Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11482–11486, November 1994
Immunology

The peptide p2Ca is immunodominant in allorecognition of L^d by β chain variable region $V\beta8^+$ but not $V\beta8^-$ strains

JANET M. CONNOLLY*

Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110

Communicated by Herman N. Eisen, July 12, 1994 (received for review May 3, 1994)

ABSTRACT **An explanation for the vigorous allograft rejection that results from the recognition by $CD8^+$ T cells of allogeneic major histocompatibility complex (MHC) molecules has long eluded immunologists. Recent evidence has demonstrated that alloreactivity involves recognition of both the allogeneic MHC molecule and its associated peptide ligand, suggesting the current theory that the strength of the allogeneic response results from the participation of numerous peptides. However, I report here that a single peptide, p2Ca, is immunodominant in allorecognition of the murine MHC class I molecule H-2L^d. The majority of L^d-alloreactive T-cell clones are specific for L^d–p2Ca and this immunodominance is not due to peptide cross-reactivity. Generation of L^d-alloreactive cytotoxic T lymphocytes in a strain tolerant to p2Ca did not affect the peptide immunodominance, demonstrating that tolerance to p2Ca is MHC-restricted. The p2Ca-specific clones express β chain variable region $V\beta8$ T-cell receptors, however, L^d-alloreactive cytotoxic T lymphocytes generated in $V\beta8^-$ mice are not dominated by recognition of p2Ca, suggesting that the T-cell receptor repertoire is a factor in determining peptide immunodominance.**

The molecular basis of T-cell recognition of allogeneic cells remains controversial in terms of both the composition of the responding clones and the nature of the epitope that positively selects for alloreactive T cells. The original model of Matzinger and Bevan (1), to explain the high frequency of alloreactive T cells, proposed that several different antigens, in addition to major histocompatibility complex (MHC) determinants, were being recognized by a large number of T cells. Recent evidence suggests that alloreactive T cells recognize a complex of both class I and endogenous peptide ligand on the allogeneic tissue (2–4) and that alloreactive T cells are capable of the same degree of specificity as are self-MHC-restricted T cells (5). Thus, a current interpretation of the original model would predict that the high precursor frequency observed in allorecognition is the sum of several responding clones, each specific for a different peptide. Such diversity of antigenic epitopes recognized by alloreactive cytotoxic T lymphocytes (CTLs) would be reflected in a diversity of the T-cell receptor (TCR) repertoire produced in response to alloantigen. However, some studies have reported preferential TCR usage in alloreactive responses (6–8), whereas other studies observed diverse TCR usage (9–11).

Recent evidence has also revealed that thousands of endogenous peptides can bind to MHC class I molecules and potentially be presented to the immune system (12). However, it is not known how many different peptides are involved in allorecognition, whether alloreactive CTLs are more cross-reactive with other peptides than are MHC-restricted CTLs, and what fraction of the endogenous peptides involved are tissue-specific. The difficulty in addressing these questions stems from the facts that, with one exception, the peptides that are involved in allorecognition are unknown and the specific sequences of endogenous peptides are to a large extent unknown. A significant finding relevant to this issue was the identification and sequencing of the endogenous peptide, p2Ca, recognized by the L^d-alloreactive clone 2C (13). The 2C clone was generated from a BALB.B mouse stimulated with H-2^d (14) and was subsequently shown to express a β chain variable region $V\beta8^+$ TCR positively selected on H-$2K^b$ (15). Previous studies from this laboratory (8, 16) have shown that responses restricted by and specific for L^d preferentially use $V\beta8$ TCR. Indeed, *in vivo* elimination of the $V\beta8^+$-responding T-cell population with antibody or treatment of mice with $V\beta8$ peptides has been shown to significantly prolong L^d-disparate skin and cardiac allograft survival (8, 17).

Results reported here demonstrate that p2Ca is immunodominant in L^d-allorecognition by CTLs generated from $V\beta8^+$, but not $V\beta8^-$, strains of mice. Tolerance to p2Ca did not affect the peptide immunodominance in responses generated by L^{q+} mice. The demonstration of the peptide specificity of these L^d-alloreactive clones was dependent upon the availability of the L^d-transfected target cell, T2-L^d, that does not present this peptide. Thus p2Ca-specific clones are detectable only after peptide sensitization of T2-L^d. The data presented here indicate that peptide immunodominance can occur in allorecognition and that this immunodominance may be influenced by the available TCR repertoire.

