

Recognition of the Major Histocompatibility Complex Restriction Element Modulates CD8⁺ T Cell Specificity and Compensates for Loss of T Cell Receptor Contacts with the Specific Peptide

By Johan K. Sandberg, Klas Kärre, and Rickard Glas

From the Microbiology and Tumor Biology Center, Karolinska Institute, S-17177 Stockholm, Sweden

Summary

Triggering of a T cell requires interaction between its specific receptor (TCR) and a peptide antigen presented by a self-major histocompatibility complex (MHC) molecule. TCR recognition of self-MHC by itself falls below the threshold of detection in most systems due to low affinity. To study this interaction, we have used a read-out system in which antigen-specific effector T cells are confronted with targets expressing high levels of MHC compared with the selecting and priming environment. More specifically, the system is based on CD8⁺ T cells selected in an environment with subnormal levels of MHC class I in the absence of β_2 -microglobulin. We observe that the MHC restriction element can trigger viral peptide-specific T cells independently of the peptide ligand, provided there is an increase in self-MHC density. Peptide-independent triggering required at least four times the natural *in vivo* level of MHC expression. Furthermore, recognition of the restriction element at expression levels below this threshold was still enough to compensate for lack of affinity to peptides carrying alanine substitutions in major TCR contact residues. Thus, the specificity in TCR recognition and T cell activation is fine tuned by the avidity for self-MHC, and TCR avidities for peptide and MHC may substitute for each other. These results demonstrate a functional role for TCR avidity for self-MHC in tuning of T cell specificity, and support a role for cross-reactivity on "self" during T cell selection and activation.

Key words: T cell • specificity • major histocompatibility complex restriction • major histocompatibility complex class I • peptide

Triggering of a CD8⁺ T cell requires TCR interaction with a specific peptide bound to an MHC class I molecule. The requirement for the selecting MHC molecule in recognition of antigen is known as MHC restriction (1, 2). Although interactions between TCR and the restriction element have been detected only in the presence of specific peptides, it is believed that thymic positive selection imposes a low self-MHC affinity onto the peripheral T cell pool (3–6). Early studies of TCR–MHC interactions involved analyses of MHC mutants for their ability to disrupt T cell reactivity (7–9). Mutations in the α -helical regions in the $\alpha 1/\alpha 2$ domains of the MHC molecule were found to perturb recognition of specific antigen, indicating that the TCR physically contacts the α -helices of MHC molecules. However, measurements of affinity between TCR and peptide–MHC complexes have revealed relatively low affinities for MHC in complex with agonist peptides and slightly lower affinities for MHC in complex with antagonist peptides, whereas no detectable affinities have been

found for MHC in complex with unrelated peptides (10–13). Thus, the idea of TCR affinity to self-MHC in the absence of specific antigen has been hard to support biochemically.

Recently, crystallographic studies have shown a physical interaction between TCR and MHC in the presence of specific peptide (14, 15). Of the total surface area contacted by the TCR in the MHC complex, approximately one third is provided by the peptide while MHC $\alpha 1/\alpha 2$ domains contribute two thirds of the contact area. Based on alanine scanning mutagenesis of the 2C TCR, it has been estimated that almost two thirds of the binding energy in antigen-specific binding is attributable to contacts with the α -helices (16). Does the interaction between TCR and MHC as observed in crystallographic and biochemical studies also contribute to the avidity required for T cell triggering, and can it be detected in the absence of the specific peptide? Further, how does this interaction influence T cell specificity for the antigenic peptide? The answers to

these questions are of major importance for the understanding of several aspects of physiological T cell function, including MHC restriction (1, 2), thymic selection (17), and maintenance of T cell memory (18). Interestingly, recent evidence indicates that T cells require interaction with endogenous peptide-MHC complexes for long-term survival, suggesting continuous cross-recognition of self for maintenance of immunological memory (19–21).

T cell avidity for self has been demonstrated in functional assays. CTLs triggered by allogeneic MHC can specifically kill bystander cells of self-MHC haplotype, indicating that self-MHC can mediate binding to CTLs that are efficiently triggered by their antigen, although it cannot trigger CTLs directly (22). Second, mice with low MHC class I expression select T cells adapted to that environment, whose avidity to self-MHC is revealed when assessed in the context of normal MHC class I levels. For example, CTLs generated against allogeneic cells in such mice specifically cross-react with cells expressing high levels of self-MHC class I (23–25). Similarly, T cell hybridomas derived from CD3 ζ / η -deficient mice expressing very low levels of cell surface TCR also react against cells expressing self-MHC class I molecules, once a normal level of TCR expression has been restored by CD3 ζ / η transfection (26). Thus, in systems where either the MHC or the TCR are expressed at subnormal levels, it is possible to generate T cells for the study of TCR affinity/avidity for self-MHC, which are not present in normal mice with high MHC expression due to negative selection.

To study the role of self-MHC avidity in MHC-restricted CTLs, we immunized β_2 -microglobulin-deficient ($\beta_2m^{-/-}$)¹ mice, which have low expression of conformed MHC class I (27, 28), with the H-2D^b-restricted immunodominant lymphocytic choriomeningitis virus (LCMV) GP 33-41 (GP33) peptide. We obtained CD8⁺ CTLs which were specific for the GP33 epitope when loaded onto cells with low MHC class I expression (MHC^{low}). However, unlike GP33-specific C57Bl/6 (B6) CTLs, these CTLs also killed cells expressing high levels of self-MHC class I (MHC^{high}) in the absence of specific peptide. Further experiments revealed a dual specificity of these CTLs: peptide specificity against MHC^{low} targets, and peptide-independent specificity for the restriction element when tested against MHC^{high} targets. By mAb blocking of the MHC restriction element, we found that peptide-independent triggering of a CTL clone with high avidity for self-MHC required at least four times the in vivo expression of MHC. Most interestingly, at lower MHC expression an increased recognition of self-MHC could compensate for lack of TCR interaction with the specific peptide, as evaluated with peptides carrying alanine substitutions in major TCR contact residues. These results indicate that TCR avidity for the MHC molecule functionally contributes to T cell specificity, and that TCR

affinities for MHC and peptide are interchangeable. Our results have important implications for the understanding of T cell specificity and the role of self-MHC in peripheral T cell function.

