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Immunology

Identification of functional regions on the I-A^b molecule by site-directed mutagenesis

(class II major histocompatibility complex molecules/antigen presentation/T-cell activation)

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ABSTRACT **Functional analysis of mutant class II major histocompatibility complex molecules has begun to identify regions important for antibody binding and for T-cell activation. By using *in vitro* mutagenesis directed at the β_1 domain of the A^b_β gene we have constructed three structurally distinct mutant A^b_β genes. Each of these genes, as well as the wild-type A^b_β gene, was cotransfected together with the wild-type A^b_α gene into the Ia-negative B-lymphoma cell line M12.C3. Transfection resulted in the successful synthesis and cell surface expression of three mutant class II antigens that showed serological and functional alterations as compared to the I-A^b antigens from the M12.C3 cell transfected with the wild-type gene. The variable patterns of both I-A^b-specific monoclonal antibody binding and activation of I-A^b-specific T-cell hybridomas show that the mutations result in the loss of structural epitopes required for both monoclonal antibody binding and for T-cell recognition. The data suggest that there are multiple sites on a single Ia molecule that are recognized by T helper cells and also that the tertiary conformation of the Ia molecule can be critical in the formation of such sites.**

The induction of an immune response requires that the helper T cell recognize an antigen in the context of a class II Ia molecule on the surface of an antigen-presenting cell (APC) (1). The location and number of sites on the Ia molecule that interact with T-cell receptor proteins and with foreign antigen, however, remain unknown. One approach to identifying such sites has been to compare the amino acid sequences as predicted from the cloned genes of the four class II molecules, A_α, A_β, E_α, and E_β, from different haplotypes. Thus it has been found that the amino acid sequence differences between different alleles of α and β chains are focused predominantly in the N-terminal α_1 and β_1 domains (2, 3). Exon-shuffling experiments that exchange β_1 domains of one haplotype for another have shown that monoclonal antibody (mAb) binding sites and T-cell recognition sites are localized to this first external domain (4, 5). Hypervariable clusters within this variable region are marked by substitutions of amino acids with different charges (3). These amino acid alterations are likely to cause significant structural differences between the allelic Ia molecules. Though useful in predicting important structural regions on the Ia molecule, allelic comparisons become too complex when attempting to identify the relationship between Ia structure and function.

Another approach to this issue has been to examine the effect of structural alterations of the Ia molecule on T-cell activation. Studies of cloned populations of APCs that bear mutated I-A^k molecules (6, 7) together with observations obtained from the one existing spontaneous *I*-region mutant mouse strain, B6.C-H-2^{bm12} (bm12) (8) suggested the existence of several determinants on a single class II molecule. Two of the A^k_β mutants described have single amino acid changes in the β_1 domain of A^k_β (22), and the three amino acid differences between bm12 and B6 are also located in the β_1 domain of the A^b_β gene (9). These mutant systems attempt to identify important structural regions on the Ia molecule by correlating amino acid changes with changes in T-cell activation.

In an attempt to further identify functionally important sites on the Ia molecule, we have constructed altered A^b_β genes by site-directed mutagenesis in the β_1 domain. These genes were introduced by transfection into an antigen-presenting Ia-negative B-cell lymphoma that now expresses the mutant Ia molecules on the cell surface. We present here the functional and serological characteristics of these cloned I-A^b mutant APCs.

MATERIALS AND METHODS

Synthetic Oligonucleotides. The three mutagenic deoxyribonucleotides, 5′ TTCGTGCACCAGTTCATG 3′ (18.1), 5′ TCATGCCCGAGTGCTACT 3′ (18.3), and 5′ GAACAGCCAGTACCTGGAGCGAA 3′ (23.1) were synthesized with an Applied Biosystems (Foster City, CA) 380A DNA synthesizer, using the silica-based solid-phase method (10) and the proton-activated nucleoside phosphoramidite method (11). The purified oligodeoxynucleotides were isolated from the crude synthetic mixtures by polyacrylamide gel electrophoresis.