MATERIALS AND METHODS

Mice. BALB/c Kh ($K^dD^dL^d$), BALB/c H-2^{dm2} (dm2, K^dD^d), (dm2 × B10.AKM)F_1 ($K^{d/k}D^{d/q}L^q$), and B10.BAR8 ($K^bD^dL^d$) were bred in the animal facility of Donald C. Shreffler (Washington University School of Medicine, St. Louis, MO). C57BL/10 (K^bD^b), and C57L (K^bD^b) were obtained from The Jackson Laboratory.

Peptides. The amino acid sequence of p2Ca is LSPFPFDL (13). Sequences of the control L^d peptide ligands are as follows: MCMV (YPHFMPTNL) (18), LCMV (RPQASGVYM) (19), tum^- (TQNHRALDL) (20), and β-gal (TPHPARIGL) (21). Sequences of the other L^d-derived endogenous peptide ligands (22) are as follows: P29 (YPNVNIHNF), P24B (APQPGMENFK), and P25A (QPQRGRENF). Peptides were synthesized using Merrifield's solid-phase method (23) on a peptide synthesizer (model 431A, Applied Biosystems). Peptides were purified (≥95%) by reverse-phase HPLC and purity was assessed (24).

Cell Lines. R1.1-L^d is a C58/J (H-2^k) thymoma cell line transfected with the L^d gene. P815 is a DBA/2 (H-2^d) mastocytoma cell line. T2-L^d is a human lymphoblastoid cell line

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Abbreviations: MHC, major histocompatibility complex; CTL, cytotoxic T lymphocyte; TCR, T-cell receptor; mAb, monoclonal antibody; IL-2, interleukin 2.

*To whom reprint requests should be addressed at: Department of Genetics, Box 8232, Washington University School of Medicine, St. Louis, MO 63110.

deficient in peptide transport, transfected with the L^d gene (25, 26) (gift of P. Cresswell, Yale University, New Haven, CT). Cell lines were grown in RPMI medium 1640 supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), 2 mM L-glutamine, and 10% (vol/vol) newborn calf serum (HyClone Laboratories). Transfected cell lines were carried in medium containing G418.

Flow Cytometry. Cells were incubated for 30 min at 4°C in Hanks' balanced salt solution containing 0.2% bovine serum albumin and 0.1% sodium azide in the presence of the murine γ2a monoclonal antibody (mAb) 30-5-7 specific for the L^d α2 domain (27), the murine γ2a mAb F23.1 specific for Vβ8 (28), clonotypic mAb 1B2 specific for the 2C TCR (40), or medium alone. The cells were washed and incubated with a fluorescein-conjugated Fc-specific affinity-purified $F(ab')_2$ fragment of goat anti-mouse IgG (Organon Teknika, West Chester, PA) for 30 min at 4°C. The cells were analyzed on a FACScan (Becton Dickinson) equipped with an argon ion laser tuned to 488 nm and operating at 150 mW of power. Fluorescence histograms were generated with logarithmic amplification of fluorescence emitted by single viable cells.

Generation of L^d-Alloreactive CTLs and CTL Clones. Approximately 7.5×10^6 responding spleen cells were cocultured with 3.5×10^6 stimulating spleen cells (2000R) in 24-well Linbro trays (Flow Laboratories) containing 2 ml of RPMI medium 1640 supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, nonessential amino acids (0.1 mM), penicillin (100 units/ml), streptomycin (100 ug/ml), 5×10^{-5} M 2-mercaptoethanol, and 10% (vol/vol) fetal calf serum (HyClone). After 5 days at 37°C in the presence of 5% CO_2/95% air, effector cells were analyzed for cytolytic activity as described below and/or were restimulated at 3×10^6 cells per well with 5×10^6 stimulating spleen cells (2000R) per well. After the second week, CTLs were plated at 2×10^5 cells per well with 5×10^6 irradiated stimulating spleen cells and recombinant interleukin 2 (IL-2; BioSource, Camarillo, CA; 10 units/ml). Lines were maintained by weekly restimulation in the presence of recombinant IL-2 (10 units/ml). After 1–3 weeks in IL-2, clones were generated by limiting dilution in 96-well plates. After an additional 3–4 weeks, all of the wells were analyzed for recognition of various target cells by split-well analysis. Selected clones were expanded in 24-well plates.

