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Immunology

Direct demonstration of critical amino acid residues required for cytotoxic T-lymphocyte allorecognition of H-2 class I antigens

(major histocompatibility complex/site-directed mutagenesis/protein design)

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ABSTRACT **To identify critical amino acid residues recognized by alloreactive cytolytic T lymphocytes (CTL) generated between $H\text{-}2K^b$ and $H\text{-}2K^{bm1}$, we have derived a series of cloned L-cell lines expressing the following mutant $H\text{-}2K^b$ class I genes. Cell line L-$K^{bTyr\text{-}Tyr}$ expresses a mutant gene in which positions 155–156 of the K^b molecule have been changed from Arg-Leu to Tyr-Tyr, leaving position 152 unchanged. Cell line L-K^{bAla} expresses the reciprocal mutant gene that has position 152 of the K^b molecule mutated from glutamic acid to alanine, leaving positions 155–156 unchanged. Electrophoretic mobilities of the mutant K^b molecules reflect only those changes predicted by the mutations. Mutant-specific (anti-K^{bm1}) and native-specific (anti-K^b) CTL lyse L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla} target cells equally well. Unlabeled target inhibition of lysis revealed a pattern of recognition and inhibition that suggests that the amino acid differences between K^{bm1} and K^b create at least two discrete determinants that can be recognized by different populations of CTL. The results suggest that these determinants consist, at least in part, of a linear amino acid sequence from which critical amino acid residues can be identified.**

The class I genes of the murine major histocompatibility complex (*H-2*) encode the serologically defined transplantation antigens that serve as major recognition elements in cytolytic T-lymphocyte (CTL) responses to foreign antigens (1). Class I antigens are M_r 45,000 integral membrane glycoproteins that exhibit three extracellular domains, α_1, α_2, and α_3, a transmembrane region, and a short cytoplasmic tail. Alloreactive CTL recognize non-self class I antigens directly, whereas H-2-restricted CTL recognize self-class I structures in association with a nominal foreign antigen—e.g., viral, tumor, or chemical (2). Several approaches have been used to localize the precise regions and determinants on class I molecules involved in CTL recognition (3–15). These studies all clearly indicate that the extracellular α_1 and α_2 domains of class I molecules express the predominant determinants recognized by CTL, whereas the α_3, transmembrane, and cytoplasmic domains appear to be uninvolved. Sequence analysis has shown that as few as one or two amino acid substitutions in either of the α_1 or α_2 domains are sufficient to alter CTL recognition (6, 16). Although a pattern of crossreactive allorecognition can be determined for different class I mutants that correlates with common elements of primary amino acid sequence, the majority of CTL appear to recognize determinants that are dependent on domain interactions. Therefore, although the location of the determinants has been effectively "domain mapped," the relationship between linear sequence and determinant formation remains unknown.

Studies involving *in vitro*- and *in vivo*-derived mutations of class I genes provide model systems for establishing the minimal biochemical requirements necessary to effect CTL recognition. However, *in vivo*-derived spontaneous mutations are limited in number, and those that exhibit unique noncrossreactive determinants, such as the K^{bm1} mutation, express multiple amino acid substitutions that complicate critical residue identification. *In vitro*-derived mutations are equally limited in that they are generated randomly and are negatively selected on the basis of the lack of expression of either serological or CTL-specific epitopes. It is possible to avoid these limitations by using site-directed mutagenesis to alter the sequence of a well-characterized class I mutation that displays significant functional changes in recognition such that critical residues can be identified. This is the rationale and the approach that we have taken to study the K^{bm1} mutation. The K^{bm1} gene differs from the native K^b gene by a cluster of seven nucleotides that result in three amino acid substitutions (position 152, glutamic acid → alanine; positions 155–156, Arg-Leu → Tyr-Tyr) (17–20). These substitutions are sufficient to elicit bidirectional graft rejection *in vivo* and mixed lymphocyte responses *in vitro* between the parent C57BL/6 and mutant B6.C-H-2^{bm1} mouse strains (16, 21). CTL that specifically recognize K^b antigens do not recognize K^{bm1} antigens and vice versa. Similarly, CTL that are restricted to the K^b antigen and a nominal antigen (e.g., viral or chemical) do not recognize target structures comprising K^{bm1} and the same nominal antigen (22–25).

