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 N-terminal domains of the paired α and β chains that are allelically divergent. These are referred to as α1 and...
βI, and within each one, polymorphism is even further clustered into what we have termed "allelically hypervariable regions" (16, 17). For the murine Aα 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 Aα but not an Aβ 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-
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 allore cognition 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 co-workers (23). On the other hand, evidence has been presented that spatially distinct regions of a class I molecule specify allore cognition (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 allore cognition 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'-reactive T cell. We first evaluate the contribution of the individual A^a and A^b chains and then delineate, through site-directed mutagenesis, determining residues on the A^x 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^a and A^b 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^a 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 NruI and Bgl II sites artificially introduced into the A^a cDNA (32).

Transfected L cells were selected by cytofluorimetric sorting, after staining with the appropriate mAb, usually one of the anti-A^b reagents 10-2-16, 39E, or 40B (30). The resulting lines showed surface A^a:A^b levels that vary by a factor of 5 or less; no correlation was noted between the density of A^a:A^b on a given line and the pattern of T hybridoma cell (THC) 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 x 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 x 10^-3 M 2-ME, antibiotics, and 2 mM MOPS (Sigma Chemical Co.). In some experiments, the alloactivated blasts were harvested on day 8, and 5 x 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^- or E^- bearing L cell transfecteds. 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 x 10^4) in the presence of increasing numbers (from 2.5 x 10^4 to 10^5) of stimulator L cell transfecants 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 ± [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 transfecant 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 transfecant lines used as targets for the alloreactive hybridomas is depicted schematically in Fig. 2. Certain of the transfecant lines have been de-

| Table I |
|---------|
| **THCs Used in this Study** |
| Fusion experiment | Strain combination | In vitro allosensitization* | Cloned THC† | H^2^ subregi on specificity§ |
| T40, T41 | A.TH anti-A.TL (K^D^D^α anti-K^D^D^a) | Primary MLR | T40-51, T41-351 T40-73 | A^k |
| T50 | BALB/c anti-BALB.K (K^D^D^α anti-K^D^D^a) | 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^D^D^α anti-K^D^D^a) | 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).
§ As determined by transfecant analysis (e.g., see Fig. 2).
Figure 2. Sequences of wild-type, chimeric and single site-mutated Aα chains. The k-haplotype sequence for the first domain of Aα 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 Aα and Aβ 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 Aα chains in association with chimeric Aβ 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 Aα molecule from which the ahv I, II, and III regions (respectively) were derived. The last set of transfectants expresses the wild-type Aβ chain in association with Aα chains bearing single site replacements. The first letter of each name indicates the haplotype of the Aα 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). Aα and Aβ 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 Aα:Aβ heterodimer were selected by cytofluorimetric sorting. The panel consists of three sets of transfectants, displaying (a) haplotype-matched or haplotype-mismatched wild-type Aα:Aβ complexes, (b) Aβ chains associated with Aα:b chimeric molecules, or (c) Aα chains associated with single site-substituted Aα molecules (always substituted with the corresponding Aα residue).

Chain Specificity of the Aα-reactive THCs. To establish the specificity of the THCs, we first examined their responses to L cell transfectants expressing either haplotype-matched (Aα:Aβ, Aα:Aβ, Aα:Aβ, Eα:Eβ) or haplotype-mismatched (Aα:Aβ or Aβ:Aα) wild-type class II molecules (Fig. 3). All of the hybrids were triggered by the Aα (KK) but not the Aβ (BB) complex. Some showed cross-reactivity to Aα or Eα. All L cell transfectants activate the control hybridoma T40-73, which recognizes the H-2Kk 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 Aα and Aβ chains be of the
THC Recognition of Haplotype-Matched or Haplotype-Mismatched Wild-Type Aα and Aγ Chains. THCs (7.5 × 10⁴) were cultured with increasing numbers (from 2.5 × 10⁴ to 10⁵) of untransfected L cells (Ltk⁻) or transfectant L cells expressing wild-type Aα and Aγ chains of k or b haplotype (see Fig. 2). L cell transfectants expressing Aα:Aγ, Eα:Eβ, and Eα:Eγ 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 [³H]Tdr uptake from duplicate CTL.L cultures. The results are summarized in the right panel, where the IL-2 levels were scored as + (<5 × 10³ cpm), ++ (between 5,000 and 10,000 cpm), and +++ (>10,000 cpm).