Limiting Dilution Analysis of Primary CTLs. Naive spleen cells were cultured in 96-well microtiter plates in replicates of 24 at six 1:2 serial dilutions beginning at 2×10^5 cells per well. Optimal conditions were determined to be 1×10^6 irradiated stimulator spleen cells and 20% (vol/vol) Con A supernatant as a source of IL-2. After 7 days, 100 μl of medium was replaced with fresh stimulator cells in medium containing 20% Con A supernatant and 4 days later 40–50 μl from each well were tested on three or four different targets simultaneously. A well was considered negative if the lysis was 10% or lower, based on analysis of unstimulated cultures and lysis of negative control target cells by stimulated cultures. In some cases, the remaining cells were restimulated and clones were selected for expansion in 24-well plates.

^{51}Cr Release Assay. Approximately $1–2 \times 10^6$ target cells were labeled for 1 hr with 150–200 μCi of ^{51}Cr ($Na^{51}CrO_4$, DuPont/NEN; 1 Ci = 37 GBq) in 100 μl of RPMI medium 1640/10% newborn calf serum with or without peptide for 1 hr at 37°C in 5% CO_2/95% air. In some experiments, cells were cultured overnight with peptide then labeled with ^{51}Cr in the presence of peptide. For established clones, 5×10^3 target cells per well were added to 96-well microtiter plates and effector cells were added at various concentrations. For 96-well cloning plates, 100 μl from each well was combined with 100 μl of fresh medium containing 1×10^3 target cells. The plates were centrifuged at $50 \times g$ for 1 min and incubated for 4 hr at 37°C in 5% CO_2/95% air. Radioactivity in 100 μl of supernatant was measured in an Isomedic γ counter (ICN). The mean of triplicate samples was calculated and percent ^{51}Cr release was determined as described (29).

RESULTS

Peptide p2Ca Is Immunodominant in L^d Allorecognition. Peptide p2Ca is the octamer LSPFPFDL recognized by the L^d-alloreactive clone 2C (13). To determine whether other T cells specific for L^d alloantigens also recognized p2Ca, CTLs were generated in dm2 mice against BALB/c. The dm2 strain was derived from a spontaneous mutation in BALB/c resulting in the deletion of the entire L^d gene and no other known functional class I genes (30). Clones generated in dm2 mice anti-BALB/c that recognized P815 target cells were tested for p2Ca specificity by using T2-L^d, a cell line defective in peptide transport (25, 26). Surprisingly, the majority of L^d-alloreactive clones generated from different cultures were specific for p2Ca when tested on peptide-fed T2-L^d (Fig. 1). These clones did not recognize T2-L^d in the absence of peptide or T2-L^d fed a different endogenous L^d ligand, P29. Both peptide ligands induce equivalent levels of L^d expression on T2-L^d (Fig. 2), eliminating the possibility that increased determinant density of L^d accounts for the recognition of T2-L^d–p2Ca. To determine whether the L^d-alloreactive clones specific for p2Ca were peptide cross-reactive, they were tested for the ability to recognize L^d complexed with other ligands. A total of six L^d ligands (MCMV, LCMV, P29, tum$^-$, β-gal, and p2Ca) were tested for recognition by seven clones, after peptide feeding T2-L^d overnight. None of the other L^d–peptide complexes were recognized by the p2Ca-specific clones (data not shown), indicating that they are truly peptide-specific, and the peptide immunodominance cannot be explained by the presence of cross-reactive clones.

Limiting dilution analysis was performed on cultures generated from naive spleen cells and from cultures expanded in IL-2 for 2 weeks prior to cloning to determine whether p2Ca-specific clones could be detected. The clones were tested simultaneously by split-well analysis on T2, T2-L^d,

