	B6 [‡]	B10.A(4R) [‡]	EL4/NY	EL4/Mar	RDM4
CTL line KB5	75	7.5	80	3.6	NT [§]
Clone KB5-B1	70	7.1	98	-0.9	NT
Clone KB5-C20	68	5.4	32	-0.16	NT
B. Anti-D ^b effector cells					
K4R Clones	B10.BR [†]	B10.A(4R) [†]	EL4/NY	EL4/Mar	RDM4
-E1	4.3	48	74	71	1.1
-E4	2.8	44	81	79	4.2
-E8	7.1	65	74	76	14
-G6	-1.4	57	70	76	3.2

* Effector cells: H-2K^b-specific CTL line KB5 and clones KB5-B1 and C20 have been described (8). H-2D^b-specific CTL clones (K.4R series) are from a B10.BR anti-B10.A (4R) immunization. Results shown are for an effector to target cell ratio of 2 to 1. Cell-mediated cytotoxicity was performed and the percent specific release calculated as previously described (8).

[‡] Con A-induced splenic blast cells.

[§] Not tested.

[†] Lipopolysaccharide-induced splenic blast cells.

all CTL clones derived from a B10.BR anti-B10.A (4R) immunization (anti-D^b) lysed the EL4/Mar target cells as well as EL4/NY (Table II).

Discussion

Previous studies (1-3) have demonstrated that there is an association between MHC class I heavy chains and $\beta 2m$. The observations that the Daudi cell line does not express any HLA class I antigens (4) and that the R1 variant is H-2⁻ TL⁻ (5, 6) due to defects in $\beta 2m$ synthesis suggest that $\beta 2m$ is required for cell surface expression of class I molecules. The data presented in this paper, using several criteria for detection of surface molecules, demonstrate that on a variant of the EL4 tumor line, EL4/Mar, H-2D^b is expressed on the cell surface in the absence of detectable $\beta 2m$. In the human MHC, results of sequential immuno-

precipitation of HLA molecules with monoclonal antibodies directed against the HLA heavy chain or $\beta 2m$ suggested that a subset of cell surface HLA heavy chains is not associated with $\beta 2m$ (11). Thus, the expression of H-2^b on EL4/Mar in the absence of $\beta 2m$, while novel for the murine system, may not be entirely unique for MHC class I antigens.

The failure to express both K^b and $\beta 2m$ on the cell surface could be due to a primary defect either in $\beta 2m$ or K^b synthesis; however, it is clear that if there is defect in $\beta 2m$ synthesis this does not affect D^b expression. If the defect actually resides in K^b heavy chain synthesis and $\beta 2m$ is produced, the failure to express $\beta 2m$ on the cell surface in association with H-2D^b raises the possibility that there is no association between D^b and $\beta 2m$, and that H-2D^b does not require $\beta 2m$ for membrane insertion. Alternatively, it may be that H-2D^b is inserted in association with $\beta 2m$ but the complex dissociates soon after the appearance of the antigen on the cell membrane. In either case, it would seem that the association of H-2D^b with $\beta 2m$ is more tenuous than that of other class I antigens. It is of interest to note that in contrast to K^b, K^k, and D^d, which have two carbohydrate side chains, there are three associated with the D^b molecule (12). This additional glycosylation site is on the C2 domain of the molecule, which has been suggested to be the $\beta 2m$ -binding region (13). In addition, precipitation with anti-D^b brings down very little $\beta 2m$ compared with precipitation with anti-K^b (14). It is therefore tempting to speculate that the third carbohydrate chain may replace $\beta 2m$ on D^b.

It is noteworthy that the $\beta 2m$ -free D^b molecule expressed by EL4/Mar could be detected by the monoclonal antibody 28-14-8. The epitope recognized by this antibody has been mapped to the C2 domain of H-2L^d (15). As noted above, this is also the domain that has been suggested to be the $\beta 2m$ -binding domain (13). Whether the additional carbohydrate group present in the C2 domains of the D^b and L^d molecules influences $\beta 2m$ association is not known.

The results presented in this report may have some relevance to the attempts of groups studying the nature of the many class I genes in the murine MHC. One assay involves the transfection of an unknown class I gene into LTK⁻ cells, which are then evaluated for increased expression of $\beta 2m$ (16). The failure of the transfected gene to cause an increase in $\beta 2m$ expression may be interpreted to mean that the gene is a "pseudogene" or otherwise unable to direct synthesis of a cell surface class I protein. The fact that H-2D^b (this paper) and some human class I gene products (11) can be expressed on the cell surface without detectable $\beta 2m$ raises the possibility that other murine class I antigens may also exhibit this property. Therefore, a negative result in the $\beta 2m$ assay (16) must be interpreted with caution.

Summary

In this report we describe a variant of the C57BL/6 T lymphoma EL4 (EL4/Mar) which, in contrast to the parental cell line, expresses neither H-2K^b nor $\beta 2m$ but which does express H-2D^b detectable by serology and by alloreactive cytotoxic T lymphocytes (CTL). This observation raises the possibility that H-2D^b and perhaps other major histocompatibility complex class I molecules are normally not associated with $\beta 2m$ on the cell surface. In addition, this report is the first to indicate that alloreactive CTL can interact with a $\beta 2m$ -free class I antigen.

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