Materials and Methods

Mice. B6, transporter associated with antigen processing (TAP)1^{-/-} (29), $\beta_2m^{-/-}$ (30, 31), and TAP1/ $\beta_2m^{-/-}$ (32) mice of the H-2^b haplotype were bred at the Microbiology and Tumor Biology Center, Karolinska Institute. Animal care was in accordance with institutional guidelines. TAP1^{-/-}, $\beta_2m^{-/-}$, and TAP1/ $\beta_2m^{-/-}$ mice used were back-crossed to B6 background 6, 10, and 7 times, respectively. For thymectomy experiments, $\beta_2m^{-/-}$ mice were 735 rad-irradiated and thymectomized or sham-thymectomized, and reconstituted with $\beta_2m^{-/-}$ fetal liver hematopoietic cells.

Cell Lines. RMA is a subline of the Rauscher virus-induced B6 lymphoma RBL-5, and RMA-S is a TAP2-deficient variant of RMA (33). T2 is a hybrid between the two human cell lines 0.174 and CEM, and has an antigen-processing deficiency due to a deletion on the MHC class II region including the TAP1 and TAP2 genes. T2D^b is an H-2D^b transfectant of T2. All cell lines were maintained at 37°C and 5% CO₂ in RPMI 1640 tissue culture medium supplemented with 5% FCS, 50 μ g/ml streptomycin, 100 μ g/ml penicillin, and 2 mM l-glutamine. Con A-activated blasts were generated by culturing erythrocyte-depleted splenocytes in 5 μ g/ml of Con A for 2 d in tissue culture medium as described above with 10% FCS.

Synthetic Peptides. The following synthetic H-2D^b-presented peptides were synthesized using solid phase F-moc chemistry: LCMV GP33 KAVYNFATM (34, 35); LCMV GP 33-4A (GP33-4A) KAVANFATM; LCMV GP 33-34A (GP33-34A) KAAANFATM; LCMV GP 33-348A (GP33-348A) KAAANFAAM; influenza PR8 NP 366-374 (NP366) ASNENMETM (36); and *Yersinia enterocolitica* Yop51 249-257 (Yop249) IQVGNTRTI (37).

Generation of LCMV GP33-specific CTL Cultures and Clones. GP33-specific CD8⁺ CTLs were elicited in B6 and $\beta_2m^{-/-}$ mice by peptide immunization (38). 100 μ g peptide was dissolved in distilled water and mixed with IFA in a 1:1 ratio by sonication, then injected subcutaneously in the base of the tail. 12 d after immunization, 25 \times 10⁶ immune spleen cells were cocultured with 25 \times 10⁶ 2,000 rad-irradiated B6 or $\beta_2m^{-/-}$ splenocytes in the presence of 0.05 μ M peptide in 12 ml complete medium (RPMI 1640 supplemented with 10% FCS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 5 \times 10⁻⁵ M 2-ME, 2 mM l-glutamine, and 50 μ g/ml streptomycin, 100 μ g/ml penicillin) at 37°C and 5% CO₂. 6–7 d later, these cells were used as effector cells in a ⁵¹Cr-release assay. CTL clones were generated by limiting dilution cloning. Long-term CTL clones and lines were maintained in complete medium (see above) based on MEM- α and further supplemented with Hepes buffer and 20 IU/ml IL-2. CTL clones and lines were restimulated in 12-d intervals with 2,000 rad-irradiated splenocytes in the presence of 0.05 μ M peptide.

mAbs and FACS[®] Analysis. B22-249.1 is a mouse mAb which binds to a conformation-dependent epitope on the α 1 domain of the H-2D^b molecule, and Y3 is a mAb which binds to conformed H-2K^b molecules. For flow cytometry with these mAbs, cells were incubated with mAb for 30 min on ice, washed, and then stained with goat anti-mouse Oregon Green conjugate

¹Abbreviations used in this paper: β_2m , β_2 -microglobulin; B6, C57Bl/6; LCMV, lymphocytic choriomeningitis virus; TAP, transporter associated with antigen processing.

(Molecular Probes) on ice for 30 min. For measurement of CD8 and TCR expression, CTLs were stained for 30 min with the FITC-conjugated anti-CD8 α mAb 53-6.7 (PharMingen) and the PE-conjugated anti-TCR- α/β mAb H57-597 (PharMingen), respectively. After washing, analysis was performed using a FAC-Scan[®] (Becton Dickinson) with CellQuest software.

CTL Assay, Cold Target Competition, and CTL Blocking with mAb. CTL activity was measured in a standard ⁵¹Cr-release assay. In brief, peptide-coated target cells were prepared by incubating cells with indicated concentrations of peptide for 1 h at 37°C. Coated cells were labeled with 10 μ l 10 mCi/ml ⁵¹Cr for 1 h at 37°C. Titrated amounts of effector cells were incubated with 3 \times 10³ ⁵¹Cr-labeled target cells for 4 h at 37°C, 5% CO₂. After incubation, released radioactivity was measured and specific lysis was calculated according to the formula: % specific release = [(experimental release - spontaneous release)/(maximum release - spontaneous release)] \times 100. Cold temperature-treated target cells were generated through culture of RMA-S at 26°C for 12 h (39). For cold target competition experiments, effector T cells and unlabeled (cold) competitor cells were mixed and preincubated at 37°C for 30 min before addition of labeled (hot) target cells. The assay was then run as a standard ⁵¹Cr-release assay for 4 h. For blocking of CTL recognition with the B22-249.1 mAb, titrated amounts of mAb were included in the CTL assay medium.

Results

Expression of Properly Conformed H-2D^b in $\beta_2m^{-/-}$ Cells Is TAP Dependent. We first compared the levels of properly conformed H-2D^b molecules on the cell surface of $\beta_2m^{-/-}$, TAP1/ $\beta_2m^{-/-}$, and B6 (TAP1/ $\beta_2m^{+/+}$) control cells stained with an mAb directed against H-2D^b (B22-249.1) or H-2K^b (Y3) conformation-sensitive epitopes. A substantial level of conformed cell surface H-2D^b molecules was found on $\beta_2m^{-/-}$ cells, whereas conformed H-2K^b was detected at lower levels, in line with previously published results (27, 28; Fig. 1). This is compatible with H-2D^b being more independent of β_2m during folding and transport of MHC class I free heavy chains (27, 28). On Con A blasts deficient for both β_2m and TAP1, the staining with B22-249.1 was virtually at background levels, which implies that the pool of conformed H-2D^b molecules on $\beta_2m^{-/-}$ cells is dependent on a functional TAP complex.