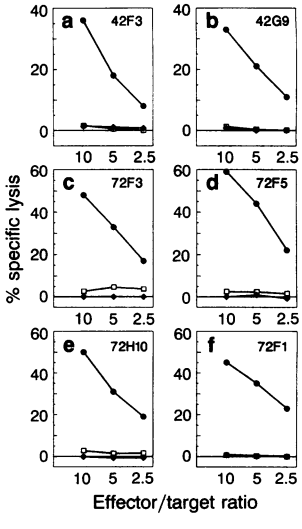

FIG. 1. L^d-alloreactive clones generated from dm2 anti-BALB/c cultures are specific for the same peptide, p2Ca. Clones were tested for peptide specificity using T2-L^d target cells that had been incubated with 100 μM p2Ca overnight (●) and washed prior to addition of effector cells. Control target cells included T2-L^d in the absence of exogenous peptide (□) and T2-L^d incubated overnight with 100 μM P29 (◆). Clones 42F3 and 42G9 were generated from one culture and clones 72F3, 72F5, 72H10, and 72F1 were generated from another culture. (*a*) Clone 42F3. (*b*) Clone 42G9. (*c*) Clone 72F3. (*d*) Clone 72F5. (*e*) Clone 72H10. (*f*) Clone 72F1.

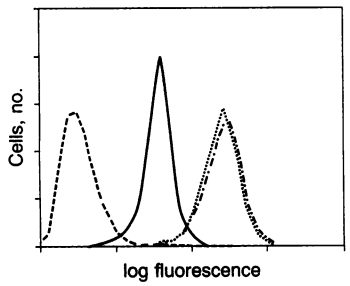

FIG. 2. Expression of L^d by T2-L^d was detected using the conformation-dependent mAb 30-5-7 (27). Cells were cultured overnight in the absence of peptide (solid line) or in the presence of 100 μM p2Ca (dotted line) or 100 μM P29 (dotted and dashed line). Background fluorescence is from cells incubated only with developing antibody (dashed line). The mean fluorescence values are as follows: background, 3; L^d, 44; L^d–p2Ca, 311; L^d–P29, 281.

T2-L^d–p2Ca, and P815. Whereas polyclonal L^d-alloreactive primary CTLs lyse T2-L^d, p2Ca-specific clones do not recognize T2-L^d in the absence of exogenous peptide. Thus, the presence of clones in a given well, with specificity for this peptide, can be distinguished by stronger lysis of the p2Ca-peptide-fed target. As a control peptide, the abundant endogenous L^d ligand P29 (22) was included in some experiments and gave results similar to non-peptide-fed target cells (data not shown). Results from CTL clones that were positive on T2-L^d and/or T2-L^d–p2Ca were plotted on a single graph (Fig. 3). Approximately half of the wells from primary cultures showed stronger lysis of the p2Ca-peptide-fed target, indicating that a significant number of peptide-specific clones can be detected (Fig. 3*a*). In addition, the presence of p2Ca-specific clones is dramatically apparent in short-term cultures (Fig. 3*b*). A majority of the wells now contained p2Ca-specific clones. Therefore, p2Ca-specific CTL clones are detectable in primary cultures and it is these clones that are preferentially expanded.

Tolerance to p2Ca Does Not Affect the Immunodominance of p2Ca in Responses by an L^{q+} Strain. To determine whether tolerance to the p2Ca peptide influences the peptide immunodominance, L^d-alloreactive CTLs were generated in L^q-bearing mice. H-2L^d and H-2L^q differ by only six amino acids in the α2 domain; positions 95, 97, and 116 in the peptide binding groove, positions 155 and 157 on the α-helix predicted to interact with the TCR, and a serologic determinant at position 107 on a loop between the α-helix and β-sheet (27). In addition, L^q binds p2Ca and L^q expression is up-regulated by peptide binding (unpublished observation). L^d-alloreactive CTLs were generated in a strain combination that differs only at the *L* locus, (dm2 × B10.AKM)F_1 anti-BALB/c. These F_1 responder mice express both L^q and the BALB/c-derived gene product from which the p2Ca peptide originates (31). The majority of clones generated from the F_1 mice were specific for p2Ca. Indeed, even the primary CTL response was strongly dominated by p2Ca-specific CTL (Fig. 4 *a* and *b*) and the clones generated after short-term culture are almost exclusively p2Ca-specific (Fig. 4*c*). Of the wells analyzed in Fig. 4, 22 wells were positive on P815, 20 were positive on T2-L^d, and 50 were positive on T2-L^d–p2Ca. The disparity between the number of wells positive on T2-L^d–p2Ca vs. P815 is likely due to the fact that T2-L^d was peptide-fed overnight and, therefore, expresses a high determinant density of L^d–p2Ca complexes. P815 expresses the p2Ca peptide endogenously and, therefore, at a lower determinant density. In any case, L^d-alloreactive CTL responses generated in (dm2 × B10.AKM)F_1 mice are even more strongly dominated by recognition of p2Ca. Interestingly, the precursor frequency of L^d-alloreactive CTLs is higher in (dm2 × B10.AKM)F_1 mice than in dm2 mice (data not shown), apparently due to the presence of a higher number of p2Ca-specific clones. These data demonstrate that tolerance of L^{q+} responders to peptide p2Ca did not reduce the peptide immunodominance of L^d-alloreactive CTLs.

