

Alloreactive Cytotoxic T Lymphocytes Recognize Epitopes Determined by Both the α Helices and β Sheets of the Class I Peptide Binding Site

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Summary

A chimeric class I glycoprotein was created to investigate the functional contribution of the α helices and the β -pleated sheets in forming the antigen recognition site (ARS) of antigen-presenting molecules. This novel molecule was generated by replacing the DNA sequences encoding the α helices of the L^d gene with the corresponding sequences from the K^b gene. Serologic analysis of transfected L cells that expressed the chimeric molecule ($K^b_\alpha L^d_\beta$) revealed that the engineered class I glycoprotein retains two conformational epitopes associated with the α helices of K^b , as defined by monoclonal antibodies K10.56 and 28-13-3. These results demonstrate that the α helices of K^b can associate with the β -pleated sheets of L^d to form a stable structure, which is expressed on the cell surface. To address the role of the α helices of the ARS in determining T cell crossreactivity, alloreactive cytotoxic T lymphocytes (CTL) were used to analyze L cells expressing $K^b_\alpha L^d_\beta$. CTL raised against K^b or L^d as alloantigens showed little, if any, ability to lyse L cells expressing $K^b_\alpha L^d_\beta$. Thus, alloreactive CTL did not recognize structures determined by the α helices alone or by the β sheets of the ARS alone. However, bulk and cloned alloreactive CTL that were generated against the mutant K^b glycoprotein K^{bm8} reacted strongly with $K^b_\alpha L^d_\beta$. In addition to the K^b α helices, the K^{bm8} ARS shares a single polymorphic amino acid at position 24 with $K^b_\alpha L^d_\beta$. Amino acid 24 is located on the $\beta 2$ strand that forms part of the floor of the ARS and has been identified as a component of pocket B in the HLA class I ARS. The substitution of Glu to Ser at this position was shown previously to be the central determinant of the K^{bm8} mutant alloantigenicity. The functional significance of this position in determining crossreactivity between $bm8$ and $K^b_\alpha L^d_\beta$ identifies pocket B as a strong anchor for allogenic self-peptides. These findings demonstrate that determinants recognized by CTL on class I alloantigens are formed by interactions involving both the α helices and β sheets of the ARS. These interactions are best explained by the influence of the α helices and β sheets on the peptide-binding properties of these antigen-presenting molecules.

Discerning the molecular basis of the interactions between MHC class I glycoproteins and TCRs is central to understanding both antigen-specific and alloreactive immune responses. Current models explain alloreactivity at the molecular level in the context of the three-dimensional crystal structure of HLA-A2 (1). According to this view, T cell alloreactivity is caused by an altered antigen recognition site (ARS)¹ along the top face of polymorphic class I glycoproteins. The polymorphic amino acids whose side chains impinge on the ARS alter the orientation and spectrum of self-peptides presented to T cells by the class I glycoproteins (2).

The altered orientation and spectrum of self-peptides may account for the activation of a high proportion of the available T cell repertoire (3, 4). The importance of amino acid side chains oriented into the ARS with regard to alloreactivity has been tested by site-directed mutagenesis (5-9). These investigations confirm that natural polymorphisms in amino acid side chains oriented away from the ARS are silent, while amino acids with side chains oriented into the ARS influence alloreactivity.

An added component of complexity to allorecognition comes from the polymorphic amino acids located on the α helices with side chains oriented outward from the top face of the class I glycoprotein (2). These amino acids may influence alloreactivity without altering self-peptides bound in the ARS

¹ Abbreviation used in this paper: ARS, antigen recognition site.

by directly interacting with the TCR. Thus, several different interactions between MHC molecules and the TCR could result in allorecognition (2, 3, 4, 10). Alloreactive T cells could respond to the polymorphic amino acids located in the α helices, which are directly accessible to TCR binding. Some T cells may recognize peptides in the ARS with no interaction with the α helices being necessary. Still other T cells may require a combination of altered self-peptide and interaction with the α helices for allorecognition to occur.

To investigate the role of the α -helical regions of the ARS in allorecognition, we constructed a chimeric class I protein using a novel technique based upon the PCR (11). Using this technique, a new MHC class I molecule was designed in which the DNA encoding the α helices of K^b have been spliced into the L^d gene. The protein expressed by this construct has a chimeric ARS with K^b α helices and L^d β -pleated sheets ($K^b_\alpha L^d_\beta$). This new class I construct was expressed in L cells and analyzed serologically with a panel of mAbs and for cell-mediated crossreactivity using a series of alloreactive CTL.