$\beta_2m^{-/-}$ Mice Have Thymus-dependent CD8⁺ T Cells that Mount an H-2D^b-restricted and Peptide-specific CTL Response against the LCMV GP33 Epitope. B6 and $\beta_2m^{-/-}$ mice were immunized with antigenic peptides restricted to either H-2D^b or H-2K^b. All tested peptides primed responses in B6 mice (data not shown), whereas only one, the H-2D^b-restricted LCMV GP33, primed a response in $\beta_2m^{-/-}$ mice. This is in line with the low but significant levels of folded H-2D^b molecules on the surface of $\beta_2m^{-/-}$ cells (27; Fig. 1). CTLs from both B6 and $\beta_2m^{-/-}$ mice primed with the GP33 peptide killed RMA-S cells pulsed with the GP33 peptide used for priming but not a control influenza NP366 peptide (Table I). CTL responses were mediated by CD8⁺ cells as determined by mAb- and complement-mediated depletion of effector populations in vitro (data not shown). FACS[®] analysis of both polyclonal bulk populations and clones confirmed the TCR⁺CD8⁺ phenotype of

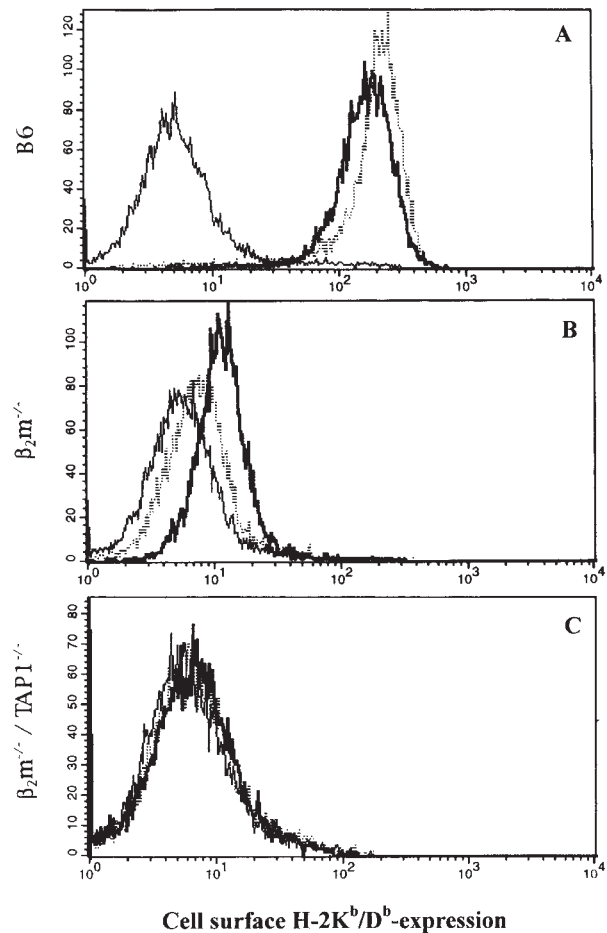


Figure 1. H-2D^b expression in the absence of β_2m is TAP dependent. FACS[®] analysis of H-2D^b and H-2K^b expression on Con A-activated blasts from B6 (A), $\beta_2m^{-/-}$ (B), and TAP1/ $\beta_2m^{-/-}$ (C) mice using the B22-249.1 and Y3 mAbs, respectively. Bold and dotted lines represent H-2D^b and H-2K^b expression, respectively. Solid thin line represents staining with secondary antibody only.

the responding CTLs in $\beta_2m^{-/-}$ as well as B6 mice (Fig. 2). Cell surface expression levels of both TCR and CD8 were similar in CTLs from $\beta_2m^{-/-}$ and B6 mice. However, a marginal increase in CD8 expression levels was detected in some $\beta_2m^{-/-}$ CTLs compared with B6 CTLs. This increase may be due to the low levels of H-2D^b expressed during selection and priming in the absence of β_2m . However, lysis performed by $\beta_2m^{-/-}$ CTLs did not show increased dependency on CD8 compared with B6 CTLs, as determined by mAb blocking of CD8 (data not shown).

No priming of CTLs was observed in thymectomized $\beta_2m^{-/-}$ mice that had been irradiated and reconstituted with fetal liver (Table I). Control mice (receiving irradiation and fetal liver reconstitution without thymectomy) generated a normal antipeptide CTL response, showing that the responding CD8⁺ T cell population in $\beta_2m^{-/-}$ mice was dependent on the thymus for development. In addition, a normal antipeptide response was also found in mice that had been thymectomized but not irradiated, showing that the thymus was not necessary during the

Table I. Development of Peptide-specific $\beta_2m^{-/-}$ CTLs Is Thymus Dependent

| Treatment | Target | E:T | Responder mouse strain | | | | |
|--------------------------------|-------------------|------|------------------------|----|------------------|------------------|------------------|
| | | | $\beta_2m^{-/-}$ * | B6 | $\beta_2m^{-/-}$ | $\beta_2m^{-/-}$ | $\beta_2m^{-/-}$ |
| Thymectomy [†] | | | – | – | – | + | + |
| Irradiation and reconstitution | | | – | – | + | + | – |
| | RMA-S plus GP33 | 45:1 | 49 | 79 | 47 | 1 | 41 |
| | | 15:1 | 38 | 55 | 37 | 1 | 20 |
| | | 5:1 | 23 | 34 | 15 | 0 | 12 |
| | RMA-S plus NP366 | 45:1 | 3 | 1 | – | – | – |
| | | 15:1 | 2 | 0 | – | – | – |
| | | 5:1 | 2 | 0 | – | – | – |
| | RMA-S, no peptide | 45:1 | 3 | 2 | 0 | 0 | 2 |
| | | 15:1 | 3 | 1 | 2 | 0 | 0 |
| | | 5:1 | 0 | 0 | 4 | 0 | 3 |

*Mice immunized with synthetic LCMV GP33 peptide were restimulated in vitro and tested in a ⁵¹Cr-release assay against RMA-S cells coated with the indicated peptides as described in Materials and Methods.

[†] $\beta_2m^{-/-}$ mice were thymectomized or sham-thymectomized, 735 rad-irradiated, and reconstituted with fetal liver hematopoietic cells as indicated.

priming of the response (Table I). The latter control indicated that the generated CTLs were not positively selected in the thymus in response to the peptide injected for immunization. We conclude that $\beta_2m^{-/-}$ mice have a peripheral pool of CD8⁺ T cells which are thymus dependent, H-2D^b restricted, and peptide specific despite expressing very low levels of H-2D^b molecules, corresponding to a few percent of those expressed by B6 mice.

