I-Aα POLYMORPHIC RESIDUES THAT DETERMINE ALLOREACTIVE T CELL RECOGNITION

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Alloreactivity is one of the most puzzling phenomena in immunology. Quite some time ago it was discovered that an individual's T lymphocytes are highly reactive to cells from another individual when they express different (allelic, allogeneic) MHC molecules (1, 2). The reactive T lymphocytes must occur at high frequency, as 1-4% of virgin T cells are capable of responding across any given MHC disparity. These T cells do not constitute a distinct subpopulation since they have repeatedly proven identical to foreign antigen-specific T cells, even using the same TCRs (3-9, and references therein). Allogeneic MHC class I molecules primarily activate $CD8^+$ CTL; in contrast, allogenic class II molecules mainly trigger $CD4^+$ T cells of "helper" or "inflammatory" phenotype (for reviews and references, see reference 10). Obviously, alloreactivity is of little relevance in a normal immune defense, but this phenomenon is of enormous clinical importance because it leads to graft rejection or to graft-versus-host disease in incompatible tissue transplants. Furthermore, alloreactivity probably reflects the shaping of the T cell repertoire through MHC restriction and self-tolerance (for review see reference 11), and thus might be expected to furnish clues to the mechanisms involved in repertoire selection.

T cell alloreactivity focusses on MHC class I and II molecules, heterodimeric glycoproteins displayed at the cell surface and known to play a directorial role in the presentation of antigen to T cells (for reviews see references 9, 12, 13). Sequence analysis has established that an MHC complex has four extracellular domains: two are membrane-proximal and are essentially invariant; the other two are membrane-distal and show allelic variation (for sequence compilations and references see references 14, 15). For the class II complexes, it is the NH_2-terminal domains of the paired α and β chains that are allelically divergent. These are referred to as $\alpha1$ and

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β1, and within each one, polymorphism is even further clustered into what we have termed "allelically hypervariable regions" (16, 17). For the murine A_α chain, the focus of this study, there are three such regions: α-ahvI, α-ahvII and α-ahvIII (16). A hypothetical tertiary structure for class II molecules has recently been proposed (18) on the basis of the crystallographic structure of an MHC class I molecule (19). In both the class I and class II structures, the membrane-distal domains are intertwined to form a single entity: a platform of β-pleated sheets supporting two parallel α-helices. The antigens presented by the MHC molecules appear to fit into the groove between the two helices, above the β sheets (18, 20). According to the hypothetical class II structure, the polymorphic amino acids of α-ahvI would be located in one of the β-strands on the floor of the groove, while α-ahvII and α-ahvIII seem to map to one of the α-helices.

An important and unresolved question is: What structure does an alloreactive T cell actually recognize? Speculation over the last 15 years has prompted several models of allorecognition. Before discussing them, we wish to emphasize an important distinction: we refer to MHC *contact* residues as those amino acids that interact directly with amino acids of the TCR to stabilize the TCR/MHC complex, but define MHC *determining* residues as polymorphic amino acids that are responsible for the specificity of TCR/MHC interactions. Thus, determining residues are those positions that dictate that an A^k but not an A^b molecule is recognized by a given alloreactive T cell clone. Determining residues may, but need not necessarily be, contact residues and vice-versa.

For clarity of discussion, we have sketched in Fig. 1 the most representative models addressing the mechanism of alloreactivity; presented are a set of cartoons showing cross-sections of TCR/MHC complexes engaged in allorecognition. The snail-like elements represent the two α-helices of the MHC molecule, delimiting the antigen-binding groove, supported underneath by the β-sheets. The proposed determining regions on the allogeneic MHC molecule are crosshatched. In model I, perhaps the most traditional view of alloreactivity, an intact allogeneic MHC molecule is recog-

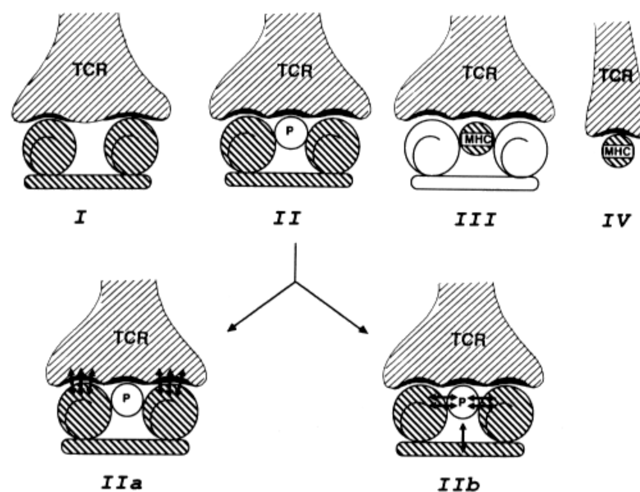


FIGURE 1. Models of allorecognition. The cartoons depict various models of the recognition of an allogeneic MHC molecule by T cells. The snail-like structures represent the two large α-helices of the MHC molecule, supported by a platform of β-sheets (18–20). Polymorphic regions of the MHC molecule that determine recognition by alloreactive T cells are coarsely hatched. The TCR, placed above the MHC molecules, is finely hatched. Thick black lines denote regions of the TCR that are actual contact areas. The small open circle labeled P signifies a non-MHC-encoded peptide. The coarsely hatched circle represents a peptide derived from the allogeneic-MHC molecule. The double-headed arrows indicate MHC/ TCR or MHC/peptide interactions. For an explanation of the various models, see the Introduction.