Materials and Methods

Mice. Mice used in these studies that were purchased from The Jackson Laboratories (Bar Harbor, ME) were C57BL/6ByJ (H-2^b), B6.C-H-2^{bm10}/KhEg (bm10), B6.C-H-2^{bm11}/KhEg (bm11), C57BL/6J-H2^{bm5}/KhEg (bm5), B6.C-H-2^{bm1}/ByJ (bm1), C57BL/6Kh-H-2^{bm8}/KhEg (bm8), C3H/HeJ (H-2^k), and C3H.SW (H-2^b). B10.GD/DfDv (H-2^{g2}), B10.AQR/KIDv (H-2^{q1}), and B10.RQDB (H-2^{qbl}) recombinant mice were obtained from Dr. Chella David's Immunogenetic colony at the Mayo Clinic (Rochester, MN).

Monoclonal Antibodies. The origin and specificity of the H-2K^b-reactive mAbs K10.56 (γ 2b, κ) and B8-24-3 (γ 1a, κ), as well as the H-2L^d-reactive mAbs 64-3-7 (γ 1a, κ), 30-5-7 (γ 2a, κ), and 28-14-8 (γ 2a, κ), were described previously (12–17).

Immunofluorescence Analysis. The serologic profile of the transfected L cell lines were determined as previously described (8). Briefly, L cells were incubated with either the specific mAb or an isotype control for 30 min on ice. The cells were then washed three times with HBSS containing 0.2% Na-azide and 1% BSA. The washed cells were incubated with an appropriate dilution of FITC-conjugated goat anti-mouse antibody (Tago, Inc., Burlingame, CA). Viable cells, as determined by exclusion of propidium iodide exclusion, were analyzed on a FACS IV[®] (Becton Dickinson & Co., Mountain View, CA).

Construction of the Chimeric Class I Gene. The chimeric class I gene was constructed by splicing the DNA encoding the α helices from the K^b gene to DNA encoding the β -pleated sheets of L^d . K^b and L^d were chosen for the construction, because these two molecules differ at virtually all polymorphic positions on the β strands that form the floor of the ARS and by 10 amino acids along the α helices that influence peptide binding and/or that interact directly with the TCR (Fig. 1). The chimera was assembled using the PCR-based method (11) of splicing by overlap extension. The oligonucleotide primers used for constructing the chimeric gene are listed in Table 1 along with the corresponding region that each pair of primers amplified. The four individual PCR fragments (1–4) were amplified and gel purified using Gene Clean (Bio 101, Inc., La Jolla, CA). In a separate PCR, the first fragment corresponding to the first intron to amino acid 50 of L^d was fused to the second fragment corresponding to amino acids 50–87 of K^b using the flanking primers A and D. Similarly, the third fragment corre-

sponding to amino acids 87–129 of L^d was fused to the fourth fragment corresponding to amino acids 129–181 of K^b using the flanking primers E and H. These two fragments were then gel purified and fused in a final PCR using primers A and H to create the chimeric construct consisting of DNA encoding the α helices of K^b and the β -pleated sheets of L^d ($K^b_\alpha L^d_\beta$). The construct was cloned into the class I expression vector described by Pullen et al. (8) and sequenced before transfection.

L Cell Transfection. The $K^b_\alpha L^d_\beta$ construct was transfected into L cells (H-2^k) using the calcium phosphate method as previously described (8). L cells expressing K^b , K^{bm8} , K^{bm8-22} , K^{bm8-24} , and $K^{bm8-23,30}$ have been described previously (8, 9).

Generation of CTL and Analysis of L Cells by ⁵¹Cr Release. CTL lines were generated as described previously (8, 9). Briefly, primary CTLs were generated by coculture of equal numbers (5×10^6) of responder and irradiated (3,000 rad) stimulator cells in 2 ml of complete medium (RPMI 1640, 10% FCS, 5×10^5 2-ME, 0.4 mM L-glutamine, 40 U/ml penicillin "G", 50 μ g/ml streptomycin sulfate) using 24-well plates. After 5 d, cytotoxic activity was measured using a standard ⁵¹Cr release assay. CTL specific for the stimulating strain and with low background lysis of targets bearing self-antigens were cultured continuously by restimulation every 7–10 d in media containing 5 U/ml rIL-2 (Amgen Biologicals, Thousand Oaks, CA). Some bulk CTL lines were cloned at five cells/well in 96-well plates. To determine cell lysis, transfected L cell targets were labeled with 100 μ Ci of sodium [⁵¹Cr]chromate (Amersham Corp., Arlington Heights, IL) per 10^6 cells. Labeled cells were washed three times and 5×10^3 cells were incubated with serial dilutions of effector CTL in 200 μ l of complete medium using 96-well round-bottomed plates. After a brief centrifugation, plates were incubated for 8 h at 37°C, supernatants were harvested, and radioactivity was determined. Maximum release of radioactivity was determined by detergent lysis (1% Triton X-100) and specific lysis was calculated using the formula: percent specific lysis = (cpm released by CTL – cpm of spontaneous release)/(cpm released by detergent – cpm of spontaneous release). Spontaneous release was <20% of detergent lysis in all experiments.