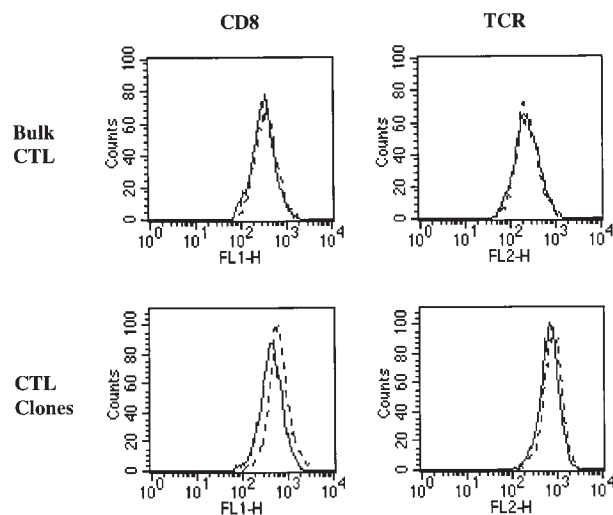


Figure 2. TCR and CD8 coreceptor expression in $\beta_2m^{-/-}$ and B6 CTLs. FACS[®] analysis of TCR- α/β and CD8 α in GP33-specific CTLs, using the PE-conjugated anti-TCR- α/β mAb H57-597 and the FITC-conjugated anti-CD8 α mAb 53-6.7 (PharMingen), respectively. Solid lines represent B6 CTLs; dotted lines represent $\beta_2m^{-/-}$ CTLs. In staining of CTL clones, the representative clones 2C10 (B6) and 3C5 ($\beta_2m^{-/-}$) were used. The histograms show CD8 expression and TCR expression in TCR⁺ and CD8⁺ cells, respectively.

GP33-specific $\beta_2m^{-/-}$ CTLs Have an Increased Avidity for H-2D^b Molecules. Alloreactive CTLs from mice with low MHC class I expression have an increased avidity to self-MHC (23). By priming peptide-specific, self-MHC-restricted CTLs in $\beta_2m^{-/-}$ mice it is possible to study the role of self-MHC avidity for T cell specificity in MHC-restricted T cells. To compare the avidity for self-MHC class I in B6 and $\beta_2m^{-/-}$ CTLs specific for the GP33 peptide, these CTLs were tested for their ability to kill RMA target cells (MHC^{high}) in the absence of loaded peptides. B6 CTLs failed to kill RMA cells, whereas $\beta_2m^{-/-}$ CTLs efficiently killed these target cells (Fig. 3, A and B, respectively). Cold target competition experiments made with bulk CTLs showed that the $\beta_2m^{-/-}$ CTL killing of labeled RMA cells was completely inhibited by RMA-S pulsed with GP33 but not by RMA-S without peptide (Fig. 3 C). This showed that a majority of the clones in the $\beta_2m^{-/-}$ CTL population were specific for both self-MHC expressed at high levels and the peptide antigen presented by MHC at low levels.

Three GP33-specific $\beta_2m^{-/-}$ CTL clones were generated, all of which also killed RMA cells. However, the clone C10 was more efficient in killing RMA-S loaded with GP33 compared with RMA (Fig. 3 D), whereas clone 27/30 killed RMA slightly better than RMA-S plus GP33 (Fig. 3 E), and clone 3C5 killed both of these target cells to a similar extent (Fig. 3 F). This pattern is compatible with a clonal variation in TCR avidity for peptide versus MHC within the $\beta_2m^{-/-}$ CTL population. In line with the results obtained with bulk cultures, the GP33-specific B6 CTL clone 2C10 did not recognize RMA in the absence of GP33 peptide (Fig. 3 G).

To test the reactivity of the CTLs against MHC class I in the absence of peptides, we used RMA-S cells incubated at

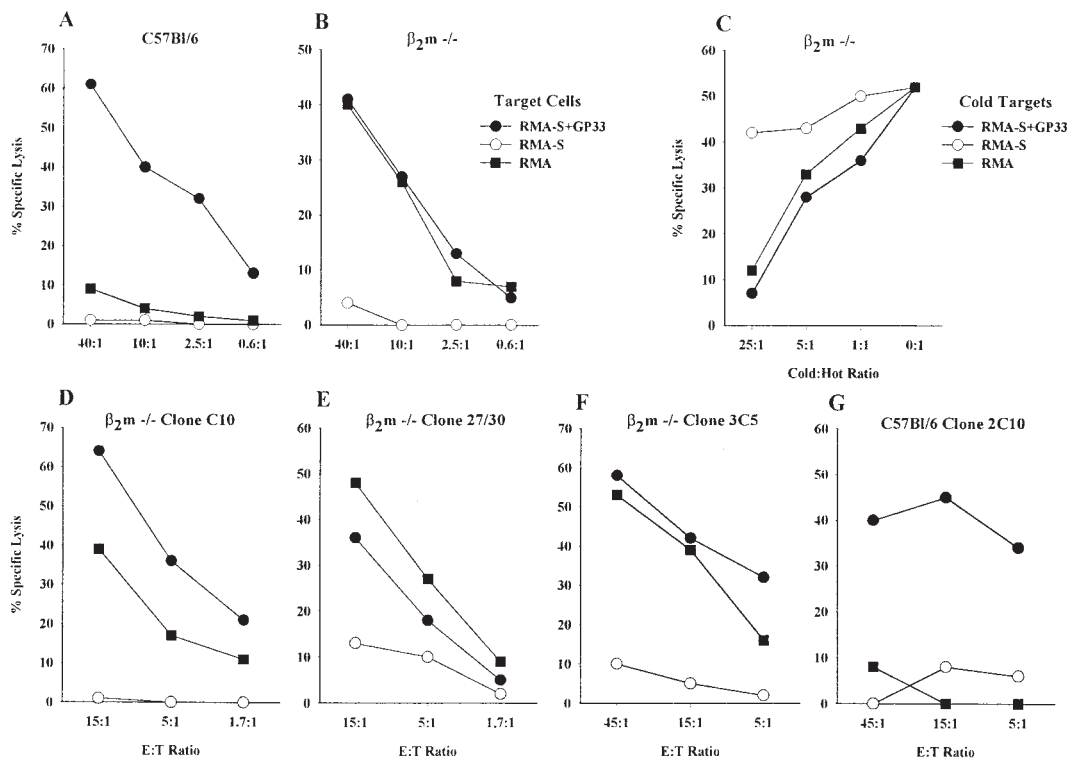


Figure 3. Elevated self-MHC reactivity in $\beta_2m^{-/-}$ CTLs. CTLs generated by synthetic LCMV GP33 peptide immunization (as described in Materials and Methods) in B6 (A) and $\beta_2m^{-/-}$ (B) mice were tested against RMA-S, RMA, and RMA-S loaded with GP33 in a ^{51}Cr -release CTL assay. (C) $\beta_2m^{-/-}$ GP33-specific CTL lysis of ^{51}Cr -labeled (hot) RMA target cells was subjected to cold target competition using cold (unlabeled) RMA-S, RMA, and RMA-S loaded with GP33. $\beta_2m^{-/-}$ CD8⁺ CTL clones C10 (D), 27/30 (E), and 3C5 (F), and the B6 CTL clone 2C10 (G) were obtained by limiting dilution and tested against RMA-S, RMA, and RMA-S loaded with GP33.