nized independently of bound peptide; a peptide may actually be present but has no relevance for the specificity of recognition. The determining residues would, then, reside on the intact MHC molecule and serve as (or indirectly influence) MHC/TCR contact residues. Model II also emphasizes the intact allogeneic MHC molecule, but differs from the first model by invoking an obligatory contribution to the specificity of recognition from a bound peptide (P). The peptide would originate from some cellular, non-MHC-encoded protein. Determining residues on the MHC molecules could dictate specificity either by participating in MHC/TCR contacts (IIa) or by controlling peptide binding (IIb). Models III and IV are quite different, in that they propose the recognition of a processed form of the allogeneic MHC molecule. In model III, presentation of the MHC peptide occurs in the context of an intact MHC molecule. There are multiple contacts within the allo-MHC peptide/MHC/TCR trimolecular complex; complex formation is determined, however, by allelic variation in the MHC peptide. An extreme variant of this model would be model IV, which proposes that the processed allogeneic MHC molecule is recognized independently of the responder's MHC molecule (which may actually be involved in presentation, but is not relevant to specificity and does not contact the TCR).

Which of these models, if any, actually explain allorecognition remains largely unknown. In the case of MHC class I molecules, recognition of an allogeneic MHC peptide by CTL (models III or IV), first suggested by the experiments of Maryanski et al. (21) and Parham et al. (22), has been directly demonstrated by Song and coworkers (23). On the other hand, evidence has been presented that spatially distinct regions of a class I molecule specify allorecognition (24-26), and it has been shown that soluble class I molecules do not induce tolerance in transgenic mice (27). A recent study on chemically induced cell mutants provided evidence for multiple contact sites between the TCR and an allogeneic class I molecule, in effect eliminating model IV as an explanation for allorecognition by CTL (28).

There is even less known about the mechanics of alloreactivity to MHC class II molecules. To address this problem, we have attempted to define what structural features dictate that an A^k but not an A^b molecule is attractive to an A^k-reactive T cell. We first evaluate the contribution of the individual $A\alpha$ and $A\beta$ chains and then delineate, through site-directed mutagenesis, determining residues on the $A\alpha$ chain. The results prompt us to favor model IIb as an explanation for alloreactivity.

Materials and Methods

Mice. The origin of the inbred strains used in this study was detailed previously (29).

Expressible cDNAs and L Cell Transfectants. A_α and A_β cDNAs of the k or b haplotype were expressed in L cells in pKCR-based vectors, as described previously (30). The transfectants displaying chimeric A_α chains have also been reported (31). To introduce the various single-site mutations [K53(G) to K76(V) in Fig. 2], we used oligonucleotide-directed mutagenesis on single-stranded templates (31) or double-stranded cassette mutagenesis between Nru I and Bgl II sites artificially introduced into the A^k_α cDNA (32).

Transfected L cells were selected by cytofluorimetric sorting, after staining with the appropriate mAb, usually one of the anti-A^k_β reagents 10-2-16, 39E, or 40B (30). The resulting lines showed surface A_α:A_β levels that vary by a factor of 5 or less; no correlation was noted between the density of A_α:A_β on a given line and the pattern of T hybridoma cell (THC)[1] response.

[1] *Abbreviations used in this paper:* THC, T hybridoma cell; tk, thymidine kinase.

Allosensitization and the Derivation of T Cell Hybridomas. Mouse splenocytes were stimulated in conventional MLRs as follows (33): 25 × 10^6 responder cells were cultured with the same number of X-irradiated (2,000 rad) stimulator cells in DME supplemented with 10% heat-inactivated FCS, 10 mM sodium Pyruvate, 5 × 10^{-5} M 2-ME, antibiotics, and 2 mM MOPS (Sigma Chemical Co.). In some experiments, the alloactivated blasts were harvested on day 8, and 5 × 10^6 of these cells were restimulated in a secondary MLR for a 3-d period under the same conditions. Alloactivated blasts from primary or secondary MLRs were harvested by centrifugation on a Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden) Telebrix 38 (Guerbet Laboratories, Paris, France) gradient and then were fused with the AKR thymoma BW5147 as described (34). THC were selected in HAT-containing medium and tested for IL-2 responses (see below) either untransfected Ltk^- cells or A^k- or E^k-bearing L cell transfectants. Selected THC were serially cloned at limiting dilution to ensure their clonality and stability, and frozen in large-scale before further study.