THC Recognition of Chimeric Aα Chains. To assess the contribution of polymorphic sites on the Aα chain to allo-determinants recognized by Aκ-reactive THCs, we examined hybridoma responses to a series of L cell transfectants that express a wild-type Aγ chain in association with chimeric Aα chains. In the various chimeric molecules, single Aα and Aγ 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 Aα 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...
FIGURE 4. THC recognition of chimeric Aα molecules. The transfectant stimulators display Aα in association with chimeric Aα 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 Aα-reactive THCs. The plotted points represent the mean [3H]Tdr uptake from duplicate cultures. In the right panel, the IL-2 responses were scored as in the legend to Fig. 3.

THC Recognition of Single-Site Mutated Aα Chains. To more precisely localize the polymorphic residues on Aα that contribute to allo-determinants recognized by Aα-reactive THCs, we stimulated the various hybridomas with a series of L cell transfectants expressing an Aα chain in association with Aα molecules that carry single site replacements. At each of the eight positions in αhvi and αhviII that differ
between $A_k^a$ and $A_k^b$, 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 $A_k^b$ molecules; that is, if a THC failed to react to an $A_k^a$ 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 $\alpha$-ahvII or $\alpha$-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 $\alpha$-ahvII, the $k\rightarrow b$ 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 $\alpha$-ahvIII the critical positions were 69 and 70, while mutations at positions 75 and 76 had hardly any effect.

### Table II

**THC Recognition of $A_k^a$ Chains Bearing Single Site Mutations**

| THC       | IL-2 responses | $\alpha$-ahvII Mutations | $\alpha$-ahvIII Mutations |
|-----------|----------------|---------------------------|----------------------------|
| Requirements | $KK$ | $K53G$ | $K56A$ | $K57S$ | $K59D$ | $K69V$ | $K70V$ | $K75G$ | $K76V$ |
| 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          | 384 | 208 | 324 | 297 | 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   | III         | 335 | 208 | 333 | 158 | 209 | 302 | 379 | 328 | 384 |
| T50-345   | II or III   | 344 | 202 | 377 | 233 | 344 | 343 | 152 | 257 | 473 |
| T50-199   | II or III   | 327 | 283 | 327 | 167 | 327 | 371 | 342 | 361 | 392 |
| T50-118   | III         | 311 | 18 | 51 | 236 | 227 | 470 | 499 | 384 | 497 |

*THCs ($7.5 \times 10^6$) were cultured with $5 \times 10^4$ L cell transfectants displaying wild-type $A_k^a$ and $A_k^b$ chains (KK) or $A_k^a$ molecules that carry $k \rightarrow b$ 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 $[\text{H}]$TdR uptake (cpm $\times 10^{-2}$) 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.

1 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 Aα molecules are not monstrously deformed; all changes involve replacement by amino acids normally found at the corresponding positions in allelic Aα 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 Aα/Aβ pairing, as has been described by Germain and co-workers (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, Aα and Aβ 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 Aβ, as well as in several Aα ahv regions; another hybridoma will respond if k-haplotype residues occur in either Aα or Aβ. 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 Aα or Aβ. Yet, for most of them, the data presented in Fig. 4 and Table II suggest a complex interplay of determinant residues; even the apparently Aβ-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 Aα:Ag (Reske-Kunz, A., et al., manuscript submitted for publication). In the latter study as well, contributions from both the Aα and Aβ 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 $\alpha$-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 $\alpha$-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 $\alpha$-ahvII are not as easy to orient, because the class I model makes few predictions in this region (the pairwise alignment of $\alpha_\alpha$ and HLA-A2 breaking down due to the chemically heterogeneous nature of the $\alpha_\alpha$ 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 $A^\beta$ 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 $\alpha$ 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 $\alpha\beta$ molecules with wild-type, chimeric or single site-mutated $\alpha$ 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 $\alpha$ and $\beta$ chains, and from several regions or amino acids of the $\alpha$ 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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