cold temperature (26°C), which induce high levels of functionally “empty” MHC class I molecules (39), and T2D^b cells expressing D^b molecules mostly devoid of peptides. Clone 27/30 also killed RMA-S cells after cold temperature incubation (Fig. 4 A). Furthermore, T2D^b was recognized irrespective of loaded peptide, whereas T2 control targets were not killed (Fig. 4 B). Thus, the $\beta_2m^{-/-}$ CTL clone 27/30 displays an avidity for the restriction element of the GP33 peptide, even in the absence of specific peptide. It should be noted that these CTLs were primed to respond only against GP33, indicating that the ability to rec-

ognize self-MHC had been selected for during thymic development and priming. Although these results do not exclude that $\beta_2m^{-/-}$ CTLs also have an increased avidity for self-peptides, we use the term “peptide independent” to describe the specificity for the restriction element displayed by the GP33-specific $\beta_2m^{-/-}$ CTLs.

Triggering of GP33-specific $\beta_2m^{-/-}$ CTLs Uses a Higher Number of MHC Ligands to Compensate for Absence of Specific Peptide. We next compared the avidity of $\beta_2m^{-/-}$ CTLs for self-MHC with and without added GP33, by blocking of the H-2D^b ligands. We used an mAb specific for prop-

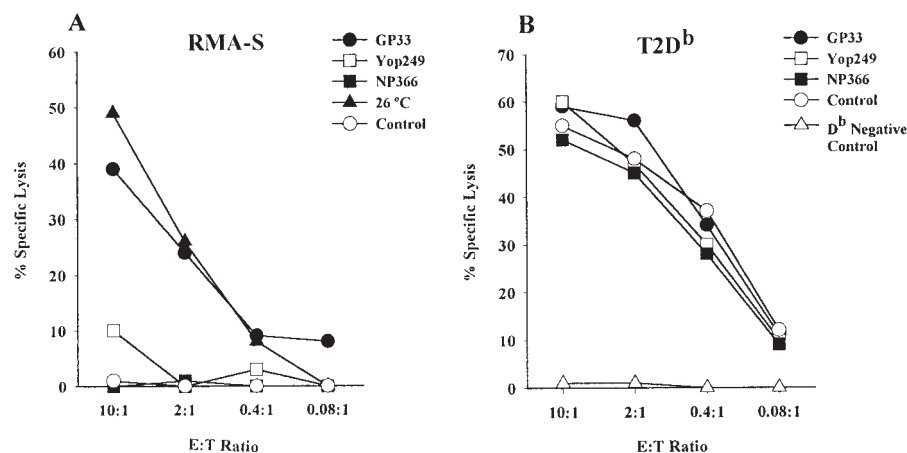


Figure 4. Recognition of self-MHC at high ligand density by the CTL clone 27/30 is largely independent of peptide. (A) The $\beta_2m^{-/-}$ CTL clone 27/30 was tested in a ^{51}Cr -release CTL assay against RMA-S target cells loaded with LCMV GP33 peptide, influenza NP366 peptide, Yop249 peptide, unloaded RMA-S, or RMA-S incubated for 12 h at 26°C without peptide to stabilize “empty” MHC class I on the cell surface. (B) Clone 27/30 was tested against T2D^b target cells loaded with GP33, NP366, Yop249, and unloaded T2D^b. The T2 cell line was included as a D^b negative control.

erly conformed H-2D^b molecules (B22-249.1), the binding of which is not affected by GP33 (data not shown). RMA target cells were killed at similar levels regardless of the presence or absence of specific peptide (Fig. 5). However, in the absence of GP33 peptide the CTL killing activity was efficiently blocked by 5.0 μg/ml of B22-249.1, whereas virtually no blocking was observed when the RMA target cells were prepulsed with the GP33 peptide at 37°C (Fig. 5 A). FACS[®] analysis of B22-249.1 binding to RMA cells did not allow accurate quantitation of the fraction of free H-2D^b ligands at half-maximal lysis, since half-maximal blocking of CTL lysis was achieved at a concentration at which staining was close to maximal (Fig. 5 B). However, these experiments clearly demonstrate that triggering of β₂m^{-/-} CTLs in the absence of GP33 peptide requires a considerably higher number of H-2D^b ligands.

We next made a similar series of experiments using T2D^b cells as targets. These cells express the B22-249.1 epitope at levels <10% those of RMA (Table II) but are still killed by the β₂m^{-/-} GP33-specific CTL clone 27/30 in the absence of specific peptide (Fig. 4 B). Lysis of T2D^b cells was blocked already at a 1.0 μg/ml concentration of B22-249.1 in the CTL assay (Fig. 5 C), indicating that a large fraction of cell surface H-2D^b molecules is required for triggering of this CTL clone. Pulsing of T2D^b cells with

GP33 peptide prohibited blocking at all mAb concentrations tested (Fig. 5 C). This effect was peptide specific, since neither NP366 nor Yop249 had any effect on avidity as determined by blocking with mAb. By titration of B22-249.1 in FACS[®] analysis of T2D^b (Fig. 5 D), the fraction of free H-2D^b ligands on T2D^b at half-maximal peptide-independent lysis was estimated at ~80% (comparing Fig. 5, C and D). Thus, ~80% of the H-2D^b molecules on T2D^b were necessary for peptide-independent recognition by the high-avidity CTL clone 27/30, which corresponds to about four times the level of folded H-2D^b on β₂m^{-/-} cells (Table II). Taken together, these data indicate that β₂m^{-/-} CTLs use peptide-independent recognition of a high number of H-2D^b ligands to compensate for the absence of specific peptide.