THC Stimulation Assays. THCs were harvested in log phase and cultured (7 × 10^4) in the presence of increasing numbers (from 2.5 × 10^4 to 10^5) of stimulator L cell transfectants according to the culture conditions previously described (34). 50 μl of culture supernatant was harvested 24 h later and tested for IL-2 content using the CTL-L assay. Results were expressed as the mean ^{3}[H]TdR uptake (cpm) of duplicate CTL-L cultures. Standard errors were within 10% of the mean.

Results

Alloreactive T Hybridoma Cells and Target L Cell Transfectants. To sample the T cell repertoire alloreactive to the A^k molecule, we assembled a panel of 13 A^k-reactive THC. They were generated in several fusion experiments, using T lymphocyte blasts sensitized against H-2^k-bearing stimulator cells (Table I). These hybridomas all produce IL-2 when specifically activated. The A^k specificity of the THC was established by stimulating them with different L cell transfectant lines (see below); all 13 react to stimulators expressing the A^k, but not the A^b, molecule. A fourteenth hybrid, T40-73, is specific for a class I molecule (Table I), and serves as a control line.

The panel of L cell transfectants used as targets for the alloreactive hybridomas is depicted schematically in Fig. 2. Certain of the transfectant lines have been de-

TABLE I
THCs Used in this Study

Fusion experiment	Strain combination	In vitro allosensitization*	Cloned THC‡	*H-2*^{k} subregion specificity§
T40, T41	A.TH anti-A.TL ($K^sI^sD^d$ anti-$K^sI^kD^d$)	Primary MLR	T40-51, T41-351	A^k
			T40-73	K/D^k
T50	BALB/c anti-BALB.K ($K^dI^dD^d$ anti-$K^kI^kD^k$)	Secondary MLR	T50-45, T50-72, T50-118, T50-183, T50-199, T50-231, T50-243, T50-244, T50-345, T50-368	A^k
Z0	B10.S(7R) anti-B10.BR ($K^sI^sD^d$ anti-$K^kI^kD^k$)	Secondary MLR	Z0-1	A^k

Alloactivated T cell blasts were fused with the $HGPRT^-$ AKR thymoma BW5147.

* Fusions were performed either after a primary mixed lymphocyte reaction (MLR) (3 d of culture) or after a secondary MLR (8 d of primary culture, followed by 3 d of restimulation).

‡ Stable IL-2-producing THCs established after serial subcloning.

§ As determined by transfectant analysis (e.g., see Fig. 2).

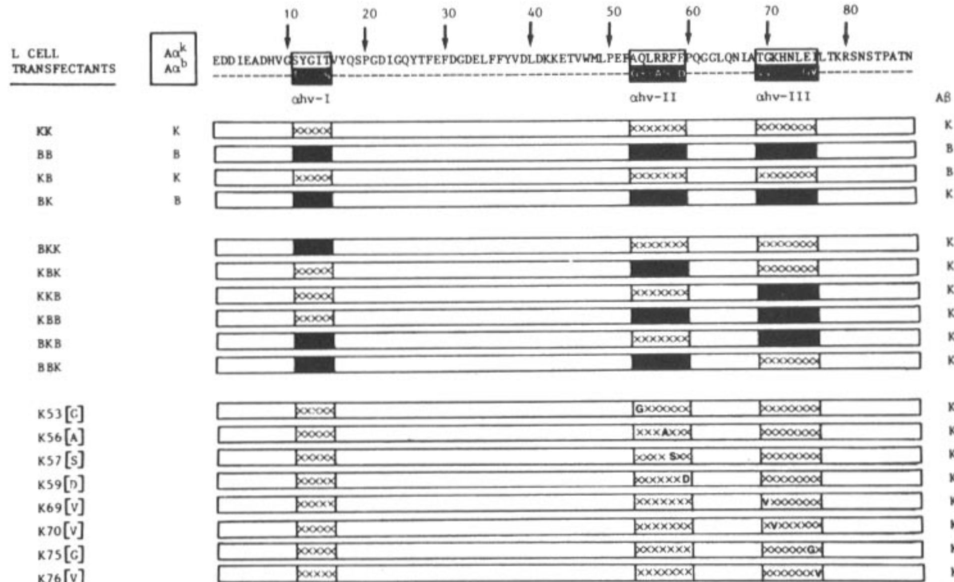
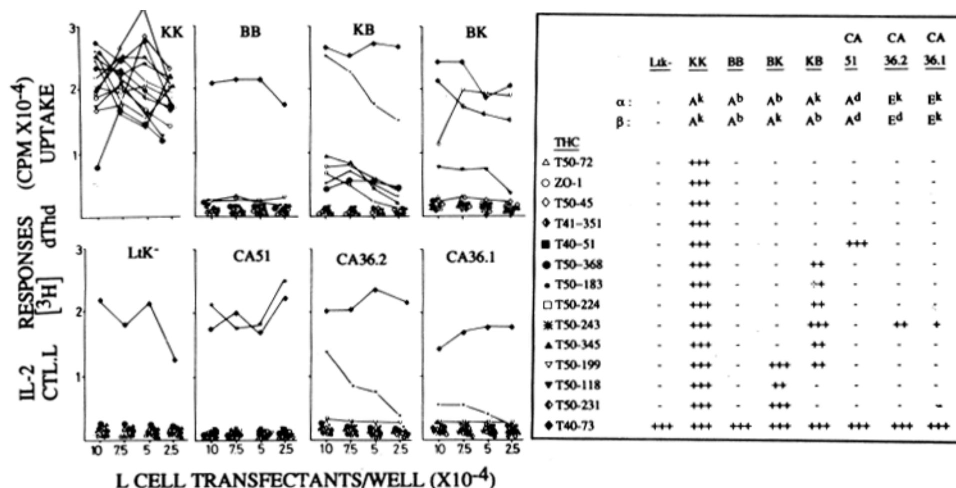