Mutagenesis of the A_β Gene. A 0.6-kilobase *Nae* I/*Nar* I restriction fragment containing the β_1 exon of the A^b_β gene was inserted into the *Sma* I/*Acc* I sites of the single-stranded DNA vector M13mp9. This subclone was used as a template for the oligonucleotide-primed mutagenesis according to described conditions (12). The DNAs from the oligonucleotide-primed mutagenesis reactions were transformed into competent JM103 cells and screened by plaque hybridization with the mutagenic oligonucleotides (12). The positive M13 mutant clones were sequenced using the dideoxy chain-termination method to confirm the mutations (13, 14). A 352-base-pair *Sst* II restriction fragment, which encodes the entire β_1 exon, was isolated from the replicative form DNA of each M13 subclone containing mutant β_1 exons. This fragment was religated into a *Sst* II site on an A_β gene construct (15) from which the analogous *Sst* II fragment containing the wild-type β_1 exon had been deleted. The orientation of the *Sst* II fragment in the A_β gene was determined by restriction mapping. Hybridization with the

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Abbreviations: mAb, monoclonal antibody; APC, antigen-presenting cell; IL-2, interleukin 2; bm12, B6.C-H-2^{bm12}.

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Table 1. DNA sequences of synthetic oligonucleotides showing altered nucleotide positions and resulting amino acid mutations in the β_1 domain of the A^b_β gene.

Oligonucleotide	Amino acid		
	Position	Domain	Change
5′ TTCGTG*CAC*CAGTTCATG 3′ (T)	9	β_1	Tyr → His
5′ TCATG*CCC*GAGTGCTACT 3′ (GG)	13	β_1	Gly → Pro
5′ GAACAGCCAG——*TAC*——CTGGAGCGAA 3′ (CCGGAGATC)	65, 66, 67	β_1	Pro, Glu, Ile → Tyr

mutant oligonucleotides confirms the presence of the mutations on the expected restriction fragment (data not shown).

Cell Lines. M12.C3 (C3) is an adherent Ia-negative variant of the homozygous, antigen-presenting Ia^d-positive BALB/c lymphoma, M12.4.1 (23). The lack of surface *I-A*^{d} expression by M12.C3 is secondary to an absence of RNA specific for the A^d_β gene: the A^d_α gene is transcribed and translated and can form a functional I-A molecule when provided with a normal A_β gene (see below). A20.A^{bm12} (*bm12*) was produced by transfection of the A20.2J Ia^d-bearing lymphoma with A^b_α and A^{bm12}_β genes (15).

DNA Transfection. The Ia-negative B-cell lymphoma line M12.C3 (2–3 × 10^6 cells) was cotransfected by the calcium phosphate technique (15) with 10 μg of mutant A^b_β (described above) [10 μg of A^b_α—that is, 6 μg of A^b_α plus 4 μg of A^b_α containing a metallothionine promoter in an effort to enhance transcription (E.C., unpublished work)] and 2–2.5 μg of pMSV-neo containing the neomycin resistance gene as a selectable marker (16). Prior to transfection the plasmids were linearized at the unique *Sal* I site in the vector pBR327. Twenty-four to 48 hr after transfection, a selection medium containing the antibiotic G418 at 125 μg/ml (GIBCO) was added to cultures. Two to 3 wks later, neomycin-resistant colonies were expanded for analysis. Positive colonies were cloned and then subjected to electronic cell sorting to obtain cells with a high density of I-A^b molecules.

Fluorescence-Activated Cell Sorter Analysis and Electronic Cell Sorting. FACS analysis using a Becton-Dickinson Analyzer was performed on 1 × 10^6 cells incubated with 20 μl of a previously titrated quantity of the mAb indicated. After mAb binding, cells were incubated with a fluorescein-conjugated Fab fragment of goat anti-mouse heavy and light chain immunoglobulin (GαMIg) (Cappel Laboratories, Cochranville, PA). The specificity control for staining by anti-Ia mAb followed by fluoresceinated GαMIg was the fluorescence histogram obtained with the latter reagent alone. Electronic cell sorting was performed using an Ortho Diagnostics fluorograph 50-H system. Approximately 3 × 10^6 cells were stained by treatment with 100 μl of mAb followed by the fluorescein-conjugated GαMIg reagent, and the 2% brightest staining cells were selected by sorting.

Antigens and mAbs. The random polymer poly(Glu^{60}Ala^{30}-Tyr^{10}) (all L) (Vega Biochemical, Tucson, AZ) was used at 200 μg/ml in culture. Ovalbumin (Sigma) was used at a final concentration of 1 mg/ml in culture.

The following I-A^b-specific mAbs were used: Y-3P and Y-8P (17); 34-5-3, 25-9-17, and 28-16-8 (18); M5/114 (19); and AF6120, 2A2-A11, 3F12-35, IE9-4, 4D59, and 3B95 (B. Beck, J. M. Buerstedde, and D. McKean, personal communication).