GP33-specific β₂m^{-/-} CTLs Are Less Dependent on the Exact GP33 Peptide Sequence. Finally, we analyzed the consequences of self-MHC avidity in GP33-specific B6 and β₂m^{-/-} CTLs in terms of peptide specificity, when MHC was expressed at lower levels. Neither B6 nor β₂m^{-/-} CTLs kill RMA-S (MHC^{low}) in the absence of specific peptide, and RMA-S cells require a pulse of ~100 pM GP33 peptide to become sensitive targets to both B6 and β₂m^{-/-} CTLs (Fig. 6). This shows that B6 and β₂m^{-/-} CTLs require roughly equal amounts of H-2D^b/GP33

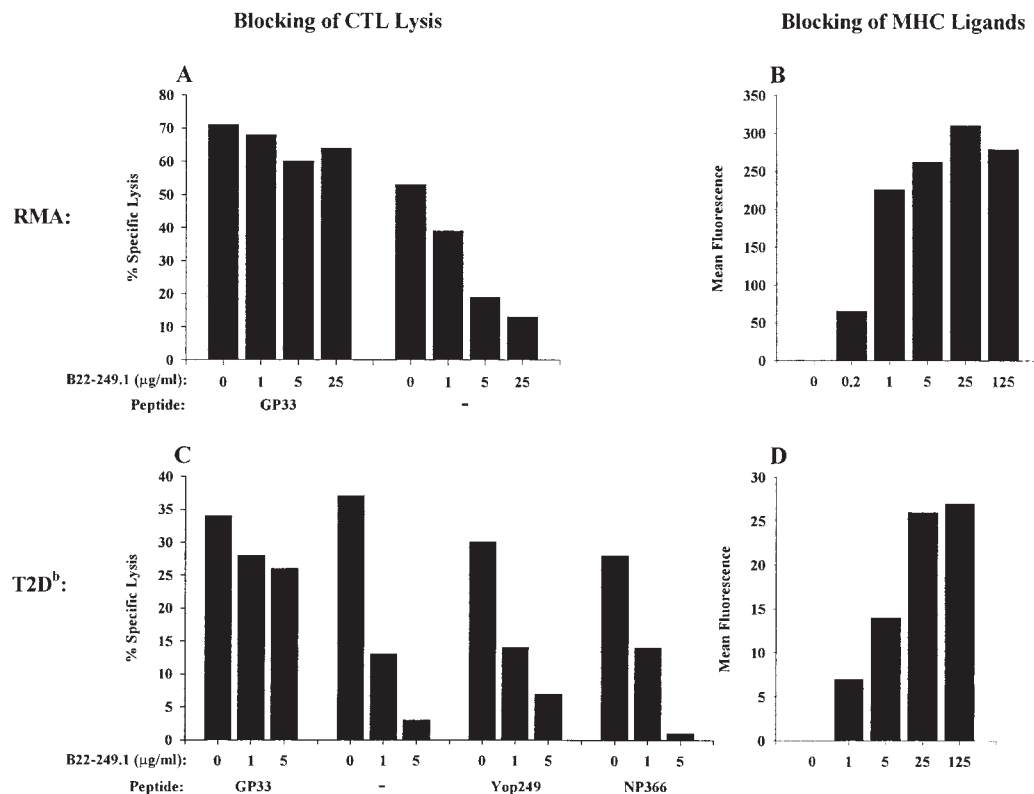


Figure 5. CTL triggering by self-MHC is a low-avidity event that requires interaction with a high density of ligands. (A) Recognition by β₂m^{-/-} LCMV GP33-specific polyclonal CTLs of RMA in the absence or presence of GP33 peptide was blocked using the anti-H-2D^b conformation-specific mAb B22-249.1. (B) FACS[®] analysis of B22-249.1 titration on RMA cells. (C) Recognition by the GP33-specific β₂m^{-/-} CTL clone 27/30 of T2D^b loaded with GP33, Yop249, NP366, or T2D^b without peptide was blocked using B22-249.1. (D) FACS[®] analysis of B22-249.1 titration on T2D^b. The preincubation of target cells with peptide at 37°C did not affect expression of H-2D^b in these experiments (data not shown).

Table II. Surface Expression of H-2D^b on Cells with Defects in TAP or β_2m

| Cell type | H-2D ^b expression (mean FL1)* | H-2D ^b surface density (FL1/FSC) [‡] |
|---------------------------------|--|--|
| B6 [§] | 227 | 398 |
| TAP1 ^{-/-} | 14 | 24 |
| β_2m ^{-/-} | 4 | 6 |
| TAP1/ β_2m ^{-/-} | 0 | 0 |
| RMA | 396 | 568 |
| RMA-S | 15 | 21 |
| T2D ^b | 28 | 31 |
| T2 | 0 | 0 |

*H-2D^b expression was measured by FACS[®] analysis using the conformation-dependent B22-249.1 epitope.

[‡]As forward scatter (FSC) is proportional to the cross-sectional area of the analyzed cell, FL1/FSC was used as an estimate of the surface density of H-2D^b.

[§]B6, TAP1^{-/-}, β_2m ^{-/-}, and TAP1/ β_2m ^{-/-} Con A-activated blasts were generated by culturing splenocytes for 3 d in RPMI supplemented with 10% FCS and 5 μ g/ml Con A.

complexes to get a triggering signal. However, when using GP33 peptide variants alanine-substituted at one or several TCR contact residues (reference 40, and data not shown), the β_2m ^{-/-} CTLs recognized RMA-S cells pulsed with up to 1,000-fold less peptide than B6 CTLs (Fig. 6). Substitution at one position (GP33-4A) already drastically reduced the efficiency of B6 CTLs against peptide-pulsed RMA-S targets, whereas β_2m ^{-/-} CTLs could still recognize peptides with alanine substituted at two positions. Thus, although β_2m ^{-/-} CTLs were still peptide specific, they were less dependent on the TCR contact residues in the peptide when the peptide was loaded on RMA-S target cells (which express about three times the levels of H-2D^b found on β_2m ^{-/-} cells [Table II]). These data indicate that the avidities for peptide and MHC both contribute functionally to the triggering of CTLs, and that they can be considered separately. Further, increased avidity for the restriction element compensates for a reduced avidity for the peptide.