Results

Surface Expression and Structural Integrity of $K^b_\alpha L^d_\beta$. The ability of the chimeric protein encoded by the $K^b_\alpha L^d_\beta$ construct to fold properly and be expressed on the cell surface in a functional form is critical for the analysis of its alloantigenic properties. The surface expression and structural integrity of $K^b_\alpha L^d_\beta$ was assessed using mAbs that recognize conformationally dependent epitopes in conjunction with a mAb that can provide conformationally independent measurements.

The class I vector used to express $K^b_\alpha L^d_\beta$ in L cells incorporates the α 3 domain of L^d , which determines the antibody epitope detected by mAb 28-14-8. The L^d α 3 domain is expressed on the cell surface in the absence of β 2-microglobulin or in the absence of functional α 1 and α 2 domains (16). Since the α 3 domain of L^d is expressed promiscuously on the cell surface, the 28-14-8 epitope can be used to monitor the level of protein expression by the $K^b_\alpha L^d_\beta$ construct regardless of any changes in folding patterns created by generating an α 1 and α 2 chimera.

The serologic epitope identified by mAb K10.56 is dependent on amino acids in both the α 1 and α 2 helices of K^b as well as by the amino acids on the β sheets forming the

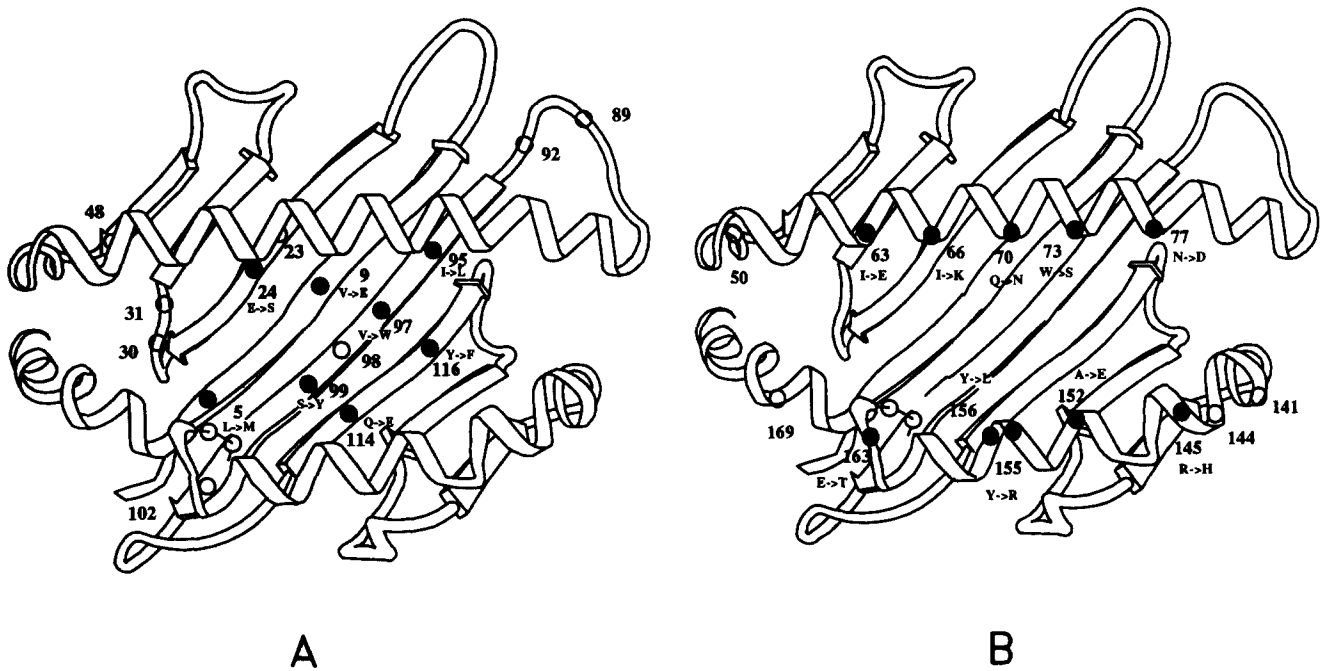


Figure 1. Structural differences between K^b and L^d . The position of the amino acid differences in the $\alpha 1$ and $\alpha 2$ domains between K^b and L^d are displayed by circles on the class I ribbon diagram. The solid circles indicate amino acid positions with the potential to influence ligand binding or TCR interactions with the α helices. The open circles indicate amino acid positions predicted to be silent with regard to ligand binding and TCR interaction. (A) Differences in the amino acids on the β sheets that form the floor of the ARS. The first single-letter code identifies the K^b amino acid followed by the single-letter code for the amino acid found in L^d . (B) Differences in the amino acids along the α helices. The first single-letter code identifies the L^d amino acid followed by the single-letter code for the amino acid found in K^b . In both panels, only the single-letter codes for amino acids predicted to alter ligand binding or TCR interaction are identified. Note that the second amino acid indicated in each case is present in the chimera.