T-Cell Hybridomas and Assay of T-Cell Hybridomas for Activity. T-cell hybridomas used have been designated by antigen specificity and Ia restriction element and were produced as previously described (20). Several of the hybridomas were the gifts of P. Marrack (National Jewish Hospital, Denver, CO), J. Bluestone (National Cancer Institute, Bethesda, MD), and K. Rock (Harvard Medical School, Boston, MA).

T-hybridoma cells (1 × 10^5) were cultured with various numbers of APC cells in the presence of the relevant antigen in 0.2 ml of RPMI 1640 medium containing 8% fetal calf serum, penicillin, 2-mercaptoethanol, and L-glutamine. After 24 hr in culture, supernatant was collected and assayed for interleukin 2 (IL-2) content in a secondary culture using HT-2 cells, an IL-2-dependent T-cell line. HT-2 cells were cultured for 24 hr in the presence of serial dilutions of primary culture supernatant and amounts (units) of IL-2 per ml of supernatant were calculated according to Kappler *et al.* (21).

RESULTS

Construction of A^b_β Mutant Genes. Three mutant A^b_β genes have been constructed using oligonucleotide site-directed mutagenesis. The choice of the mutation sites was based on the location of allelic polymorphisms in the *b* and *k* haplotypes. The three mutagenic oligonucleotides and the resulting amino acid substitutions are shown in Table 1 and Fig. 1. Two of these mutants, 18.1 and 18.3, convert single amino acid residues at positions 9 and 13 to those in the *k* haplotype. A third mutant, 23.1, involves the replacement of three residues at positions 65, 66, and 67 (Pro-Glu-Ile) with a single tyrosine residue. This change from the *b* to the *k*

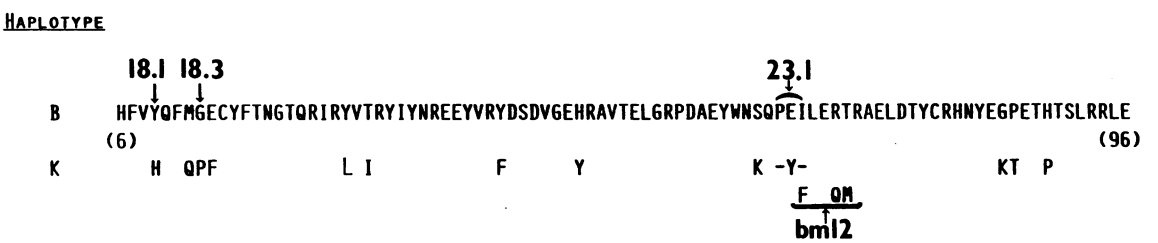

FIG. 1. Mutational sites in the β_1 domain of the I-A^b_β protein chain corresponding to the sequences of the oligonucleotides described in Table 1. The amino acid sequence of the β_1 domain (amino acids 6–96) of the A^b_β chain (2) and the amino acid residues of the *k* haplotype that differ from the *b* haplotype are shown. ↓, Site-directed mutations that are changes from the *b* to the *k* haplotype; ↑, the bm12 mutation that has three amino acid changes from the *b* haplotype. Dashes represent deletions. (A, alanine; R, arginine; N, asparagine; D, aspartic acid; c, cysteine; Q, glutamine; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine.)

Table 2. Reactivity pattern of A^b_β mutants with a panel of I-A^b mAbs

Mutant cell line	Reactivity with mAb											
	34-5-3	25-9-17	28-16-8	Y-3P	Y-8P	AF6-120	IE9	3F12-35	2A2-A11	3B9-5	4D59	M5/114
C3.A^b.I (wild type)	+	+	+	+	+	+	+	+	+	+	+	+
M12.C3 (parent cell)	−	−	−	−	−	−	−	−	−	−	−	−
18.1	+	+	+	+	+	+	+	+	+	+	+	+
18.3	+	+	+	+	+	+	+	+	+	+	+	+
23.1	−	−	−	+	+	+	+	+	+	+	+	+
C3.β^b	+	+	+	−	−	−	−	−	−	−	−	−

FACS analysis of the designated APCS with a series of αI-A^b mAbs. +, Staining approximately equivalent to that of the wild-type C3.A^b.1 cell; −, no staining above background.

haplotype results in the net deletion of two amino acids and a substitution for the third amino acid. The mutagenic oligonucleotides that were hybridized to a restriction fragment containing the wild-type β_1 domain inserted into M13mp9 served as a primer for the *in vitro* mutagenesis. The wild-type β_1 domain in an A_β gene construct was replaced by the mutant β_1 domain to make a mutant A_β gene. To ensure that inadvertent mutations were not created elsewhere in the A_β gene we used an M13 subclone that contains only the β_1 exon during the *in vitro* mutagenesis reactions. The mutations were confirmed by sequencing the entire β_1 exon in the M13 subclone.