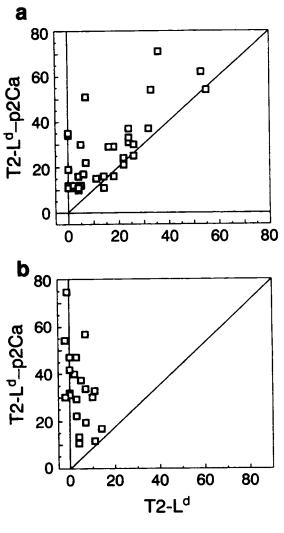

FIG. 3. Split-well analysis of limiting dilution cultures from dm2 anti-BALB/c primary cultures (*a*) and cultures that were expanded in IL-2 for 2 weeks (*b*). Negative wells are not plotted. For the data in *a*, a total of 144 wells were analyzed. Input cell numbers started at 2 × 10^5 cells per well and 1:2 serial dilutions in replicates of 24 were made. A total of 34 points are plotted that are positive on T2-L^d and/or T2-L^d–p2Ca. For the data in *b*, 96 wells were analyzed at a single input cell number of 100 cells per well. A total of 21 points are plotted.

The Vβ8 Dominance of L^d-Alloreactive CTLs Correlates with Recognition of Both L^d and p2Ca. All nine of the p2Ca-specific clones from dm2 that were analyzed express Vβ8 TCR (six are shown in Fig. 5), consistent with our previous observation (8). In addition, the 2C TCR generated in H-2^b is Vβ8$^+$ and L^d-alloreactive polyclonal lines generated in (dm2 × B10.AKM)F_1 mice contain a high proportion of Vβ8$^+$ T cells (data not shown). Recently, p2Ca-specific self-L^d-restricted CTLs were shown to be dominated by Vβ8$^+$ T cells and to use similar β chain joining segments (32, 33). None of the dm2 or (dm2 × B10.AKM)F_1 clones or lines were positive with the clonotypic antibody 1B2 (40), specific

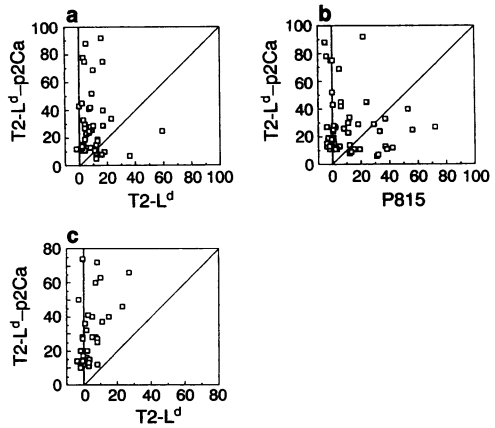

FIG. 4. Split-well analysis of limiting dilution cultures from (dm2 × B10.AKM)F_1 anti-BALB/c primary cultures (*a* and *b*) and cultures that were expanded in IL-2 for 1 week (*c*). Negative wells are not plotted. For the data in *a* and *b*, three 1:2 serial dilutions in replicates of 24 were made starting with input cell numbers of 2.5 × 10^4 cells per well. A total of 72 wells were analyzed. For the data in *c*, 96 wells were analyzed at a single input cell number of 30 cells per well. A total of 34 points are plotted.