floor of the antigen binding site (Pease et al., manuscript in preparation). Similarly, the epitope identified by mAb 28-13-3 is determined by amino acids in the $\alpha 2$ helix and by the β sheets of the ARS. Interestingly, the identified amino acids that are important in determining these epitopes contain side chains that are oriented into the antigen recognition site and, therefore, probably influence the structure of the surface of the molecules indirectly. Amino acid substitutions at these conformationally interactive sites can in some cases disrupt the conformation of glycoproteins even though

they represent normal structural polymorphisms of functional antigen-presenting molecules.

The structural integrity of the K^bL^d chimera was assessed by comparing the relative expression of the epitopes detected by mAbs K10.56 and 28-13-3 with the epitope encoded in the $\alpha 3$ domain with 28-14-8. The results of this analysis showed that these two conformational epitopes were expressed similarly by K^b and K^bL^d (Table 2). While K^bL^d bound the mAbs with conformationally sensitive epitopes, another K^b -specific mAb (B8-24-3), which recognizes an epitope deter-

Table 1. Oligonucleotides Used for Fusing K^bL^d

Fragment	5' Oligonucleotide primer	3' Oligonucleotide primer	Template	Amplified region
1	(A) 5'GCGTCCTCGGGTCAACCACCGGACCC	(B) 5'CGGCCTGCGGCTCATA	L^d	First intron to amino acid 50
2	(C) 5'TATGAGCCGCAGGCCG	(D) 5'GGTTGTAGTAGCCGAGCA	K^b	Amino acids 45-87
3	(E) 5'TGCTCGGCTACTACAACC	(F) 5'CTTCGTTTCAGGGCGATG	L^d	Amino acids 82-129
4	(G) 5'CATCGCCCTGAACGAAG	(H) 5'CCTGGGCACTCTCGAGGGAGAAGGCT	K^b	Amino acid 124 to third intron

Table 2. Serologic Profile of K^bL^d

Cell lines	Peak channel fluorescence					
	28-14-8	K10.56	28-13-3	B8-24-3	30-5-7	64-3-7
K^b	171	162	ND	175	42	45
L^d	144	44	ND	42	141	159
K^bL^d	168	168	ND	46	42	44
	28-14-8	K10.56	28-13-3	IgG2a	IgG2b	IgM
K^b	186	197	183	37	39	44
K^bL^d	177	185	165	38	51	47

mined in part by the loop connecting the α helix with the β -pleated sheets (8), did not bind the chimera. The mAb 30-5-7 that recognized an epitope determined by the displaced $L^d\alpha_2$ helix (our unpublished observation), and an epitope recognized by mAb 64-3-7, which has been reported to be associated with L^d molecules that contain empty ARS (17), also did not bind to the chimeric molecules. Thus, the serologic profile of the expressed molecules was consistent with the designed construct, and the conformation of the chimeric class I protein encoded by K^bL^d appeared to be intact.

Failure of K^b - or L^d -specific CTL to Recognize K^bL^d . CTL were raised against the K^b and L^d alloantigens to determine whether they could recognize epitopes formed primarily by the α helices or by the β sheets of the ARS (Fig. 2). B10.GD (K^d,D^b) anti-B6 (K^b,D^b) CTL lysed L cells expressing the K^b ARS efficiently, but did not lyse L cells expressing K^bL^d above background. Similarly, C3H(K^kD^k) anti-C3H.SW (K^bD^b) CTL lysed cells expressing K^b but not those expressing the chimera. B10.RQDB (K^q,D^d,D^b) anti-B10.AQR (K^q,D^d,L^d) CTL lysed L cells expressing L^d but also failed

to lyse the cells expressing K^bL^d . Together, these three CTL populations provided evidence that the alloantigenic structures recognized by T cells are not determined by either the α helices nor by the β sheets of K^bL^d acting alone.

In an attempt to focus CTL recognition on specific regions of the K^b molecule, a series of bm mutants (bm1, bm8, bm10, and bm11) was used to generate anti- K^b (B10) CTL. Three of the CTL lines used in this study were raised against epitopes determined by amino acid differences located directly on the K^b α helices (18) (K^{bm1} anti- K^b , K^{bm10} anti- K^b , and K^{bm11} anti- K^b). Since K^bL^d shares the helical structures with K^b , these CTL in particular might be expected to crossreact with the chimera, if the α helices themselves were capable of forming antigenic determinants recognized by T cells. The panel of mutant anti- K^b CTL lysed L cells that expressed K^b , but failed to lyse cells expressing K^bL^d (Fig. 3). This pattern of recognition was consistent with the pattern seen using fully allogeneic CTL and demonstrates directly that the alloantigenicity of the K^b molecule was not determined to any significant extent by the α helices alone.