We have generated two mutant K^b genes by oligonucleotide-directed mutagenesis. When expressed in mouse L cells, these genes produce the following mutant K^b gene products. The $K^{bTyr\text{-}Tyr}$ molecule has positions 155–156 mutated from Arg-Leu to Tyr-Tyr, leaving position 152 unchanged from the native K^b sequence. The K^{bAla} molecule has position 152 mutated from glutamic acid to alanine, leaving positions 155–156 unchanged from the native K^b sequence. Each of these class I molecules represents a reciprocal mutation expressing elements of native K^b and mutant K^{bm1} sequences. The ability of either anti-K^b or K^{bm1}-specific CTL to recognize these molecules provides a unique opportunity to study the importance of primary amino acid sequence, particularly the role of individual residues, in CTL recognition. On the basis of the data presented here, we propose that the recognition of K^{bm1} allodeterminant(s) by CTL is dependent on the presence of critical amino acid residues located within the sequence that defines the K^{bm1} mutation. These residues comprise a complex determinant with at least two CTL specificities defined by different amino acid sequences.

MATERIALS AND METHODS

Mice. Some C57BL/6 ($H\text{-}2^b$) and B6.C-H-2^{bm1} ($H\text{-}2^{bm1}$) mice were a gift of Peter Wettstein (Wistar Institute, Phila-

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Abbreviations: CTL, cytotoxic T lymphocyte(s); 2D, two-dimensional.

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delphia); others were obtained from The Jackson Laboratories. C57BL/10 ($H\text{-}2^b$) and BIO.BR ($H\text{-}2^k$) mice were obtained from The Jackson Laboratories.

Oligonucleotide-Directed Mutagenesis. Oligonucleotides were synthesized on a Systec Microsyn 1450 automated DNA synthesizer and purified by HPLC according to published procedures (26). Oligonucleotide-directed mutagenesis was carried out as described (27, 28). The sequences of the various genes and their expressed products are shown in Table 1.

Generation and Identification of Transfected L-Cell Lines. Mouse L cells deficient in thymidine kinase were cotransformed with the ptK5 plasmid and a plasmid vector containing one of the four different class I genes by using a standard calcium phosphate precipitation method (29); this was followed by selection in hypoxanthine/aminopterin/thymidine medium. Surviving colonies were cloned and assayed for expression of $H\text{-}2K^b$-like class I products by radioimmunoassay (RIA) according to published procedures (5) with the following monoclonal antibodies: 20-8-4, $H\text{-}2^b$ (30) and $H\text{-}2K^{bm1}$ (M.M., unpublished observation); 16-1-2, $H\text{-}2^k$ (31); 7-16.4, $H\text{-}2K^p$ (32). Cell lines (Table 1) were selected from a number of positive clones on the basis of the binding of comparable levels of 20-8-4 ($H\text{-}2^b$). The levels of binding 16-1-2 ($H\text{-}2^k$) varied among the cells. None of the cell lines bound the control, 7-16.4 ($H\text{-}2K^p$).

Isolation of Class I Molecules. The procedures for radiolabeling, immunoprecipitation, isolation of proteins, and two-dimensional (2D) gels have been described (33, 34).

Generation and Analysis of CTL Specificities. Alloreactive CTL were generated *in vitro* according to published procedures (35). H-2K^{bm1}-specific CTL were generated by using C57BL/6 responder cells with B6.C-H-2^{bm1} stimulator cells. Likewise, H-2K^b-specific CTL were generated by using B6.C-H-2^{bm1} responder cells and C57BL/6 stimulator cells. Specific cell lysis was measured by a standard ^{51}Cr-release assay (35). Percent specific lysis was calculated as (cpm experimental − cpm spontaneous/cpm maximal − cpm spontaneous) × 100. Specific cell lysis of labeled targets in the presence of various unlabeled targets was carried out by the ^{51}Cr-release assay above. Percent inhibition was calculated as (1 − % specific lysis in the presence of unlabeled targets/% specific lysis in the absence of unlabeled target) × 100.

Table 1. DNA and amino acid sequences of class I genes

Gene	Position 152	Position 155–156	Plasmid*	Cell line†
	Glu	Arg–Leu		
$H\text{-}2K^b$	GAA	AGA CTC	pC15-1	L-K^b
	Ala	Arg–Leu		
$H\text{-}2K^{bAla}$	GCT	AGA CTC	p-2.19	L-K^{bAla}
	Glu	Tyr–Tyr		
$H\text{-}2K^{bTyr\text{-}Tyr}$	GAA	TAT TAC	pA43	L-K$^{bTyr\text{-}Tyr}$
	Ala	Tyr–Tyr		
$H\text{-}2K^{bm1}$	GCT	TAT TAC	R6E2	L-K^{bm1}

DNA and amino acid sequences of the $H\text{-}2K^b$ mutants and $H\text{-}2K^{bm1}$ are compared to $H\text{-}2K^b$ in the region encoding residues 152 and 155–156 of the protein product.

*The designations of the plasmid vectors containing the class I genes used for DNA-mediated gene transfer are given.