Only Some of the Mutant Cell Lines Have Altered Reactivity with a Panel of αI-A^b mAbs. The three mutant cell lines, 18.1, 18.3, and 23.1, the wild-type transfectant C3.A^b.1, the Ia-negative parent line C3, and the C3 cell transfected with the A^b_β gene only, C3.β^b, were analyzed by flow microfluorometry using a panel of 12 anti-I-A^b mAbs. The results are shown in Table 2. The mean fluorescence of the mutant cell lines on several occasions was within a 1- to 2-fold range of that of the wild-type C3.A^b.1 cell. The pattern of mAb binding by two of the mutant cell lines, 18.1 and 18.3, was indistinguishable from that of the wild-type C3.A^b.1 cell line. This is interesting in view of the clear-cut functional changes discussed below. The 23.1 mutant cell line, however, failed to bind three of the mAbs, 34-5-3, 25-9-17, and 28-16-8, although it reacted with the remaining 9 mAbs. mAbs 34-5-3, 25-9-17, and 28-16-8 do bind to the $A^d_\beta A^d_\alpha$ molecule; however, the C3 cell line cannot form such a molecule since it makes no A^d_β protein as discussed above. These first 3 mAbs appear to be specific for sites in the A^b_β molecule as shown by the binding pattern of C3.β^b, the C3 cell line transfected with the A^b_β gene alone. This cell, which has on its surface only the hybrid molecule $A^b_\beta A^d_\alpha$, binds mAbs 34-5-3, 25-9-17, and 28-16-8 but not the 9 other mAbs. Therefore these latter mAbs most likely recognize sites on the A^b_α molecule or epitopes formed by the association of $A^b_\beta A^{b,d}_\alpha$. Thus it appears that the region of A^b_β DNA that is recognized by these 3 mAbs has been altered by the changes introduced at amino acids 65, 66, and 67 in the 23.1 mutant cell line while the amino acid changes at residues 9 and 13 do not appear to alter the binding sites of the mAbs tested. These results suggest that those three mAbs recognize the same epitope; however, to our knowledge, this has not been confirmed by competitive inhibition assays.

The Ability of the Mutant Cells to Activate a Panel of I-A^b-Specific T Cells Suggests Multiple Restriction Sites on the I-A^b Molecule. We next examined whether the mutations had resulted in the loss of sites on the I-A^b molecule important for T-cell recognition. The results of a representative experiment testing the ability of the A^b_β mutant cell lines to activate a panel of antigen-specific or alloreactive cloned T hybridomas are shown in Table 3. The wild-type C3.A^b.1 cells were able to activate all, and the parental Ia-negative C3 cells; none of the hybridoma tested. The variable ability of both the alloreactive and the foreign antigen [ovalbumin, poly(Glu^{60}-$Ala^{30}Tyr^{10}$)]-specific T hybridomas to be activated by the three site-directed mutant cell lines and the bm12 mutant cell subdivided these T cells into six groups.

Thus the group I hybridomas that contain two alloreactive and one poly($Glu^{60}Ala^{30}Tyr^{10}$)-specific T cell are activated by all three site-directed mutants but not by the bm12 mutant cell line, whereas the three alloreactive T cells in group II are activated by the 18.1, 18.3, and bm12 mutants but not by the 23.1 mutant cell line. The group III ovalbumin-specific T cell fails to be activated by the 18.3 and bm12 mutant lines while the group IV ovalbumin-specific T cell cannot be stimulated by either the 23.1 or the bm12 cell lines. The group V

Table 3. Antigen presentation by mutant cell lines to a panel of I-A^b-restricted T cells