Lysis of K^bL^d by B6 Anti-bm8 CTL. The mutation in

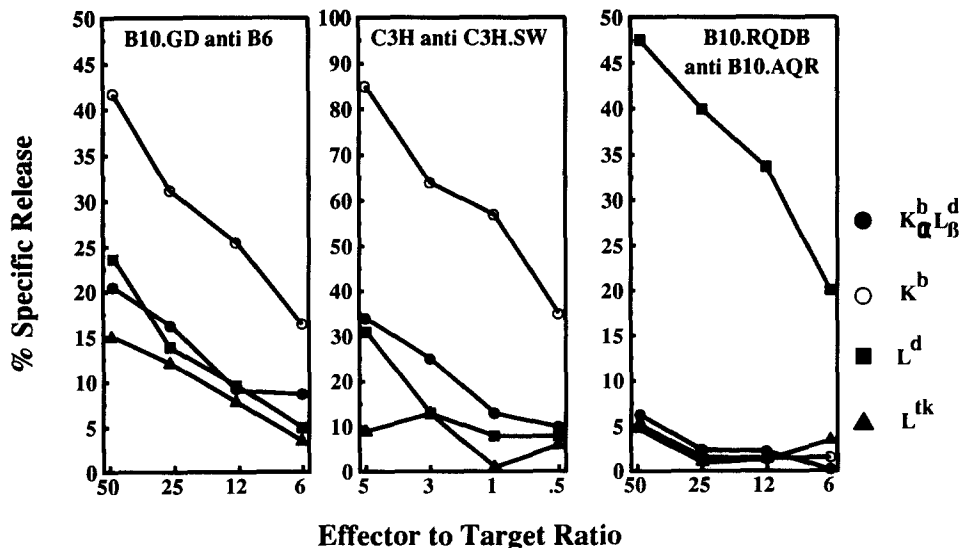


Figure 2. Anti- K^b or anti- L^d CTL lines fail to recognize the chimera. Primary CTL cultures generated in vitro against K^b (B10.GD anti-B6 and C3H anti-C3H.SW) and L^d (B10.RQDB anti-B10.AQR) were used to analyze L cells cotransfected with K^b , L^d , K^bL^d or herpes thymidine kinase gene alone (L^{tk}).

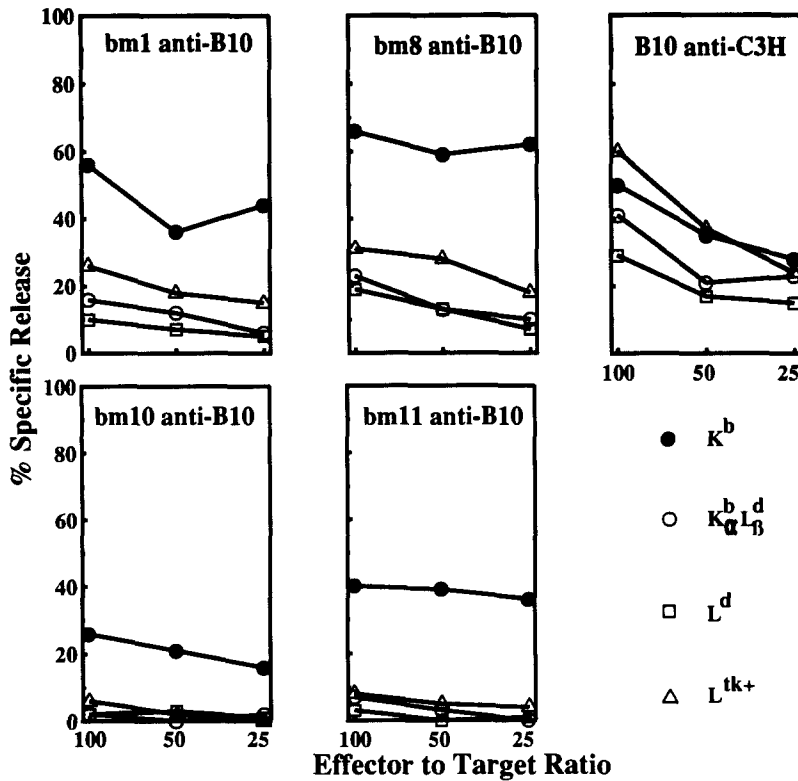


Figure 3. K^b mutant anti- K^b CTL lines fail to recognize the chimera $K_{\alpha}^b L_{\beta}^d$. The bm mutants (bm1, bm8, bm10, and bm11) were used to generate effector cells against defined regions of K^b (B10) heavy chain and used to analyze cotransfected L cells. The B10 anti-C3H (anti-H-2K) was used to determine lytic potential of the L cell (H-2K) target cells.