†The laboratory designations of these cell lines are as follows: L-K^b/L-C15-1, L-K^{bAla}/L-Ala21.17, L-K$^{bTyr\text{-}Tyr}$/L-A43.2, L-K^{bm1}/L-R6E2.3. Plasmids pC15-1, pA43, and R6E2 have been identified and characterized (28).

RESULTS

2D Gel Electrophoresis. The mutant molecules were isolated by immunoprecipitation of biosynthetically radiolabeled cell lysates and analyzed the resulting immune complexes by 2D gel electrophoresis.

Complete and partial fluorographs of the 2D gels of the transfectants are shown in Fig. 1. Fig. 1 *A* and *B* show that the K^b and K^{bm1} molecules, when expressed on L cells, give virtually identical patterns that are typical of class I molecules, having a two-chain structure, one of M_r 45,000 and one, β_2-microglobulin, having a M_r of 12,000. Pattern identity has also been observed when K^b and K^{bm1} on spleen cells are compared (M.M., unpublished data). The electrophoretic identity is in agreement with the fact that the net change in charge from K^b to K^{bm1} is zero since the glutamic acid at 152 neutralizes the arginine at 155.

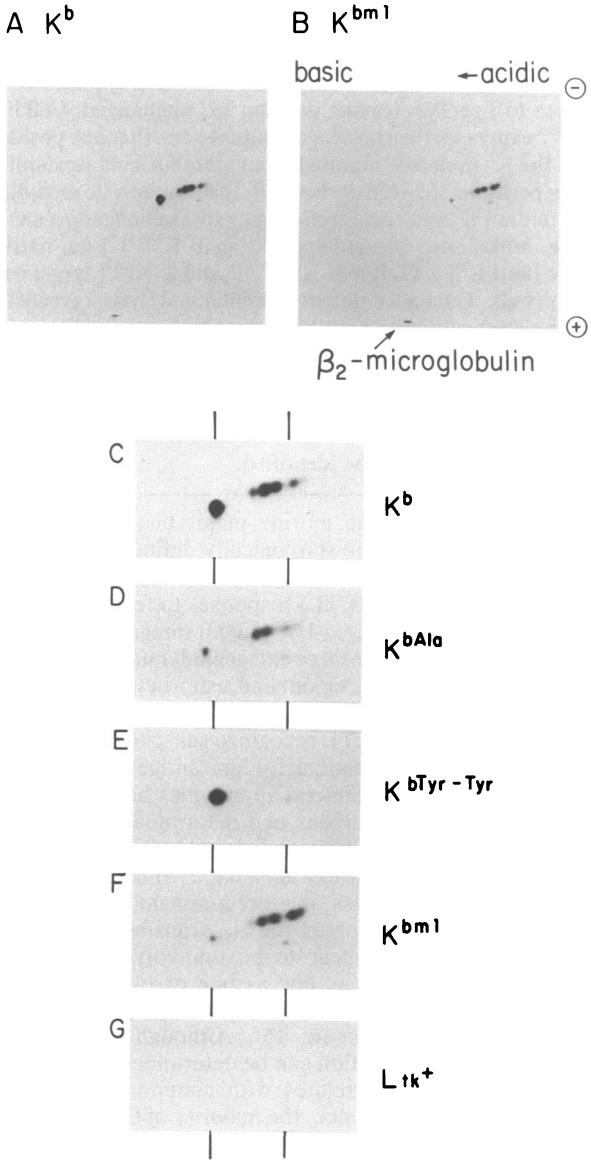

FIG. 1. Fluorographs of 2D gels of $H\text{-}2K^b$-mutated gene products immunoprecipitated from L-cell transfectants with monoclonal antibody 20-8-4. (*A* and *B*) Complete fluorographs. (*C–F*) Partial fluorographs, M_r 36,000–60,000 and pI 4.5–6.0. Actin and β_2-microglobulin were used as markers to align the prints before cutting. The most acidic spots are to the right. The vertical lines align the most basic spot of K^b and K^{bm1} (left) and actin (right). The specific target molecule is indicated beside each panel and *G* shows an analogous region for L-cells transfected with the thymidine kinase (*tk*) gene alone.