Group	T hybridoma	Specificity	Activation, units of IL-2/ml						
			C3.A^b.1*	C3	18.1	18.3	23.1	bm12	C3β^b
I	4E3	Allo: I-A^b	**640**	<20	**640**	**640**	**640**	<20	<20
	RF13.35	GAT: I-A^b	**320**	<20	**320**	**320**	**160**	†	<20
	3B8	Allo: I-A^b	**320**	<20	**320**	**160**	**160**	<20	<20
II	4A4	Allo: I-A^b	**640**	<20	**320**	**640**	<20	**640**	**160**
	AODH3.4	Allo: I-A^b	**320**	<20	**320**	**320**	<20	**160**	**160**
	FS9.6.3	Allo: $A^b_\beta A^d_\alpha$	**320**	<20	**320**	**320**	<20	**160**	**160**
III	1C4.B2	OVA: I-A^b	**160**	<20	**160**	<20	**320**	<20	<20
IV	1C4.B6	OVA: I-A^b	**640**	<20	**160**	**320**	<20	<20	<20
V	3G3	Allo: I-A^b	**320**	<20	<20	**320**	<20	<20	<20
VI	JB1C4	Allo: I-A^b	**320**	<20	40	<20	<20	<20	<20

T-hybridoma cells (10^5) were co-cultured with various numbers of wild-type and mutant stimulator cells in the presence of antigen when appropriate [poly($Glu^{60}Ala^{30}Tyr^{10}$) (GAT), 100 μg/ml; ovalbumin, (OVA), 1 mg/ml]. Twenty-four hours later, serially diluted supernatants were tested for IL-2 activity on an IL-2-dependent T-cell line, HT-2. Positive responses are set in boldface type. Allo, alloreactive.

*Wild type.

†The RF13.35 hybridoma has an independent allorecognition of I-A^d. Since the *bm12* genes have been transfected into the I-A^d-positive lymphoma, A20, we cannot evaluate the ability of the bm12 transfectant to activate the I-A^b/GAT specificity of this hybridoma.

alloreactive hybrid can be activated only by the 18.3 cell line while the group VI hybrid is not activated by any of the four mutant cells. An I-A^{bm12}-specific T-cell hybridoma is activated by the bm12 mutant cell line yet is not activated by the wild-type C3.A^b.1 cell line or by any of the site-directed mutants (data not shown). These mutant lines therefore appear to distinguish multiple sites on the I-A^b molecule used for T-cell recognition. There appears to be some correlation between the extent of the nucleotide substitution, loss of mAb binding and T-cell activation since the more extensively altered 23.1 and bm12 mutants fail to stimulate 6 of 10 and 6 of 9 hybridomas, respectively, while both the 18.1 and the 18.3 mutant lines fail to activate only 2 of 10 of the T hybridomas. Furthermore, most of these restriction sites appear to depend on the homozygous pairing of the A^b_β and A^b_α molecules. C3β^b, which expresses a hybrid $A^b_\beta A^d_\alpha$ molecule, can activate the group II hybrids. The FS9.6.3 hybridoma is known to be specific for the hybrid $A^b_\beta A^d_\alpha$ molecule (P. Marrack, personal communication); presumably, the recognition site of the other two T cells in this group can accommodate both the homozygous $A^b_\beta A^b_\alpha$ and heterozygous $A^b_\beta A^d_\alpha$ combinations.

DISCUSSION

The experiments described in this report indicate that there are multiple sites on a single Ia molecule that are recognized by T helper cells. By *in vitro* mutagenesis using synthetic oligonucleotides directed at the β_1 domain of the A^b_β gene, three structurally distinct mutant A^b_β genes have been produced. The mutation sites were chosen at points where amino acid sequences vary between the *b* and *k* haplotypes. Thus at each mutation site the wild-type A^b_β nucleotides were replaced by base pairs corresponding to the *k* haplotype. The 18.1 and 18.3 mutations in the 5′ end of the β_1 domain each contain one amino acid substitution while the 23.1 mutation, with two deletions and a substitution, is located further downstream. The spontaneous *I*-region mutant, bm12, which has nucleotide substitutions that overlap with the 23.1 mutation sites was also used in our analysis.

Serological analysis of the 23.1 mutant indicates the loss of an important antibody-binding region on the A^b_β molecule as defined by the loss of binding by three A^b_β-specific mAbs. Whether failure to bind these mAbs results directly from the alteration of primary sequence at the mutation site or whether the 23.1 mutation produces changes elsewhere in the molecule that disrupt binding cannot be determined. The 18.1 and 18.3 mutant cell lines have a mAb-binding pattern identical to the wild-type pattern despite the loss of T-cell recognition sites. Thus it appears that the sites on the Ia molecule recognized by T cells are different from those recognized by antibodies. It is certainly possible, however, that a larger panel of A^b_β-specific mAbs might detect determinants altered in these two cell lines since all three mAbs tested appear to recognize the same or a similar determinant.