bm8 consists of four amino acid substitutions at residues 22, 23, 24, and 30. In a previous report, we identified the amino acid substitutions in residues 22 and 24 as the determinants of alloreactivity between K^b and K^{bm8} (9). The substitutions at 23 and 30 were silent. Of the two productive substitutions, the Glu (K^b) to Ser (K^{bm8}) change at 24 dominated the K^{bm8} phenotype, with the substitution at 22 (Try to Phe) having minor effects. Amino acids 22 and 24 lie adjacent to or inside (respectively), a subregion of the ARS known as the 45 pocket (19) or pocket B (20). The $K_{\alpha}^b L_{\beta}^d$ chimera and K^b share the same amino acid at position 22 but differ from each other at position 24. $K_{\alpha}^b L_{\beta}^d$ has the same amino acid (Ser) as K^{bm8} at residue 24. To determine whether structural similarities in pocket B along with sequence identity in the helices of the ARS might be sufficient to cause alloantigenic crossreactivity, B6 anti-bm8 CTL were tested for their ability to lyse $K_{\alpha}^b L_{\beta}^d$. As shown in Fig. 4, B6 anti-bm8 CTL lysed targets expressing K^{bm8} and $K_{\alpha}^b L_{\beta}^d$ efficiently, but failed to lyse cells expressing K^b and L^d . Therefore, structural similarity in pocket B along with the α helices was sufficient to form shared alloantigenic epitopes.

CTL sublines from a B6 anti-bm8 CTL culture were established by limiting dilution at five cells/well to determine the contribution of amino acids 22 and 24 in the generation of alloantigenic specificities shared by K^{bm8} and $K_{\alpha}^b L_{\beta}^d$. Four of six sublines that lysed K^{bm8} -expressing L cells specifically also lysed cells expressing $K_{\alpha}^b L_{\beta}^d$ (data not shown). However, the fine specificity of these clones was not uniform. Some sublines characterized by 2D1 lysed L cells expressing a variant of the K^b molecule containing a single bm8-like substitui-

tion at position 24, while others, as line 3F1, required bm8-like substitutions at both positions 22 and 24 (Fig. 5). Our interpretation of these observations is that structural similarities in pocket B are sufficient to permit a set of similar or identical peptides to bind both molecules generating cross-reactive alloantigenic specificities in the context of the K^b helices.

Discussion

A class I chimeric molecule was created to analyze the relative contributions of the α helices and β sheets of the antigen recognition site in determining the antigenic structures

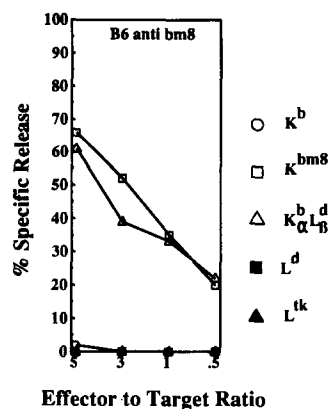


Figure 4. K^b anti- K^{bm8} CTL lyse L cells expressing $K_{\alpha}^b L_{\beta}^d$. B6 spleen cells were used to generate CTL specific for K^{bm8} in vitro. The cells were used to analyze a panel of transfected L cells.