FIGURE 2. Sequences of wild-type, chimeric and single site-mutated A_α chains. The k-haplotype sequence for the first domain of A_α is written in full, but only the polymorphic amino acids are written for the b-haplotype. Numbering begins with the first residue of the mature protein. The first set of transfectants expresses wild-type A_α and A_β chains of the k or b haplotype; the two letter names indicate the haplotype of the α and β chains, respectively. The second set of transfectants expresses wild-type A^k_β chains in association with chimeric A_α chains; the chimeric molecules consist of all six permutations of k (*open rectangles*) or b (*filled rectangles*) ahv regions. The three-letter nomenclature indicates the haplotypes of the A_α molecule from which the ahv I, II, and III regions (respectively) were derived. The last set of transfectants expresses the wild-type A^k_β chain in association with A^k_α chains bearing single site replacements. The first letter of each name indicates the haplotype of the A_α chain, the number signifies the position of the mutated amino acid, and the letter in parenthesis denotes the amino acid substituted at that position.

scribed previously (30, 31). A_α and A_β cDNAs, or mutants thereof, were inserted into an expression vector and cotransfected, along with the thymidine kinase (tk) gene, into tk^- L cells. Lines expressing high surface levels of A_α:A_β heterodimer were selected by cytofluorimetric sorting. The panel consists of three sets of transfectants, displaying (*a*) haplotype-matched or haplotype-mismatched wild-type A_α:A_β complexes, (*b*) A^k_β chains associated with $A^{k/b}_\alpha$ chimeric molecules, or (*c*) A^k_β chains associated with single site-substituted A^k_α molecules (always substituted with the corresponding A^b_α residue).

Chain Specificity of the A^k-reactive THCs. To establish the specificity of the THCs, we first examined their responses to L cell transfectants expressing either haplotype-matched (A^k_α:A^k_β, A^b_α:A^b_β, A^d_α:A^d_β, E^k_α:$E^{k,d}_\beta$) or haplotype-mismatched (A^k_α:A^b_β or A^b_α:A^k_β) wild-type class II molecules (Fig. 3). All of the hybrids were triggered by the A^k (KK) but not the A^b (BB) complex. Some showed cross-reactivity to A^d or $E^{d,k}$. All L cell transfectants activate the control hybridoma T40-73, which recognizes the H-$2K^k$ class I molecule encoded in the host genome.

The hybridomas reacted to mixed-haplotype molecules with several different specificity patterns. One group required that both the A_α and A_β chains be of the