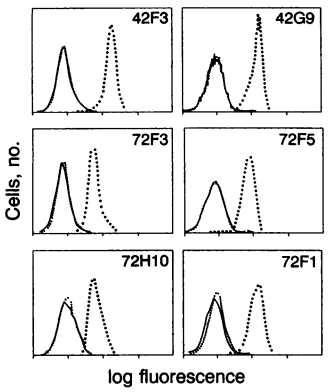

FIG. 5. All of the L^d-alloreactive clones express a $V\beta8^+$ TCR. CTL clones generated from two dm2 anti-BALB/c cultures were analyzed on a FACScan for the expression of $V\beta8$ using mAb F23.1 (large dotted lines) and for the expression of the 2C TCR epitope using the clonotypic mAb 1B2 (small dotted lines). Background represents fluorescence from cells incubated with the developing reagent alone (solid lines).

for the 2C TCR (Fig. 5 and data not shown). To determine whether the $V\beta8$ dominance correlates with the immunodominance of the p2Ca peptide in L^d-allorecognition, CTL clones were generated in the $V\beta8$-deletion strain C57L (34). Fig. 6 shows C57L clones generated by limiting dilution and tested by split-well analysis for recognition of T2-L^d and T2-L^d–p2Ca. As opposed to the results obtained with the $V\beta8^+$ strains of mice, the L^d-alloreactive CTLs generated in the $V\beta8^-$ strain are not dominated by recognition of p2Ca. One of 12 C57L clones that were expanded was specific for p2Ca. Therefore, p2Ca-specific clones are present but not preferentially expanded in cultures obtained from C57L animals. This suggests that the dominance of p2Ca is influenced by the availability of $V\beta8^+$ T cells.

Five of the C57L CTL clones were analyzed further. The p2Ca-specific clone was negative for expression of $V\beta2$, -6, and -7, whereas all four of the other clones were positive for expression of $V\beta6$, suggesting the existence of an alternative dominant population involved in L^d allorecognition. The p2Ca-specific clone recognized RMA.S-L^d in the absence of exogenous peptide but recognized T2-L^d only in the presence of p2Ca. On the other hand, the $V\beta6^+$ clones did not recognize RMA.S-L^d, even in the presence of the L^d peptide ligands MCMV, LCMV, P24B, P25A, and P29, yet they lysed T2-L^d in the absence of peptide (data not shown). Thus, both cell lines with defects in the peptide transporter, the murine RMA.S-L^d (35) and the human T2-L^d, express endogenous L^d peptide ligands and are capable of recognition by peptide-specific T cells. These data indicate that the majority of L^d-alloreactive clones are peptide-specific and that the dominant TCR usage observed in responses to L^d results from

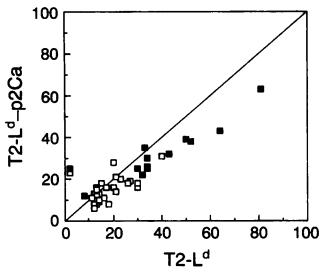

FIG. 6. Split-well analysis of clones generated from limiting dilution cultures from C57L anti-BAR.8 after expansion in IL-2. A total of 144 wells were tested at input cell numbers of 100, 30, and 10 cells per well. A total of 43 points are plotted. Those points that are represented by a solid circle were expanded for further analysis.

recognition of both L^d and peptide ligand. In addition these data suggest that peptide immunodominance can be influenced by the TCR repertoire.

DISCUSSION

The observation that a single peptide is immunodominant among alloreactive T cells was unexpected based on current theories of allorecognition. Given the large number of MHC–peptide complexes that a cell is capable of expressing (12), it is not clear why one peptide should so strongly dominate an alloreactive response. Allorecognition is believed to result from T cells selected in the thymus on a self MHC–peptide complex that cross-react with the allogeneic MHC–peptide complex. The dominance of only one or a few MHC–peptide complexes in allorecognition could result from the existence of only a few cross-reactive T-cell clones and/or only a few complexes are expressed at levels sufficient to stimulate a response. Henderson *et al.* (36) have reported that an endogenous HLA-A2.1 peptide ligand, recognized by five murine xenoreactive clones, is among the most dominant HLA-A2.1-associated species on human lymphoid cells when quantitated using mass spectrometry. Based on this observation, they propose that alloreactive T cells are stimulated by those peptides that are expressed at the highest density on the cell surface. Whether this is true for p2Ca must await surface quantitation. Although its prevalence is unknown, the p2Ca peptide has a weaker binding affinity to L^d compared with several other known L^d ligands (data not shown).