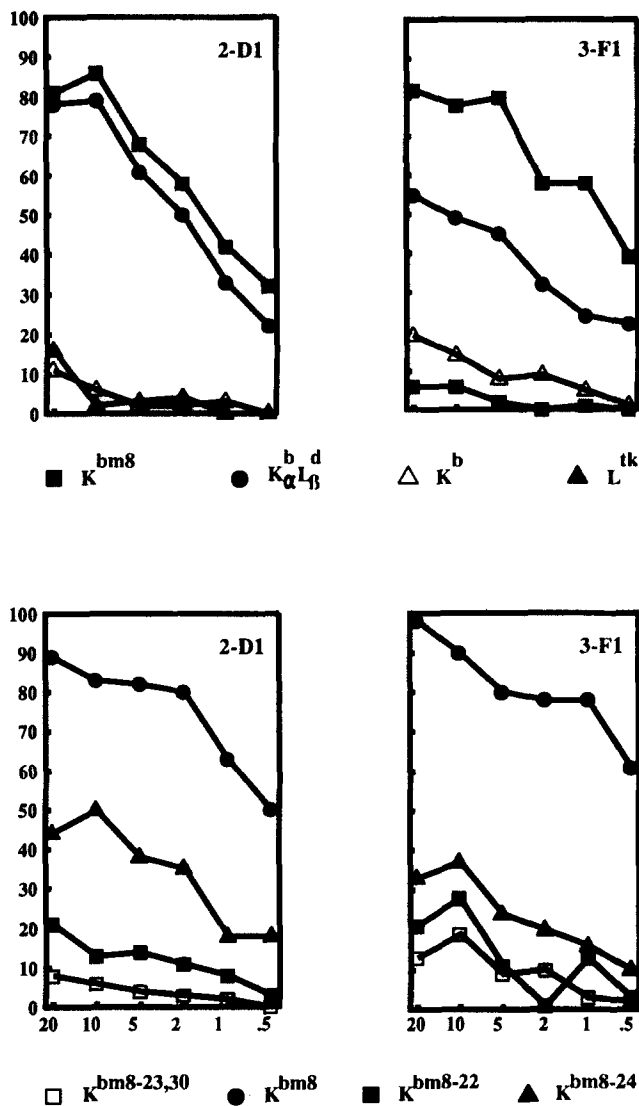


Figure 5. CTL lines that crossreact with K^{bm8} and K^bL^d have different fine specificities. CTL sublines were established by limiting dilution at five cells/well and tested against cotransfected L cell targets that included partial variants (site-directed mutants) of the bm8 mutant. Two representative K^b anti-bm8 CTL lines (2-D1 and 3F1) are shown. The target cells include partial variants of bm8 generated by site-directed mutagenesis. $K^{bm8-23,30}$ is K^b with bm8 amino acid changes at the silent positions 23 and 30. K^{bm8-22} is K^b with the bm8 amino acid change at position 22. This change produces a minor alloreactive determinant. K^{bm8-24} is K^b with the bm8 amino acid change at position 24. This change produces a major alloreactive determinant.

that interact with TCRs during allrecognition. Alloreactive CTL populations specific for molecules that shared sequences forming the α helices or the β sheets with the chimeric target were used to establish whether T cells recognize structures determined by the α helices alone, or whether the contact points between TCRs and class I molecules are influenced directly or indirectly by the amino acids on the floor of the antigen binding site. A variety of alloresponsive CTL raised in vitro against spleen cells bearing class I alloantigens that shared either the α helices (K^b) or the β sheet

structures (L^d), but not both with the chimeric class I molecule were not able to lyse targets expressing the chimeric glycoprotein. Our interpretation of these experiments is that the alloresponse is primarily directed against structures formed by interaction with both the β sheets and the α helices of the ARS. We take the fact that conformationally sensitive antibody epitopes associated with the K^b α helices are retained in the chimera to mean that the surface of the engineered molecule is not greatly perturbed by interactions of the α helices with the extensive structural differences located in the β sheets of the hybrid ARS. Therefore, we find the CTL data to support the view that the alloresponse is generated by T cells recognizing alloantigenic structures that are influenced by peptides bound to ARS and that epitopes primarily determined by the class I heavy chain play, at most, a minor role in alloantigenicity.

The array of peptides and the orientation of their side chains are determined by the polymorphic amino acids that line the floor (β sheets) and wall (α helices) of the ARS. By altering each of the polymorphic positions in the β sheets in the generation of the K^bL^d chimera, virtually all crossreactivity with CTL specific against the K^b molecule was abolished. The observation that CTL raised against the alloantigen K^{bm8} crossreacted strongly with cells expressing the chimeric alloantigen further supports this view. In addition to the K^b α helices, K^{bm8} shares a single polymorphic position on the floor of the ARS with K^bL^d . The high degree of crossreactivity between K^{bm8} and K^bL^d is remarkable in light of the remaining high degree of the structural dissimilarity along the β sheets of the two antigen-presenting molecules. It appears that shared structural properties around position 24 provide a dominant binding site that influences peptide selection and that the orientation of the peptides with respect to the TCR is determined in this case primarily by the α helices. This site may be pocket B since K^bL^d share identical residues lining the pocket. We would predict from these findings that there is extensive sharing of peptides between K^{bm8} and K^bL^d , a hypothesis that will be tested in future experiments.

Other studies have addressed whether alloreactive T cells are capable of recognizing MHC molecules directly or whether they are focused on the peptides oriented in the antigen recognition site of the molecules. Several of these have concluded that T cells can recognize diversity in the class I heavy chains directly. In one analysis, class I molecules were denatured and separated into their heavy chain and β_2 -microglobulin subunits (21). Upon renaturation of the heavy chain with excess β_2 -microglobulin, molecules were recovered that retained conformationally sensitive serologic epitopes and the ability to stimulate an allospecific CTL line. Another study addressed this issue by reducing the frequency of antigen-presenting molecules containing alloantigenic peptides on the cell surface and by assessing the impact of the changes on alloantigenicity (22). In still another report, the variant cell line RMA-S was analyzed for its antigen-presenting properties, and a distinction was observed in the ability of RMA-S cells to induce immunity and to serve as a target for CTL

specific for major and minor alloantigenic differences (23). These findings suggested that processing of minor class I-restricted antigens was an essential step, whereas, not all major alloantigenic epitopes resulted from processing of peptides. Each of these studies was interpreted in support of the hypothesis that allospecific CTL normally recognize structures determined directly by the polymorphic class I heavy chains.