Fig. 1 *C–F* show partial fluorographs of the mutated molecules and compare the K^b (Fig. 1*C*) and the K^{bm1} (Fig. 1*F*) molecules to the other K^b mutants. The vertical line on the right aligns actin in all of the gels, whereas the line on the left aligns the most basic spot of K^b and K^{bm1}. Clearly, in Fig. 1*D*, the analogous spot falls to the left (more basic) of this line, whereas in Fig. 1*E* the reverse is true—the most basic spot is to the right (more acidic). This is to be expected since the K^{bAla} molecule (Fig. 1*D*) possesses an arginine instead of a tyrosine at position 155, whereas the $K^{bTyr\text{-}Tyr}$ molecule has a glutamic acid instead of alanine at position 152. We also confirmed these charge differences by coprecipitating the K^b and mutant molecules from radiolabeled mixtures of B6 spleen and L-cell transfectants. These data establish that our L-cell transfectants are expressing proteins that have electrophoretic mobilities in agreement with those predicted from the mutated gene sequences. These fluorographs also establish that the mutant class I molecules show no other gross changes introduced as a consequence of the mutagenesis procedure or their expression in the L-cell environment.

The relative intensities of the spots varied considerably, despite the fact that these molecules are exceedingly closely related and the level of binding of K^b antibody 20-8-4 in RIA is consistent for each of the cell lines. The faint acidic spots in the L-$K^{bTyr\text{-}Tyr}$ molecules suggest that the complexities of 2D gel patterns of class I molecules are exquisitely sensitive to as-yet unknown subtleties in amino acid structure.

K^b Mutants Are Recognized by Anti-K^b- and Anti-K^{bm1}-Specific CTL. To determine if the K^b mutant molecules could be recognized by alloreactive CTL, each of the cell lines L-K^b, L-$K^{bTyr\text{-}Tyr}$, L-K^{bAla}, and L-K^{bm1} was used as labeled target cells for bulk cultures of CTL specific for either H-2K^b or H-2K^{bm1} molecules. As shown in Fig. 2, H-2K^b-specific (Fig. 2*B*) and H-2K^{bm1}-specific (Fig. 2*A*) CTL are able to lyse each of the mutant targets L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla} equally well while maintaining exquisite specificity for the stimulating population (Fig. 2*A*, K^{bm1}; Fig. 2*B*, K^b). However, the level of killing on each mutant cell line was less than that observed for the cell lines expressing native K^b (L-K^b) or the whole mutant K^{bm1} (L-K^{bm1}) antigens. Since the RIA data indicate that similar amounts of surface protein are present on each of the cell lines, this suggests that the reduced level of killing is unlikely to be caused by differences in the number of surface molecules but rather reflects the ability of the CTL population to recognize the unique determinants presented by the K^b mutant molecules $K^{bTyr\text{-}Tyr}$ and K^{bAla}. Fig. 2*C* shows the ability of each of the cell lines to be lysed by H-2^k-specific CTL. The level of lysis on each cell line appears to correlate with the level of expression of H-2 antigens determined by RIA with antibody 16-1-2.

H-2K^b- and H-2K^{bm1}-Specific CTL Recognize Different Determinants on $K^{bTyr\text{-}Tyr}$ and K^{bAla} Antigens. Since each population of CTL recognizes both mutants equally well, unlabeled target inhibition of lysis was performed to determine whether common or independent determinants are being recognized on the $K^{bTyr\text{-}Tyr}$ and K^{bAla} mutant structures. Fig. 3 *Upper* and *Lower* clearly show that although L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla} are able to inhibit lysis of themselves, L-$K^{bTyr\text{-}Tyr}$ is not able to inhibit the lysis of L-K^{bAla} targets, or vice versa, regardless of the specificity of the CTL population. We therefore conclude that the determinants recognized by each population of CTL are discrete and independent.

Line L-$K^{bTyr\text{-}Tyr}$ is able to partially inhibit the lysis of L-K^{bm1} by anti-K^{bm1}-specific CTL as well as partially inhibit the killing of L-K^b targets by anti-K^b-specific CTL. Likewise, L-K^{bAla} cells are able to partially inhibit the killing of L-K^{bm1} targets by anti-K^{bm1}-specific CTL as well as partially inhibit the lysis of L-K^b targets by anti-K^b-specific CTL. L-K^b and L-K^{bm1} targets also show partial inhibition on L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla} when the CTL population has specificity for the inhibitor. These data suggest that the determinant(s) recognized on the mutant structures $K^{bTyr\text{-}Tyr}$ and K^{bAla} is also present on the native K^b and K^{bm1} structures. These observations are consistent with the idea that the amino acid differences between K^b and K^{bm1} create multiple determinants that can be recognized by different subpopulations of CTL with different specificities.

DISCUSSION

In direct lysis experiments B6 anti-bm1 CTL (anti-K^{bm1}), which are generated specifically as a result of the three amino acid substitutions (position 152, glutamic acid → alanine; positions 155–156, Arg-Leu → Tyr-Tyr), are able to recognize each of the partial mutations $K^{bTyr\text{-}Tyr}$ (glutamic acid, Tyr-Tyr) and K^{bAla} (alanine, Arg-Leu) equally well while maintaining specificity for the stimulator population, K^{bm1}.