Although the 23.1 mutant defined a single antibody-binding determinant on the A^b_β molecule, six patterns of T-cell activation establish the existence of multiple sites on Ia with which the T-cell receptor interacts. A schematic model that emphasizes the complex picture that emerges from these data is shown in Fig. 2. Three general statements can be made. First, there are multiple determinants on the I-A^b molecule that can interact with T-cell receptor proteins and/or foreign antigen. Second, in agreement with previous studies (7, 8), most of these determinants depend on the homozygous pairing of the A^b_β and A^b_α molecules: only 3 of the 10 hybridomas can recognize an Ia determinant formed by A^b_β paired with either A^b_α or A^d_α (group II). Third, the data suggest that the tertiary conformation of the I-A molecule is important in the formation of at least some of these sites. Thus the group III ovalbumin-specific hybridoma 1C4.B2 is not activated by mutants 18.3 or bm12, which lie 54 amino acids apart, but it is activated by the 18.1 and 23.1 mutant cell lines, which have alterations adjacent to the mutations in the 18.3 and bm12 cells, respectively. Failure of T-cell activation in this case could reflect primary protein sequence alterations if (*i*) the 18.3 and bm12 regions are opposed in the tertiary structure of Ia^b in the process of T-cell recognition or (*ii*) the altered site, in one case, disrupts interaction with foreign antigen (ovalbumin) and, in the second instance, alters the

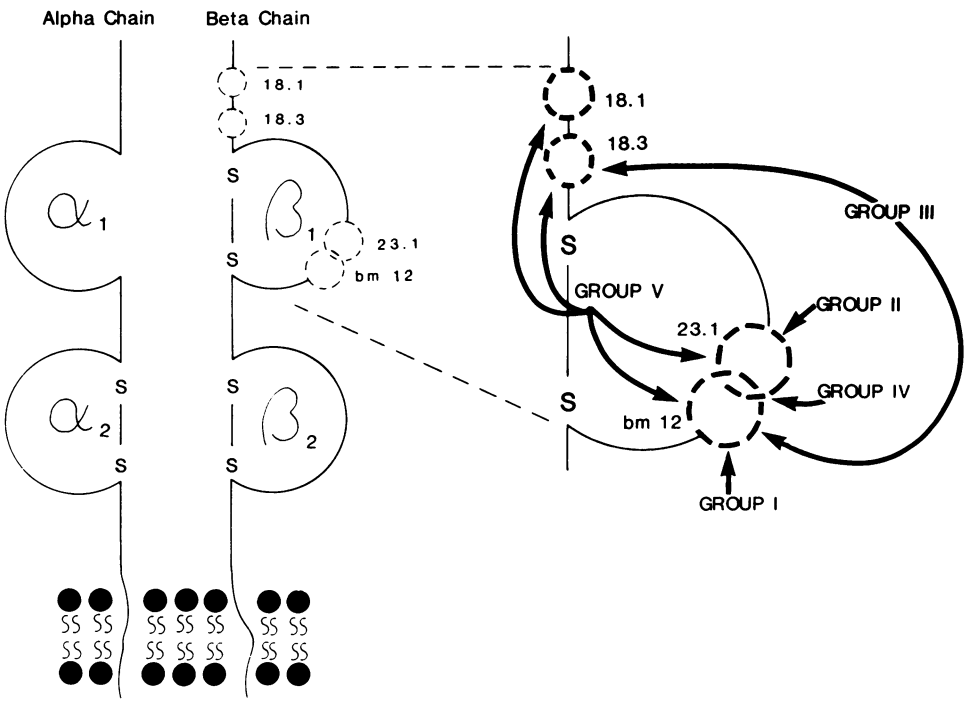

FIG. 2. Schematic representation of sites on the I-A^b molecule recognized by the T cells described in Table 3.