The observation that tolerance of L^q-bearing mice to p2Ca did not reduce the L^d-alloreactive response to this self peptide extends the observation of Grandea and Bevan (37), suggesting that tolerance to self peptides is MHC-restricted. Single-residue changes were introduced into K^b at positions predicted to interact with TCR and not peptide. K^b anti-K^{bm} alloreactive T cells recognized peptides in an HPLC fraction eluted from K^b, suggesting that these CTLs respond to self-peptide that is also bound by its own class I. However, in their study, the specific peptides were not identified. In the present study, the response to a known peptide was tested thus providing a direct demonstration that a self peptide can be presented in the context of an allogeneic class I molecule.

It is noteworthy that the L^d-alloreactive response was consistently stronger in the L^q-bearing mice, (dm2 × B10.AKM)F_1, than in dm2 mice. Perhaps this difference is a reflection of the class I alleles that are involved in positive selection of L^d–p2Ca-reactive T cells. K^b positively selects for the 2C TCRs in H-2^b mice (15), whereas in the dm2 anti-BALB/c response, it is presumably either K^d or D^d. Thus, positive selection for L^d–p2Ca-reactive T cells can involve different MHC alleles. It is tempting to speculate that L^q–p2Ca complexes also positively select for recognition of L^d–p2Ca. This laboratory has shown (5) that changing a single amino acid at position 95 in the floor of the peptide binding groove in L^q to the one in L^d can partially restore TCR recognition by peptide-specific L^d-alloreactive clones without a quantitative effect on peptide binding (5). Thus, the p2Ca bound to L^q may be presented in a different conformation than p2Ca bound to L^d and this MHC-imparted change in conformation of p2Ca may explain how p2Ca can predominate when L^q-positive mice respond to L^d alloantigens. Consistent with the notion that multiple class I alleles can positively select 2C-reactive T cells, Udaka *et al.* (13) indicated that p2Ca can bind to other MHC alleles, since acid extracts from several MHC-disparate mouse strains had identical HPLC activity profiles when tested for recognition by 2C T cells. Recently, Sykulev *et al.* (38) observed that p2Ca binds weakly to K^b, the endogenous class I that initially selected the 2C TCR, and, furthermore, found that K^b–p2Ca is recognized by the 2C clone. Whether this K^b–p2Ca com-

plex is physiologically involved in positive selection remains to be determined. Regardless, it will be interesting to determine the extent to which the p2Ca peptide is involved in positive selection of alloreactive T cells to L^d and how this impacts on p2Ca predominance in allorecognition.

Current theories of allorecognition predict diversity in the response (39). However, the degree of heterogeneity of the responding T-cell population is not a universal mechanism to explain the strength of alloreactive responses. For example, alloreactive responses to K^b are characterized by a diverse TCR repertoire (9), whereas alloreactive responses to L^d are not (8). A possible explanation for Vβ8 dominance in L^d allorecognition is that receptors that share Vβ8 are specific for several different peptide ligands bound by L^d. This is compatible with the current theories of diversity. However, the dominant Vβ8 usage in L^d allorecognition correlates with the recognition of a single peptide, p2Ca, and T cells with this specificity dominate the response. In fact, the dominance of p2Ca recognition in V$\beta8^+$ strains but not in V$\beta8^-$ strains suggests that dominant p2Ca recognition is dependent upon the presence of V$\beta8^+$ T cells. The requirement for Vβ8 to observe p2Ca dominance in L^d allorecognition may be a reflection of the intrinsic affinity of Vβ8 for L^d–p2Ca rather than the binding properties of p2Ca for L^d. A contribution by peptide ligand to Vβ8 selection could reside in the specific residues that interact with the TCR and/or in the conformation induced by peptide binding. In any case, the observation that a single peptide is dominant in allorecognition raises the possibility that peptides can be used therapeutically to enhance tumor rejection (32) or to prolong survival of tissue and organ allografts.

Note Added in Proof. It was recently observed that the alloresponse to the class I_B antigen, Qa-1 is also dominated by a single peptide (41).

I thank Rupika DeSilva and Cheryl Damico for technical assistance, John Gorka for peptide synthesis, and David McCourt for HPLC purification of peptides. I am also grateful to Drs. Ted Hansen, Joseph Smith, and William Gillanders for critical review of the manuscript. This work was supported by National Institutes of Health Grants AI-27568 and AI-19687.

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