If anti-K^{bm1}-specific CTL recognize conformational determinants revealed as a consequence of the three amino acid substitutions in K^{bm1}, then it could be argued that the double Tyr-Tyr mutation, $K^{bTyr\text{-}Tyr}$, is sufficient to induce similar steric effects on the α_2 structure, resulting in the recognition of L-$K^{bTyr\text{-}Tyr}$ targets by K^{bm1}-specific CTL. It has been noted that the presence of a similar primary amino acid sequence among different K^b mutants correlates with their ability to be recognized by alloimmune CTL (6). A similar, though less convincing, argument based on the presence of the single alanine substitution could be proposed for the recognition of L-K^{bAla} targets by K^{bm1}-specific CTL. However, the possibility that conformational constraints create the K^{bm1}-specific allodeterminant(s) becomes less likely in light of the observation that bm1 anti-B6 (anti-K^b specific)

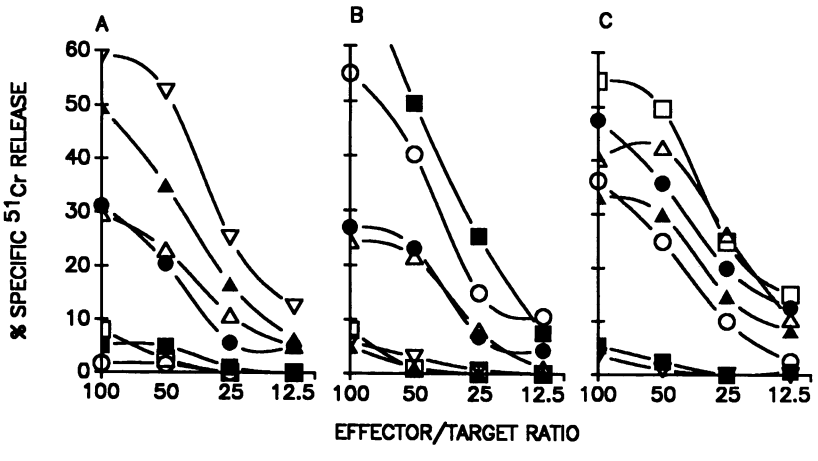

FIG. 2. Cell-mediated lympholysis of transfected cell lines expressing mutant K^b antigens. Specific cell lysis was measured in a 4-hr ^{51}Cr-release assay. All values are triplicate means with SEM ≤10%. (*A*) Effector combination C57BL/6 anti-B6.C-H-2^{bm1} (anti-K^{bm1} specific). (*B*) B6.C-H-2^{bm1} anti-C57BL/6 (anti-K^b specific), (*C*) C57BL/10 anti-B10.K (anti-H-2^k specific). Target cell lines are as follows: ▽, B6.C-H-2^{bm1} spleen; ▲, L-K^{bm1}; ●, L-$K^{bTyr\text{-}Tyr}$; △, L-K^{bAla}; □, LTK; ■, C57BL/6 spleen; ○, L-K^b.