Discussion

Contribution of Peptide-independent Recognition of MHC to the Specificity of T Cells. In this paper we have investigated the influence of TCR interaction with self-MHC in recognition of MHC-bound peptides. To address this issue, we used MHC class I-restricted CD8⁺ T cells selected and primed in an environment with high (B6) or low (β_2m ^{-/-}) MHC expression (25). We find that self-MHC can deliver a triggering signal independently of specific peptide, provided there is an increase in self-MHC density. The low surface density of H-2 expressed on RMA-S cells was not sufficient to trigger either β_2m ^{-/-} or B6 CTLs in the absence of the specific antigen, but increasing the ligand density

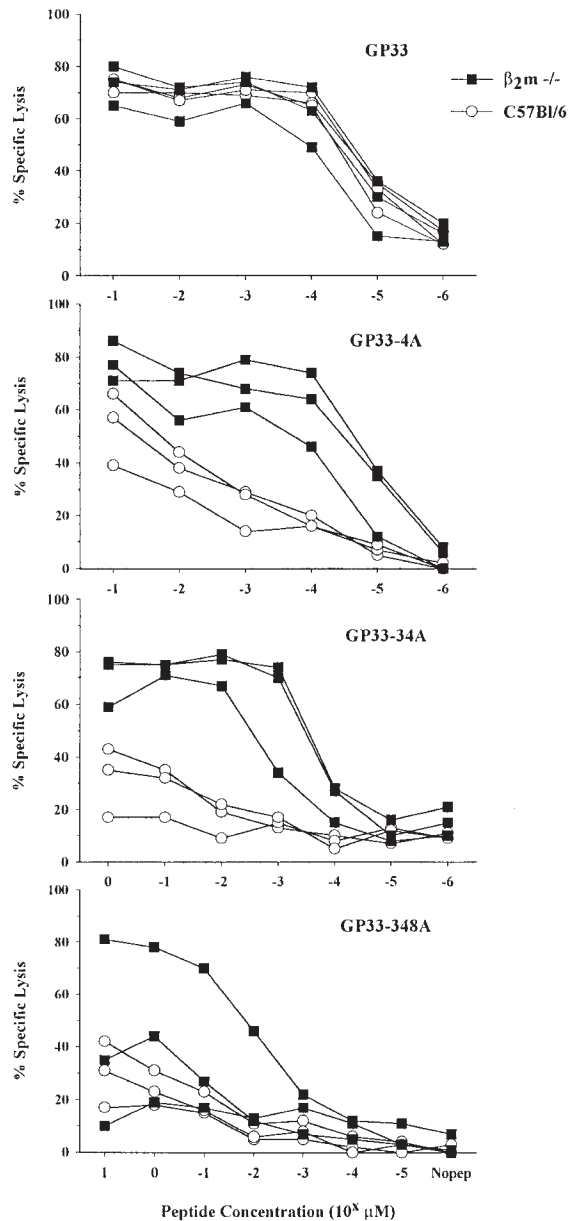


Figure 6. β_2m ^{-/-} CTLs display increased peptide promiscuity in recognition of MHC-peptide ligands. Groups of three B6 and three β_2m ^{-/-} mice were immunized with LCMV GP33 peptide as described in Materials and Methods. After 12 d, immune splenocytes were restimulated with GP33 for 6 d and tested in a ⁵¹Cr-release assay against RMA-S target cells loaded with titrated amounts of GP33 and GP33 variants: GP33 KAVYNFATM; GP33-4A KAVANFATM; GP33-34A KAAANFATM; and GP33-348A KAAANFAAM.

by cold temperature incubation could sensitize RMA-S to the β_2m ^{-/-} CTL clone 27/30. Furthermore, since T2D^b cells were also killed irrespective of loaded peptide, recognition of syngeneic class I was most probably peptide independent. These results indicate that the interaction between TCR and self-MHC as observed in crystals not only provides the structural framework of specific T cell recognition, it also contributes a part of the avidity required for CTL triggering.

Recognition of MHC^{high} targets by $\beta_2m^{-/-}$ CTLs was blocked by mAb against properly conformed D^b molecules, whereas the presence of specific peptide prohibited blocking. These results indicated that the lack of GP33 was compensated by using an increased number of low-avidity interactions with self-MHC. The results further suggest that a minimum of four times the level of MHC class I expression present during selection and priming *in vivo* were necessary for activation by self-MHC to occur *in vitro*. RMA-S expresses only about three times the level of MHC found on $\beta_2m^{-/-}$ cells (Table II), which may explain why $\beta_2m^{-/-}$ CTLs were peptide specific when tested against RMA-S targets. In the presence of specific GP33 peptide, no mAb-mediated blocking of MHC^{high} target cell killing was observed. This supports the notion that MHC-restricted CTLs have a high avidity for the peptide antigen and a low avidity for self-MHC.

Interestingly, we found that T cells with an increased avidity for MHC class I molecules had a more relaxed peptide specificity, i.e., they were less dependent on the exact peptide sequence. While both B6 and $\beta_2m^{-/-}$ GP33-specific CTLs had similar sensitivity for GP33, $\beta_2m^{-/-}$ CTLs had superior sensitivity for peptides lacking one or several TCR contact residues of the GP33 peptide. Thus, increased recognition of the restriction element compensates for lack of TCR peptide affinity in recognition of low-affinity peptide ligands.

Our data argue that recognition of the two entities of the MHC complex, peptide and MHC heavy chain, can be considered separately (41). Increased recognition of MHC could substitute for lack of peptide recognition. The indication that TCR avidity for its ligand can be subdivided in this way opens the possibility of extending the differential avidity model of T cell selection and recognition (42).

Implications for the Avidity Threshold in T Cell Recognition. The present data can be interpreted within a model for T

cell recognition based on the total avidity contributed by TCR affinity for MHC, affinity for peptide, and the number of MHC-peptide complexes. Increased avidity for MHC can compensate for lack of avidity for peptide, suggesting that these binding forces are functionally interchangeable and can be considered separately (Fig. 7 A). This would suggest a variation in the avidities for MHC and presented antigens among mature CTLs (See also Fig. 3, D–G). Interestingly, mice deficient in terminal deoxynucleotidyl transferase (TdT), which lack N-region additions in the TCR, display increased promiscuity in T cell recognition of peptide ligands (43). In this situation, MHC was suggested to provide compensatory avidity in recognition of peptide antigen by TdT^{-/-} CTLs. Further, the data in this paper support an inverse correlation between the number of MHC-peptide ligands necessary for triggering and TCR avidity for the ligand (for data, see Fig. 5; for model, see Fig. 7 B). Lower avidity for an antigen would then be compensated by an increase in antigen density. This feature becomes most important for CTLs with a relatively high MHC avidity, since self-MHC can be regarded as a high-density and low-affinity ligand.

By combining Fig. 7, A and B, a hypothetical three-dimensional diagram can be generated in which the surface depicts how the TCR affinities for peptide and MHC, and the number of ligands necessary for triggering, are interchangeable (Fig. 7 C). The diagram proposes that when the TCR avidities for both peptide and MHC are low, the number of ligands necessary to achieve triggering will be high. We would like to suggest further that the same model would apply for thymic selection, where the threshold coordinates for positive selection would be considerably lower on all axes while coordinates for negative selection would be closer to the threshold for triggering of effector functions.