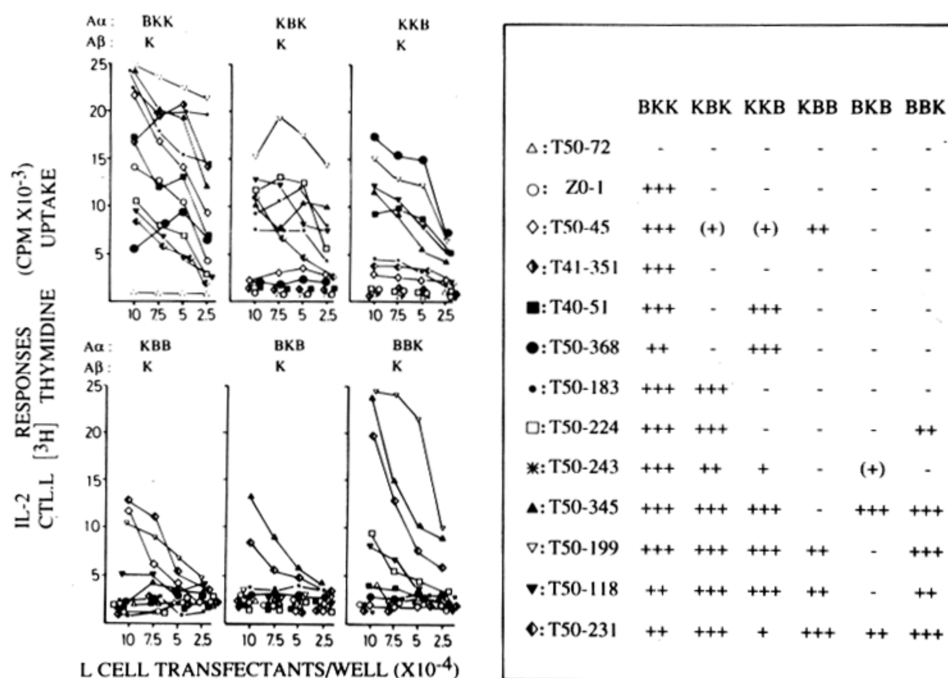
THC	Ltk-	KK	BB	BK	KB	CA 51	CA 36.2	CA 36.1
α:	-	A^k	A^b	A^b	A^k	A^d	E^k	E^k
β:	-	A^k	A^b	A^k	A^b	A^d	E^d	E^k
△ T50-72	-	+++	-	-	-	-	-	-
○ ZO-1	-	+++	-	-	-	-	-	-
◇ T50-45	-	+++	-	-	-	-	-	-
◆ T41-351	-	+++	-	-	-	-	-	-
■ T40-51	-	+++	-	-	-	+++	-	-
● T50-368	-	+++	-	-	++	-	-	-
• T50-183	-	+++	-	-	++	-	-	-
□ T50-224	-	+++	-	-	++	-	-	-
✱ T50-243	-	+++	-	-	+++	-	++	+
▲ T50-345	-	+++	-	-	++	-	-	-
▽ T50-199	-	+++	-	+++	++	-	-	-
▼ T50-118	-	+++	-	++	-	-	-	-
◆ T50-231	-	+++	-	+++	-	-	-	-
◆ T40-73	+++	+++	+++	+++	+++	+++	+++	+++

FIGURE 3. THC recognition of haplotype-matched or haplotype-mismatched wild-type A_α and A_β chains. THCs (7.5×10^4) were cultured with increasing numbers (from 2.5×10^4 to 10^5) of untransfected L cells (Ltk⁻) or transfectant L cells expressing wild-type A_α and A_β chains of k or b haplotype (see Fig. 2). L cell transfectants expressing A^d_α:A^d_β, E^k_α:E^k_β, and E^k_α:E^d_β were a kind gift from Dr. B. Malissen (CA51, CA36.1, CA36.2). IL-2 responses were measured after 24 h of co-culture using the CTL.L assay. The plotted points represent the mean [^{3}H]TdR uptake from duplicate CTL.L cultures. The results are summarized in the right panel, where the IL-2 levels were scored as + ($<5 \times 10^3$ cpm), ++ (between 5,000 and 10,000 cpm), and +++ (>10,000 cpm).

k haplotype (T50-72, Z0-1, T50-45, T41-351, and T40-51); this result is reminiscent of data from Lechler et al. (35) showing that two A^k-reactive THCs required both the A^k_α and A^k_β chains for efficient stimulation. Another group of hybridomas (T50-368, T50-183, T50-224, T50-243, and T50-345) recognized primarily A_α-dependent determinants; the haplotype of the A_β chain was less critical although not completely irrelevant, as evidenced by the distinctly lower responses to KB than to KK. Two hybridomas (T50-118 and T50-231) seemed to focus more on the A^k_β chain, and finally, T50-199 was very promiscuous, responding to cells carrying either A_α or A_β of k-haplotype sequence.

THC Recognition of Chimeric A_α Chains. To assess the contribution of polymorphic sites on the A_α chain to allo-determinants recognized by A^k-reactive THCs, we examined hybridoma responses to a series of L cell transfectants that express a wild-type A^k_β chain in association with chimeric A_α chains. In the various chimeric molecules, single A^k_α and A^b_α ahv regions have been interchanged. These k→b haplotype swaps result in the replacement of stretches containing two (ahvI) or four (ahvII and ahvIII) polymorphic residues (see Fig. 2). The transfectant lines are designated BKK, KBK, . . . etc., the three letters signifying the haplotype of the A_α chain from which the ahvI, ahvII, and ahvIII stretches were derived.