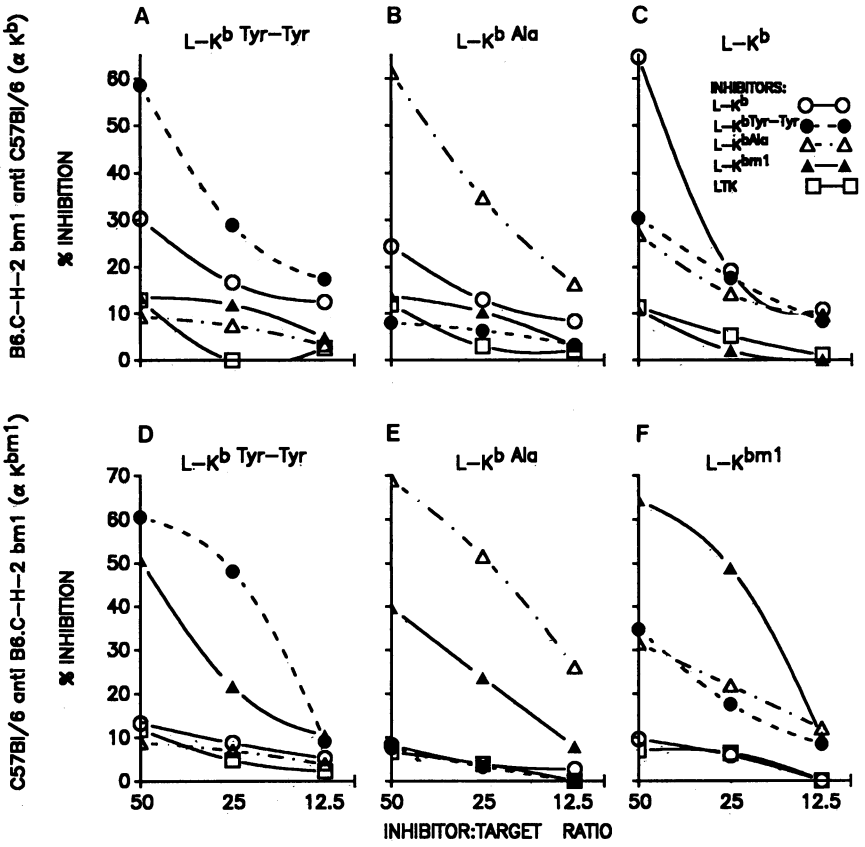

FIG. 3. Unlabeled target inhibition of lysis was assayed by using two effector populations, anti-K^b (*Upper*) and anti-K^{bm1} (*Lower*). (*A–F*) Level of inhibition of lysis on a labeled target cell in the presence of various concentrations of unlabeled cells. The labeled target cell types are L-$K^{bTyr\text{-}Tyr}$ (*A* and *D*), L-K^{bAla} (*B* and *E*), L-K^b (*C*), and L-K^{bm1} (*F*). Inhibition was assayed at an effector-to-labeled-target ratio of 50:1 in the presence of various numbers of unlabeled inhibitor cells. The % lysis in the absence of inhibitor cells was as follows: L-$K^{bTyr\text{-}Tyr}$, 31% and 28% for anti-K^b and anti-K^{bm1} effectors, respectively; L-K^{bAla}, 30% and 33% with anti-K^b and anti-K^{bm1} effector cells, respectively; L-K^b, 58% with anti-K^b effectors; and L-K^{bm1}, 56% with anti-K^{bm1} effectors, All values are triplicate means with SEM ≤ 10%.

CTL also recognize L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla} targets equally well. This implies that conformational changes induced by the double Tyr-Tyr mutation (or the single alanine substitution) are sufficient to permit recognition by anti-K^{bm1}-specific CTL but not sufficient to destroy the recognition by anti-K^b-specific CTL that does occur when the triple mutation (alanine, Tyr-Tyr) is present. In other words, conformational determinants that are gained as a result of the triple mutation (K^{bm1} specific) and those that are lost as a result of the same mutation (K^b specific) are simultaneously maintained and generated when either a single alanine substitution or a double Tyr-Tyr substitution is made. There are no indications of any significant structural changes induced in the K^b mutant molecules as assessed by their ability to bind the K^b/K^{bm1} antibody 20-8-4 (RIA and immunoprecipitation). Likewise, changes in local and extended helical structure and hydrophilicity based on Robson (36) and Hopp–Woods (37) predictions, respectively, are minimal (data not shown).

We propose that anti-K^b- and anti-K^{bm1}-specific CTL recognize $K^{bTyr\text{-}Tyr}$ and K^{bAla} structures because each mutant shares discrete determinants with the native molecules K^b and K^{bm1} within the critical region 152–156. This interpretation is supported by unlabeled target inhibition of lysis, which clearly shows that neither L-$K^{bTyr\text{-}Tyr}$ nor L-K^{bAla} targets are able to cross-compete for the same T-cell receptor with either K^b- or K^{bm1}-specific CTL. This indicates that the determinants recognized on L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla} are in fact discrete and independent. Partial inhibition of lysis on whole K^b or K^{bm1} targets is also observed with unlabeled L-$K^{bTyr\text{-}Tyr}$ or L-K^{bAla} targets and vice versa. This confirms that the determinants recognized on the native K^b or mutant K^{bm1} structures are also expressed by the partial mutants L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla}. The level of lysis on each of the mutant cell lines, L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla}, is always less than that observed on L-K^b targets by K^b-specific CTL or on L-K^{bm1} targets by K^{bm1}-specific CTL. In keeping with the above argument, one might assume that the frequency of CTL able to recognize each of the mutant structures independently is lower than the frequency of CTL able to recognize native structures. Since the frequency of CTL able to recognize native structures is expected to include the CTL that recognize each of the independent determinants represented by L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla}, as well as those additional determinants that we are unable to distinguish (e.g., CTL that recognize the whole mutation), this would result in a reduced level of lysis on targets expressing either but not both of the mutant structures. A more precise analysis of the number of additional determinants will require the recognition of these K^b mutations by cloned populations of CTL in addition to the bulk cultured CTL described here. However, it appears that the determinants defined by the K^b mutations represent a significant portion of those presented by the native K^b and whole mutant K^{bm1} molecules and may, in fact, be immunodominant since the level of lysis on each of the cell lines L-$K^{bTyr\text{-}Tyr}$ and L-K^{bAla} is almost additive regardless of the specificity of the CTL population.