interaction with the T-cell receptor. It is equally likely, however, that each of these mutations independently alters the T-cell recognition site by an effect on tertiary conformation. The critical role of tertiary conformation of the Ia molecule in T-cell activation is also demonstrated by the pairs of mutants that have adjacent amino acid changes. The 23.1 and bm12 mutation sites overlap at amino acid 67, yet the close proximity of these mutations in the primary protein sequence fails to predict how these mutant cells will function in T-cell activation. Three of six groups (I, II, and III) show that these two mutant molecules have very different functional effects. The 18.1 and 18.3 mutation sites are separated by three amino acids yet the 18.1 mutant cell fails to activate the group V hybridoma, which is, however, stimulated by the 18.3 mutant cell line. The distinct functional effects of these two mutant cells is also evident in the activation pattern of the group IV T cell. The group VI alloreactive hybridoma may be another demonstration of the role of tertiary conformation of the I-A^b molecule in the formation of restriction sites since it cannot be activated by any of the mutant cell lines.

These results demonstrate the selective loss of ability of these mutant cell lines to activate certain I-A^b-specific T cells. It would be interesting to identify a gain of *I-*A^k-specific restriction elements as well as a loss of *I-*A^b-specific sites in such mutant genes. Although cotransfection of these A^b_β mutant genes with A^k_α genes has not to date resulted in the serologic or functional acquisition of I-A^k antigens (unpublished results), larger *b*-to-*k* substitutions, as well as intra-β_1 exon shuffling experiments between the *b* and *k* haplotypes, should help to define the distinct functional sites for these alleles.

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1. Heber-Katz, E., Hansburg, D. & Schwartz, R. H. (1983) *J. Mol. Cell. Immunol.* **1**, 3–14.
2. Choi, E., McIntyre, K., Germain, R. N. & Seidman, J. G. (1983) *Science* **221**, 283–286.
3. Benoist, C., Mathis, D., Kanter, M., Williams, V., II, & McDevitt, H. O. (1983) *Cell* **34**, 169–177.
4. Folsom, V., Gay, D. & Tonegawa, S. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1678–1682.
5. Germain, R. N., Ashwell, J. D., Lechler, R. I., Margulies, D. H., Nickersen, K. M., Suzuki, G. & Tou, J. Y. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2940–2944.
6. Glimcher, L. H., Sharrow, S. O., Sachs, D. H. & Paul, W. E. (1983) *J. Exp. Med.* **158**, 1573–1588.
7. Glimcher, L. H., Serog, B., McKean, D. & Beck, B. (1985) *J. Immunol.* **134**, 1780–1786.
8. Beck, B. N., Nelson, P. A. & Fathman, C. G. (1983) *J. Exp. Med.* **157**, 1396–1404.
9. McIntyre, K. & Seidman, J. G. (1984) *Nature (London)* **308**, 551–553.
10. Matteucci, M. D. & Carruthers, M. H. (1981) *J. Am. Chem. Soc.* **103**, 3185–3191.
11. Beaucage, S. L. & Carruthers, M. H. (1982) *Tetrahedron Lett.* **22**, 1859–1863.
12. Zoller, M. J. & Smith, M. (1984) *DNA* **3**, 479–488.
13. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. (1980) *J. Mol. Biol.* **143**, 161–178.
14. Messing, J. (1983) *Methods Enzymol.* **101**, 20–78.
15. Ben-Nun, J., Choi, E., Leeman, S., Seidman, J. G. & Glimcher, L. H. (1985) *J. Immunol.* **135**, 1456–1464.
16. Mulligan, R. C. (1983) in *Experimental Manipulation of Gene Expression*, ed. Inouye, M. (Academic, New York), pp. 155–173.
17. Janeway, C. A., Jr., Conrad, P. J., Lerner, E. A., Babich, J., Wettstein, P. & Murphy, D. B. (1984) *J. Immunol.* **132**, 662–667.
18. Ozato, K., Mayer, N. & Sachs, D. H. (1980) *J. Immunol.* **124**, 533–540.
19. Bhattacharya, A., Dorf, M. E. & Springer, T. A. (1981) *J. Immunol.* **127**, 2488–2494.
20. Glimcher, L. H. & Shevach, E. M. (1982) *J. Exp. Med.* **156**, 640–645.
21. Kappler, J., White, J., Wegmann, D., Mustain, E. & Marrack, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3604–3607.
22. Brown, M., Glimcher, L. H., Nielsen, E., Paul, W. E. & Germain, R. (1986) *Science*, in press.
23. Glimcher, L. H., McKean, D. J., Choi, E. & Seidman, J. G. (1985) *J. Immunol.* **135**, 1342–1350.