Stimulation of the THCs with increasing numbers of the various transfectants provoked the responses plotted and tabulated in Fig. 4. The response patterns were complex, the focus being primarily on α-ahvII and α-ahvIII, though the precise profiles varied markedly among the different THCs. Examination of the k→b permutation data (lines BKK, KBK, and KKB) showed that some of the hybridomas required



	BKK	KBK	KKB	KBB	BKB	BBK
△: T50-72	-	-	-	-	-	-
○: Z0-1	+++	-	-	-	-	-
◇: T50-45	+++	(+)	(+)	++	-	-
◆: T41-351	+++	-	-	-	-	-
■: T40-51	+++	-	+++	-	-	-
●: T50-368	++	-	+++	-	-	-
•: T50-183	+++	+++	-	-	-	-
□: T50-224	+++	+++	-	-	-	++
✱: T50-243	+++	++	+	-	(+)	-
▲: T50-345	+++	+++	+++	-	+++	+++
▽: T50-199	+++	+++	+++	++	-	+++
▼: T50-118	++	+++	+++	++	-	++
◆: T50-231	++	+++	+	+++	++	+++

FIGURE 4. THC recognition of chimeric A_α molecules. The transfectant stimulators display A^k_α in association with chimeric A_α chains; k-to-b haplotype interchanges have been engineered at αhvI, II, or III. Increasing numbers of the transfectants were assayed for their ability to stimulate the A^k-reactive THCs. The plotted points represent the mean [^{3}H]TdR uptake from duplicate cultures. In the right panel, the IL-2 responses were scored as in the legend to Fig. 3.

that two, or even all three, of the α-ahv be k haplotype for effective recognition; interestingly, these were most of the ones that demanded contributions from both the A^k_α and A^k_β chains (T50-72, Z0-1, T50-45, and T45-351; see Fig. 3). Several other THC had less stringent demands, seeming to require k-haplotype residues in only α-ahvII (T40-51 and T50-368) or α-ahvIII (T50-183 and T50-224), as evidenced by their response to KBK and KKB stimulators. Yet, the data from the b→k permutations (KBB, BKB, BBK) suggested that more complex influences dictate specificity. Thus, loss–gain reciprocity was observed only once, for T50-224, whose reactivity was lost to KKB cells and gained to BBK, ascribing specificity to α-ahvIII. For the other cases, BKB and BBK cells failed to stimulate. One of the hybridomas, T50-345, showed an interesting response pattern: k-haplotype amino acids in *either* α-ahvII or α-ahvIII sufficed for triggering. Finally, as expected, essentially all of the A_α chain permutations stimulated the A^k_β-reactive THCs (T50-199, T50-118, and T50-231), which could thus serve as useful controls. The exceptions were the lack of responses to BKB cells by T50-118 and T50-199.

THC Recognition of Single-Site Mutated A^k_α Chains. To more precisely localize the polymorphic residues on A^k_α that contribute to allo-determinants recognized by A^k-reactive THCs, we stimulated the various hybridomas with a series of L cell transfectants expressing an A^k_β chain in association with A^k_α molecules that carry single site replacements. At each of the eight positions in ahvII and ahvIII that differ

between A^k_α and A^b_α, we replaced the k-haplotype residue with the corresponding b-haplotype amino acid [K53(G) to K76(V); see Fig. 2].

As indicated in Table II, many of these mutations had profound effects on the recognition of A^k by THCs. Several important points emerge. First, the results are quite consistent with those obtained with the set of chimeric A^k_α molecules; that is, if a THC failed to react to an A^k_α molecule carrying a given point mutation, it also failed to be triggered when the molecule bore a substitution of the entire corresponding ahv region (Fig. 4). For example, T50-72 did not react to K56(A), K59(D), K69(V), and K70(V) nor to KBK and KKB, while T40-51 did not respond to K59(D) nor to KBK. At the other end of the spectrum, T50-345 could be stimulated by all of the single site mutations and it was a hybridoma that accepted k amino acids in either of α-ahvII or α-ahvIII. A few responses were paradoxical, however: the activation of T50-224, efficiently elicited by KBK, was inefficient by K56(A) and K59(D). The second important point is that the mutant panel provoked diverse T cell response patterns; indeed every single hybridoma showed a unique profile of reactivity. Third, the individual point mutations appeared of unequal importance, as judged from the number of THCs each mutation affects. In α-ahvII, the k→b substitutions at positions 56 and 59 were the most drastic, a substitution at position 53 less so, and a mutation at position 57 hardly noticeable. Similarly in α-ahvIII the critical positions were 69 and 70, while mutations at positions 75 and 76 had hardly any effect.

TABLE II

THC Recognition of A^k_α Chains Bearing Single Site Mutations

THC	Requirements[‡]	IL-2 responses[*]								
			α-ahvII Mutations				α-ahvIII Mutations			
		KK	K53G	K56A	K57S	K59D	K69V	K70V	K75G	K76V
				cpm × 10^{-2}				*cpm × 10^{-2}*		
T50-72	I + II + III	374	97	7	137	6	7	9	376	459
Z0-1	II + III	375	19	319	214	4	271	6	95	331
T50-45	II + III	191	2	4	189	1	172	9	149	34
T41-351	II + III	336	236	1	160	322	12	207	309	303
T40-51	II	364	208	324	207	7	346	351	377	358
T50-368	II	246	20	90	38	2	NT	NT	NT	NT
T50-183	III	459	NT[§]	NT	NT	NT	28	5	423	460
T50-224	III	319	64	285	171	49	3	252	309	431
T50-243		335	208	333	158	209	302	379	321	345
T50-345	II or III	344	202	377	233	344	343	152	257	473
T50-199		327	283	327	167	327	371	342	361	392
T50-118		311	18	51	236	227	470	499	384	497