The striking parallel pattern of recognition and unlabeled target inhibition of lysis with the mutant K^b molecules is consistent with the idea that in the K^b and K^{bm1} molecules the region comprising residues 152–156 is critical in the formation of a multiple determinant that exhibits at least two discrete allorecognition sites, each of which is dependent on the presence of a different amino acid sequence. It is also evident that this sequence is involved in the generation of different populations of CTL specific for each of the different recognition sites as there are no other known genetic differences between C57BL/6 and B6.C-H-2^{bm1} (38).

The recent description by Bjorkman *et al.* (39, 40) of the x-ray crystallographic structure of a human class I antigen has provided the basis for a general model of class I structure. It appears that residues at positions 152, 155, and 156 point directly into the grove forming the putative binding site for processed foreign antigens and CTL receptor. They have

thus been identified as critical for either antigen or T-cell receptor binding.

Evidence is accumulating that suggests that class I antigens function in a similar manner to class II molecules (Ia antigens) in that they are capable of binding peptide fragments that are recognized by specific CTL (41, 42). Maryanski *et al.* (41) have shown that a syngeneic HLA class I peptide is recognized in association with the H-2K^d class I antigen and suggest that, like class II-restricted T cells, class I-restricted CTL are able to recognize peptide fragments rather than intact antigen. Since there are increasing examples of individual cloned T cells that exhibit alloreactivity and H-2-restricted recognition of peptide fragments and there are no observed sequence differences in the T-cell receptor genes between CTL and T-helper cells (43, 44), it has been suggested that the phenomenon of class I alloreactivity is also mediated by CTL recognition of peptide fragments (45, 46). The data presented here are not inconsistent with this hypothesis. It is conceivable that the region 152–156 expresses critical residues required for the binding of peptide fragments recognized by either anti-K^b or anti-K^{bm1} allospecific CTL. Further, experiments are necessary to determine whether synthetic peptides derived from the target sequence 152–156 are able to influence the CTL allorecognition described here.

Our data are in apparent conflict with the work of de Waal *et al.* (47). These authors have shown that anti-K^{bm1}-specific CTL do not appear to recognize the L^d class I antigen that shares significant homology with the α_2 domain of the K^{bm1} molecule including the region 152–156. It is possible that specific domain interactions occur that influence recognition of the allodeterminants for which region 152–156 is critical. A more detailed knowledge of the interactions between the juxtaposed α-helices that form the binding site will be necessary to interpret the influence of one domain over the other in terms of CTL allorecognition.