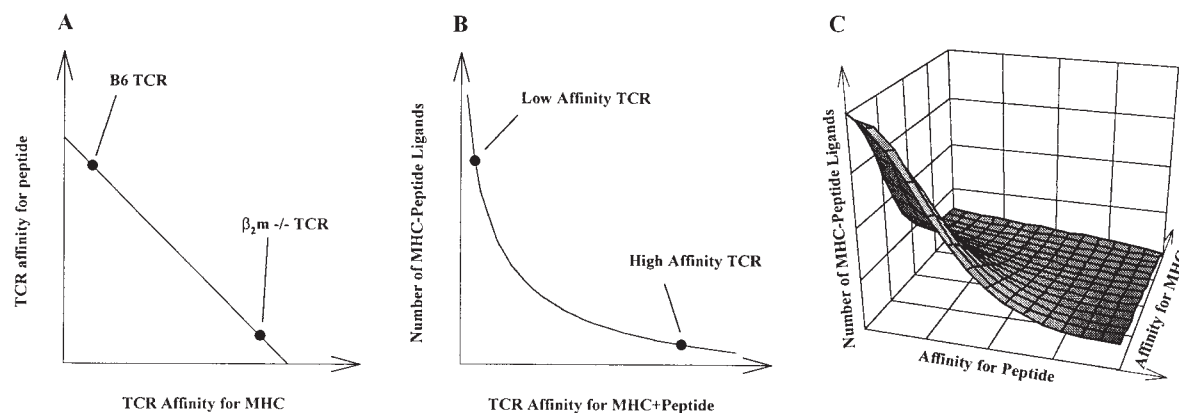


Figure 7. The TCR avidity threshold for T cell recognition. Hypothetical diagrams illustrating how the TCR affinities for peptide and MHC are interchangeable and mutually compensatory (A), and how the number of MHC-peptide ligands necessary for triggering inversely correlates with the TCR affinity for the ligand (B). (C) A and B are combined to generate a threshold composed of TCR affinities for MHC and peptide and the number of MHC-peptide complexes. In this threshold, all three contributing components can compensate for each other to generate the avidity necessary for T cell activation. The curve in A is presented as a straight line because a constant combined value of MHC-peptide avidity required for CTL triggering is subdivided into two parts, representing avidity for either MHC (x-axis) or peptide (y-axis). The shape of the curve in B is hypothetical. However, the hyperbolic shape will result if the total avidity required for triggering is constant, and if this avidity is a product of the number of ligands and the TCR affinity for each of these ligands (i.e., total triggering avidity = no. of ligands \times TCR affinity for each ligand).

Aspects of CD8⁺ T Cell Selection in the Absence of β_2m . Selection of H-2D^b-restricted T cells specific for the GP33 epitope in $\beta_2m^{-/-}$ mice is thymus dependent, and results in a peripheral repertoire biased towards recognition of the restriction element expressed with and without self-peptides. However, peptide-independent triggering occurs only when MHC is expressed at levels considerably higher than those encountered by the T cells in vivo. This indicates repertoire calibration during thymic selection to fit the self-MHC ligand density in the periphery. Our results fit very well with the notion that T cells being selected in the thymus view self-MHC as a low-affinity ligand. Self-ligands capable of triggering induce deletion, and in the periphery self-MHC never reaches the ligand density to trigger CTLs in the absence of triggering antigens. It should be noted that in addition to the increased recognition of MHC, the $\beta_2m^{-/-}$ CTLs may also display an increased avidity for self-peptides. There may be a clonal variation in recognition of MHC and peptide within the $\beta_2m^{-/-}$ CTL population, where the clone 27/30 represents the most peptide-independent phenotype.

Considering the low expression of MHC present during selection in the $\beta_2m^{-/-}$ mice, it is possible that the CD8⁺ T cells use upregulation of (co)receptor expression to achieve positive selection. Indeed, we have observed a marginal increase in CD8 expression in some of the $\beta_2m^{-/-}$ CTLs, which potentially could also contribute to the elevated recognition of self-MHC. However, in functional experiments based on recognition of GP33 and alanine-substituted variants, we have observed that $\beta_2m^{-/-}$ CTLs are less susceptible to CD8 blocking with anti-CD8 α mAbs compared with B6 CTLs. This result would suggest, rather, a decreased dependency on CD8 in triggering of $\beta_2m^{-/-}$ CTLs (data not shown).

Accumulating evidence suggests that the CD8⁺ T cells present in the $\beta_2m^{-/-}$ mice have retained important characteristics of the $\beta_2m^{+/+}$ wild-type concerning development and recognition of MHC. First, $\beta_2m^{-/-}$ mice can generate

in vivo CTL responses against MHC class I-restricted peptide (44; and this study) and viral (45) antigens, and reject skin grafts over a minor histocompatibility barrier (46). Also, $\beta_2m^{-/-}$ CTLs can recognize peptide antigens on $\beta_2m^{+/+}$ targets (this study). Second, development of $\beta_2m^{-/-}$ CD8⁺ T cells is dependent on the thymus (this study), and results in a CD8⁺ T cell repertoire biased towards recognition of syngeneic class I (23; and this study). Third, H-2D^b heavy chains expressed in the absence of β_2m are recognized by conformation-dependent mAbs (27, 28, 47; and this study), and expression is TAP dependent (32; and this study). Note also that all detectable $\beta_2m^{-/-}$ CTL reactivity against self-MHC could be blocked by an mAb specific for properly conformed H-2D^b molecules, which reduces the likelihood for a role of aberrantly conformed free D^b heavy chains in this system. Fourth, wild-type CTLs can recognize endogenously processed antigens (48) and alloantigens (27, 48) on $\beta_2m^{-/-}$ cells. Taken together, these data suggest that T cell selection follows the normal rules in $\beta_2m^{-/-}$ mice, although the selection window is dramatically shifted towards low ligand density. The confrontation of such T cells with cells expressing normal MHC levels allows detection of the T cell avidity for self-MHC. However, the functional avidity must be established already during selection and must exist during priming. Thus, we propose that T cells in normal mice have a similar avidity for self-MHC which would be detectable by exposing them to cells with supraoptimal MHC levels.

We have demonstrated that avidity for self-MHC can trigger MHC-restricted T cells independently of specific peptide if ligand density of self-MHC is sufficiently increased. This interaction compensates for lack of TCR affinity for peptide, and it depends on a TCR-MHC interaction of relatively low affinity that requires high numbers of MHC ligands. The data and the avidity threshold model discussed contribute to our understanding of T cell specificity in the periphery and during thymic selection.

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Address correspondence to Rickard Glas at his present address, Department of Pathology, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115. Phone: 617-432-4779; Fax: 617-432-4775; E-mail: glas@hms.harvard.edu

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