[*] THCs (7.5 × 10^4) were cultured with 5 × 10^4 L cell transfectants displaying wild-type Aα and Aβ chains (KK) or Aα molecules that carry *k* to *b* haplotype substitutions at single positions in the ahvII or ahvIII regions. IL-2 responses were measured after 24 h of co-culture using the CTL-L assay. Results were expressed as the mean [^{3}H]TdR uptake (cpm × 10^{-2}) from duplicate CTL-L cultures. Standard errors were <10%. The experiments actually included several different APC concentrations (usually four, occasionally two or six), as in the experiments depicted in Figs. 2 and 3. We have chosen, for the sake of simplicity, to show the data from one APC concentration only. Underlined values are <30% of control IL-2 responses. These low or nonexistent responses were also observed at the other APC concentrations.

[‡] As deduced from the data of Fig. 4 (BKK, KBK and KKB).

[§] NT, Not tested.

Discussion

Before discussing the implications of these data, one needs to consider a few points concerning the validity of the experimental system. First, the chimeric and mutant A_α molecules are not monstrously deformed; all changes involve replacement by amino acids normally found at the corresponding positions in allelic A_α chains; the mutant complexes are all recognized by a number of conformation-sensitive mAbs (30, 31, and data not shown); and all of the transfectants are capable of activating at least some T hybridomas, in this and parallel studies (Reske-Kunz, A., D. Landais, J. Peccoud, C. Benois, and D. Mathis, manuscript submitted for publication; 35a). Second, one could argue that the outcome of our experiments is influenced by restrictions on interallelic A_α/A_β pairing, as has been described by Germain and coworkers (36, 37). While pairing influences on tertiary structure could contribute to the patterns we observe, their influence is unlikely to be a major one, particularly when one considers results with the single-site replacements. Thus, A^k and A^b are the most favorable combination for interallelic pairing (36); no difficulty was encountered in obtaining L cells that express high levels of the single site–substituted A complexes (although repeated sorting was necessary for some of the α-ahv chimeric lines, such as BKB); the residues that we hypothesize to be important determinants of THC recognition are probably not, for the most part, in contact with the β-ahvI region shown to be the predominant influence on heterodimer expression (18, 37); and finally, our data are internally consistent (as regards single-site substitutions versus ahv region interchanges, for example). Therefore, we believe that the observed patterns do signify determinant roles for the amino acids of interest rather than just reflecting nonspecific perturbations of the tertiary structure of the class II complex.

Can we make any conclusions about the different models of allorecognition on the basis of our data? Five of the THCs we have analyzed require k-haplotype residues in A_β, as well as in several $A\alpha$ ahv regions; another hybridoma will respond if k-haplotype residues occur in either A_α or A_β. Both of these results are inconsistent with models whereby alloreactive T cells key on short MHC peptides, and thus argue against models III and IV (at least for these THCs). The other hybridomas appear to require contributions from one chain only, either A_α^k or A_β^k. Yet, for most of them, the data presented in Fig. 4 and Table II suggest a complex interplay of determinant residues; even the apparently A_β^k-specific THC T50-118 and T50-199 seem affected by alterations of α-ahvII at positions 53 and 56. Only for T50-224 might one make a case for recognition of a peptide from α-ahvIII. Thus, although reactivity to a processed MHC molecule cannot be ruled out in all cases, it appears that the majority of THCs rely on determining residues in the intact allogenic A molecule, as per models I and II.

Does model I or II best explain the results? Two points argue for model IIb, in which the determining amino acids act by conditioning the binding of a cellular peptide. First, striking similarities emerge between the results described here for alloreactive T cells and data obtained in similar experiments with T cells reactive to insulin in the context of A_α^b:A_β^k (Reske-Kunz, A., et al., manuscript submitted for publication). In the latter study as well, contributions from both the A_α and A_β chains, a critical role for α-ahvII and III, and a dominant role for residues 56 and 70 were documented. In other words, alloactivation appears to behave very much

like antigen presentation. This conclusion is the same as that recently reached for class I alloactivation by Jelachich et al. (38).

Second, the proposed critical amino acids have side chains pointing into the peptide binding groove according to the hypothetical structure of Brown et al. (18) (see Fig. 5). This is particularly clear for the relevant α-ahvIII residues: amino acids Thr69 and Gly70 are critical for allorecognition and are predicted to point into the groove, not towards the TCR (a prediction confirmed by the lack of influence of positions 69 and 70 on the binding of all mAbs that see an α-ahvIII epitope; Peccoud, J., unpublished data). In contrast, Glu75 is hypothesized to be solvent-accessible, is crucial for antibody recognition (31, 32, 39), but has hardly any effect on allorecognition by the panel of THCs. Most of the dominant positions in α-ahvII are not as easy to orient, because the class II model makes few predictions in this region (the pairwise alignment of A_α and HLA-A2 breaking down due to the chemically heterogeneous nature of the A_α polymorphic residues [18]). Nevertheless, the k-haplotype residue Glu59 is also hypothesized to point into the antigen-binding groove (18). Although the Brown/Wiley model remains hypothetical, we feel that consideration of both the first and second point argue for model IIb.