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1. Klein, J. (1979) *Science* **203,** 516–521.
2. Nabholz, M. & McDonald, H. R. (1983) *Annu. Rev. Immunol.* **1,** 273–306.
3. Epstein, S. L., Ozato, K. & Sachs, D. H. (1982) *J. Immunol.* **125,** 129–135.
4. Weyand, C., Hammerling, G. J. & Goronzy, J. (1981) *Nature (London)* **292,** 627–629.
5. McLaughlin-Taylor, E., Woodward, G., McMillan, M. & Frelinger, J. A. (1984) *Eur. J. Immunol.* **14,** 969–974.
6. Nathenson, S. G., Geliebter, J., Pfaffenbach, G. M. & Zeff, R. A. (1986) *Annu. Rev. Immunol.* **4,** 471–502.
7. Bluestone, J. A., Palman, C., Foo, M., Geier, S. S. & Nathenson, S. G. (1984) *UCLA Symp. Mol. Cell. Biol.* **18,** 89–97.
8. Sheil, J. M., Bevan, M. J. & Sherman, L. A. (1986) *Immunogenetics* **23,** 52–59.
9. Ozato, K., Evans, G. A., Shykind, B., Margulies, D. H. & Seidman, J. G. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2040–2043.
10. Allen, H., Wraith, D., Pala, P., Askonas, B. & Flavell, R. A. (1984) *Nature (London)* **309,** 279–281.
11. Stroynowski, I., Clark, S., Henderson, L., Hood, L., McMillan, M. & Forman, J. (1985) *J. Immunol.* **135,** 2160–2166.
12. Arnold, B., Horstman, U., Kuon, W., Burgert, H. G., Hammerling, G. & Kvist, S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7030–7034.
13. Bluestone, J. A., Foo, M., Allen, H., Segal, D. & Flavell, R. A. (1985) *J. Exp. Med.* **162,** 268–281.
14. Levy, R., Ozato, K., Richardson, J. C. & Bluestone, J. A. (1985) *J. Immunol.* **134,** 677–683.
15. Ozato, K., Takahashi, H., Appella, E., Sears, D. W., Murre, C., Seidman, J. G., Kimura, S. & Tada, N. (1985) *J. Immunol.* **134,** 1749–1758.
16. Melief, C. J. M., deWaal, L. P., van der Meulen, M. Y., Melvold, R. W. & Kohn, H. I. (1980) *J. Exp. Med.* **151,** 993–1013.
17. Schulze, D. H., Pease, L. R., Geier, S. S., Reyes, A. A., Sarmiento, L. A., Wallace, R. B. & Nathenson, S. G. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2007–2011.
18. Reyes, A. A., Schold, M., Itakura, K. & Wallace, R. B. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3270–3274.
19. Weiss, E., Golden, L., Zakut, R., Mellor, A., Fahrner, K., Kvist, S. & Flavell, R. A. (1983) *EMBO J.* **2,** 453–462.
20. Coligan, J. E., Kindt, T. J., Uehara, H., Martimko, J. & Nathenson, S. G. (1981) *Nature (London)* **291,** 35–39.
21. Bailey, D. W. & Kohn, H. E. (1965) *Genet. Res.* **6,** 330–340.
22. Blanden, R. V., Dunlop, M. B. C., Doherty, P. C., Kohn, H. E. & Mckenzie, I. F. C. (1976) *Immunogenetics* **9,** 541–548.
23. Doherty, P. C., Bennick, J. R. & Wettstein, P. J. (1981) *J. Immunol.* **126,** 131–133.
24. Levy, R. B. & Shearer, G. M. (1982) *J. Immunol.* **129,** 1525–1529.
25. Wettstein, P. J. (1982) *J. Immunol.* **128,** 2629–2633.
26. Tan, Z.-K., Ikuta, S., Huang, T., Dugaiczk, A. & Itakura, K. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **47,** 383–391.
27. Zoller, M. J. & Smith, M. (1983) *Methods Enzymol.* **100,** 468–500.
28. Schold, M., Colombero, A., Reyes, A. A. & Wallace, R. B. (1984) *DNA* **3,** 469–477.
29. Wigler, M., Pellicer, S., Silverstein, R., Axel, G., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1373–1376.
30. Ozato, K. & Sachs, D. H. (1981) *J. Immunol.* **126,** 317–321.
31. Ozato, K., Mayer, N. & Sachs, D. H. (1980) *J. Immunol.* **124,** 533–540.
32. Harmon, R. C., Stein, N. & Frelinger, J. A. (1983) *Immunogenetics* **18,** 541–545.
33. McMillan, M., Frelinger, J. A., Jones, P. P., Murphy, D. B., McDevitt, H. O. & Hood, L. (1981) *J. Exp. Med.* **153,** 936–950.
34. Cullen, S. E. & Schwartz, B. D. (1976) *J. Immunol.* **117,** 136–142.
35. Wettstein, P. J. & Frelinger, J. A. (1980) *Immunogenetics* **10,** 211–225.
36. Robson, B. (1974) *Biochem. J.* **141,** 853–867.
37. Hopp, T. P. & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3824–3828.
38. Altman, P. L. & Katz, D. D., eds. (1979) *Inbred and Genetically Defined Strains of Laboratory Animals Part I: Mouse* (Fed. Am. Soc. Exp. Biol., Bethesda, MD).
39. Bjorkman, P. J., Saper, M. A., Samraovi, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329,** 506–512.
40. Bjorkman, P. J., Saper, M. A., Samraovi, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329,** 512–518.
41. Maryanski, J. L., Pala, P., Corradin, G., Jordan, B. R. & Cerrottini, J.-C. (1986) *Nature (London)* **324,** 578–579.
42. Townsend, A. R. M., Rothbard, J., Gotch, F. M., Bahacdur, G., Wraith, D. & McMichael, A. J. (1986) *Cell* **44,** 959–968.
43. Barth, R., Kim, B., Lan, N., Hunkapiller, T., Sobieck, N., Winto, A., Gershenfeld, H., Okado, C., Hansberg, D., Weissman, I. L. & Hood, L. (1985) *Nature (London)* **316,** 517–523.
44. Acuto, O., Campen, T. J., Royer, H. D., Husey, R. E., Poole, C. D. & Reinherz, E. L. (1985) *J. Exp. Med.* **161,** 1326–1343.
45. Kourilsky, P., Chaouat, G., Rabourdin-Combe, C. & Claverie, J.-M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3400–3404.
46. Owen, M. J. & Crumpton, M. J. (1987) *Br. Med. Bull.* **43,** 228–240.
47. de Waal, L. P., Nathenson, S. G. & Melief, C. J. (1983) *J. Exp. Med.* **158,** 1720–1726.