In contrast, another set of studies argued strongly for a central role of cellular peptides in determining alloantigenic specificity (24). Human variant cell lines displaying deficiencies in class I antigen processing, similar to those seen in the RMA-S cells (25, 26), were used to evaluate the nature of structures recognized by alloresponsive CTL. The T2 hybrid cell and its transfected derivatives express class I antigen-presenting molecules on the cell surface (27). In contrast to the experimental system using RMA-S cells (23), alloreactive CTL generated in mixed lymphocyte cultures that were specific for the alloantigen K^b in the context of normal cells were not reactive with K^b -transfected targets (24). The inability of CTL to recognize the T2- K^b line could have been due to either destabilization of the antigen binding domain of K^b as a result of the absence of available peptides in the ARS and/or because peptides provide the alloantigenic specificity recognized by allospecific CTL. This unresponsiveness was reversed for a majority of the CTL lines by the addition of peptide preparations generated from cell lysates to the T2-derived target cells. Furthermore, the addition of selected peptides known to bind appropriately to the antigen-presenting molecules on the T2 cells failed to restore alloantigenicity. This latter finding was taken as evidence that the peptides were not solely providing stability to empty class I molecules, but were contributing specificity to the antigenic complex. The observation that a substantial portion of the alloresponse was specific for peptides in the context of non-self-class I is most consistent with the peptide model.

What is the basis for the disagreements emanating from these studies? One possibility is that the experimental manipulations varied in the degree they achieved the intended distinction between class I molecules containing a broad spectrum of peptides and those that contained either no peptide or defined peptides. While RMA-S cells and T2 cells appear to contain similar phenotypes (25, 26), it is not clear that their genetic lesions are identical, nor is it clear that the mouse and human antigen processing machinery is similarly sensitive to such mutations. Therefore, it is plausible that processing mutations may differentially influence the spectrum or quantity of peptides available to class I antigen-presenting molecules in the two systems. The study by Müllbaker et al. (22) used even more indirect approaches, attempting to displace peptides with high affinity peptide competitors or to disrupt antigen processing with viral infections. Although these techniques are designed to displace alloantigenic peptides, none of these approaches can be presumed 100%

efficient. We have attempted to circumvent these problems by introducing structural changes in the floor of the ARS of antigen-presenting molecules, an approach that assures that each expressed molecule is modified experimentally.

The same limitations probably do not apply to the study by Elliot and Eisen (21), although, as addressed in their study, the possible significance of residual peptide contamination in the system was difficult to assess. It would be interesting to see whether the renatured A2 molecules were capable of stimulating a wide variety of A2-specific CTL clones or whether the panning technique (28) used in the study skewed the A2-responsive T cell line, A2p.

Whatever the reasons behind these disagreements, the identification of CTL that crossreacts with structurally dissimilar alloantigens underscores the complexity of the factors determining T cell recognition of MHC class I molecules. A CTL clone has been isolated that crossreacts with two alloantigens, K^{bm3} and L^d (29). These glycoproteins differ from each other not only at each polymorphic position on the β sheets that form the floor of the ARS, but also at 10 polymorphic positions on the helices. Despite the fact that T cells of this nature exist, we have not found them to be a measurable fraction of T cells generated in alloresponses against either the K^{bm3} or L^d antigens (Z. Cai and L. R. Pease, unpublished observation); nor in our analysis of K^b alloantigenicity have we found significant numbers of CTL with receptors specific for structures determined by the α helices alone.

Our current view of the structural basis of the immune response against class I major transplantation antigens is that T cells primarily recognize determinants formed in association with peptides presented by non-self-antigen-presenting molecules. Structural diversity in the α helices and β sheets that form the antigen binding site influences the array of cellular peptides selected for presentation, and the profiles of the peptides that are exposed to the TCR. T cells probably also simultaneously recognize structures determined by the class I heavy chains (30). These associations have been demonstrated using peptides comprised of amino acid sequences in and around the ARS to inhibit CTL recognition of alloantigens (31–32). Some aspects of this direct recognition of the class I heavy chain by the TCR appears to be determined by conserved structures of the antigen-presenting molecules (31), while others involve positions of diversity in the heavy chain (32). In the event that these polymorphic structures permit strong interactions with elements of the T cell repertoire, allospecific CTL with little specificity for peptide-determined epitopes may be generated. While TCRs may exist that can recognize class I alloantigens in a manner independent of bound peptides, cells bearing these receptors do not appear to comprise a significant portion of alloresponses generated in mixed lymphocyte culture.

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