We contend, then, that the determining amino acids for alloreactivity against class II molecules are involved in peptide contacts rather than in TCR contacts. If alloreactivity is determined by differential peptide-binding, the logical extension is that the polymorphic amino acids that condition the allorecognition event act by permitting the binding of self-antigens that cannot be bound by the responder's own MHC molecules. The T cell repertoire would not be expurgated of these reactivities, since the self-MHC molecules could not have presented these peptides during thymic selection. This view is consistent with the theoretical models of alloreactivity and self tolerance recently advanced by Wederlin (40) and Kourilsky and colleagues (41). It is also reminiscent of, although somewhat different from, the hypothesis of Matzinger and Bevan (42), whereby alloreactivity is due to MHC-restricted presentation of minor histocompatibility antigens.

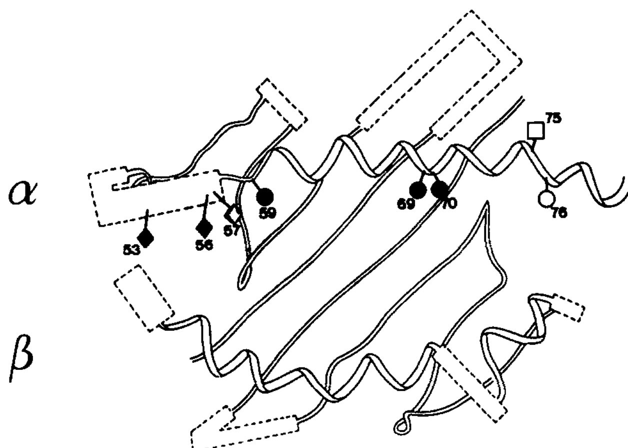


FIGURE 5. Localization of allo determinant residues within the hypothetical class II structure. The polymorphic amino acids relevant for this study have been situated on the hypothetical class II structure proposed by Brown et al. (18). The residues represented as filled symbols are important for the recognition of the A^k molecule by several alloreactive THCs; those shown as open symbols appear significantly less important (see Table II). Circles and squares signify amino acids whose side chains point into the antigen binding groove or towards the TCR, respectively. The losanges indicate amino acids whose side chains could not be reliably oriented by Brown et al. (18).

Nonetheless, we do not believe that the alloreactive TCRs contact only the presented peptide. Direct MHC/TCR contacts probably do exist, as implied by the very size of the TCR combining site (13, 18), by the natural affinity of the TCR for MHC molecules (43), and by the recent data of Ajitkumar et al. (28) on allorecognition of MHC class I mutants. These contacts just do not condition most allorecognition events and hence the discrimination between self and nonself class II histocompatibility molecules.

The model we arrive at is consistent with the detected high frequency of alloreactive T cells (the individual is or is not tolerized against innumerable self-antigens, depending on the MHC), and with the recently expounded notion of cell type-specific allostimulation (44; see also Böhme, J., K. Haskins, P. Stecha, W. van Ewijk, M. LeMeur, P. Gerlinger, C. Benoist, and D. Mathis, manuscript submitted for publication). It is also consistent with data from studies of variant or mutant class I molecules, showing that amino acids forming the peptide-binding groove can determine alloreactivity (38, 45, 46). The nature of the peptides remains obscure. The multiplicity of patterns we observe with the single site A_α mutants is certainly consistent with the idea that a given alloreactive T cell is specific for one of many possible cell-derived peptides. Identification of these peptides is the next step to full understanding of the mechanism of alloreactivity.

Summary

An individual's T lymphocytes are highly reactive to allogeneic MHC molecules. As a step in deciphering the mechanism of allorecognition by T lymphocytes, we have attempted to identify the TCR's target on MHC class II molecules, in particular the polymorphic residues that determine the specificity of recognition. We have generated a panel of A^k-reactive, A^b-nonreactive T cell hybridomas, and sets of L cell transfectants displaying $A_\alpha A_\beta$ molecules with wild-type, chimeric or single site-mutated A_α chains, with reciprocal interchanges between A^k and A^b. We then measured the stimulation of the T hybridomas in response to the transfectants. The results indicate that the hybridomas recognize diverse and complex determinants, with contributions from both A_α and A_β chains, and from several regions or amino acids of the A_α chain. The data are most consistent with a model in which alloreactivity results from the presentation of peptides to the T cell by an allogeneic MHC molecule, peptides that cannot be presented by the responder's own MHC complexes. The specificity of allorecognition seems to be imparted mainly by peptide/MHC molecule rather than TCR/MHC molecule contacts.

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