A_β POLYMORPHIC RESIDUES RESPONSIBLE FOR CLASS II MOLECULE RECOGNITION BY ALLOREACTIVE T CELLS

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Class II (Ia) molecules are polymorphic integral membrane proteins that, in association with processed antigen, comprise the ligand complex bound by antigen receptors (TCR) on $CD4^+$ T lymphocytes. The association of this ligand with the TCR on T helper lymphocytes initiates the activation program that is a requisite event for the promotion of both humoral and cell-mediated immune responses. During the past several years much has been learned about the sequences of class II molecules, peptide antigens, and TCRs. Hypothetical models of the class II three-dimensional molecular structure have also been proposed, based on the solution to the class I crystal structure (1). Although direct binding analyses indicate that a TCR ligand is formed when class II molecules bind to processed antigen (2, 3), only limited information is available concerning the molecular interactions that occur when a class II molecule associates with a peptide antigen and a TCR.

Class II molecules are glycoproteins that consist of a 34-kD α polypeptide noncovalently associated with a 29-kD β polypeptide. These two polypeptides associate to form a molecule containing two extracellular domains on the plasma membrane of a variety of different cell types. Sequence analysis of the α and β genes has shown that the polymorphic residues tend to be clustered in three or four regions of the polypeptides that comprise the membrane-distal domain (designated $\alpha 1\beta 1$) (4–6). Since all of the antibody and TCR binding sites analyzed have been mapped to the membrane-distal domain (7–9), the polymorphic residues in the $\alpha 1\beta 1$ domain are involved in determining the allele-specific antibody binding sites as well as in forming the TCR ligand.

To identify the β chain polymorphic residues that are involved in determining epitopes recognized by antibodies and by the TCR we have constructed A^k_β genes containing mutations that result in one or more *d* allele residue substitutions in the $\beta 1$ domain. The mutant A^{k*}_β polypeptides were expressed in association with either the wild-type A^k_α or A^d_α polypeptides after transfection of the genes into the B lymphoma cell line M12.C3 (9). In a previous report, we used this panel of cells bearing the mutant class II molecules to identify the polymorphic residues that determine the immunodominant serologic epitope on both the A^k_β and A^d_β polypeptides (9). In this report the class II mutants were tested for their ability to stimulate a panel of A^k or A^d alloreactive T hybridomas in order to identify the *k* and *d* polymorphic

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residues in the β1 domain that determine either the loss or the gain of epitopes recognized by these alloreactive TCRs. Our results demonstrate that both antibody and TCR binding sites can be comprised of the same β chain polymorphic residues. However, the majority of the TCR binding sites identified with this panel of alloreactive T cell hybridomas map to polymorphic residues that are predicted to be located at the bottom of the putative antigen binding site on the class II molecule.

Materials and Methods

Cell Lines. The panel of wild-type or mutant class II-bearing cloned cell lines was prepared by transfecting into M12.C3 B lymphoma cells, plasmids containing the wild-type A^k_β or mutant A^k_β (designated-A^k_β*) genes, the neomycin resistance gene *pRSVneo*, and in certain cases, with the wild-type A^k_α gene. A detailed description of the methods used to construct the A^k_β* genes and to transfect the genes into M12.C3 cells is given elsewhere (9). Cloned lines bearing class II molecules were selected by quantitative immunofluorescence for comparable levels of class II antigen expression. Nomenclature: A^k_β* and A^k_α transfected M12.C3 cells are designated T.N, with N indicating the position(s) or polymorphic region(s) substituted in the A^k_β* β1 domain with *d*-allele residues. Cells expressing A^k_β* A^d_α molecules are designated T.N-A^d_α and cells expressing wild-type A^d_α polypeptide associated with an A^k_β polypeptide containing *d* allele residues throughout the β1 domain are designated T.$A^{d/k}_\beta$ A^d_α. The transfected cells were cultured in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY), 1 mM L-glutamine, 0.1 mM 2-ME, 10 mM Hepes, and 600 μg/ml G418.

T Cell Assays. T hybridomas 59.16, 22.1, and 98.11 were produced by fusing BW5147 cells with lymph node cells from DBA/2 mice that had been primed in vivo with T.A^k cells (M12.C3 cells expressing wild-type A^k_β and A^k_α genes). T hybridoma DG11 is a CBA/J anti-BALB/c origin and hybridoma D1G10 is a C57BL/6 anti-BALB/c origin. Both hybridomas are A^d-reactive (10). IC4 is an A^d-autoreactive T cell hybrid (10). The A^d-reactive T hybrids were generously provided by Dr. Robert Chesnut (National Jewish Hospital, Denver, CO). To assay for T hybridoma activity, 10^5 T hybridoma cells were cocultured with varying numbers of wild-type or mutant class II-bearing M12.C3 cells or with mock-transfected (T.NEO) cells. After 24 h of culture, supernatants were collected and assayed for IL-2 content in a secondary culture by using HT-2 cells as previously described (11). HT-2 cells (10^4/well) were cultured for 24 h with serial dilutions of T hybridoma culture supernatants, and the degree of stimulation was measured by the incorporation of [^{3}H]thymidine into DNA during the final 6 h of the culture period. Results shown in the figures are representative experimental values of [^{3}H]thymidine incorporation obtained from HT-2 cells cultured with T hybridoma supernatants diluted 1/64.

Results

The A^k and A^d class II proteins differ significantly in both their allospecific serologic epitopes and TCR binding sites. To identify which of the 14 β1 domain positions that differ between the *k* and *d* alleles are responsible for determining the allospecificities, we have produced a panel of cloned cell lines, each of which expresses a mutant A^k_β (A^k_β*) polypeptide (containing one or more *d* allele residues in the β1 domain) associated with either a wild-type A^k_α polypeptide or a wild-type A^d_α polypeptide (see Fig. 1 for a description of the A^k_β* mutants).

Loss of k Allele Specificities. The mutant class II-bearing cells were tested for the capacity to stimulate IL-2 secretion from three different A^k-reactive (A^d-nonreactive) T cell hybridomas. Representative experiments illustrating the effects of mutations in different regions of the β1 domain are shown in Fig. 2, while the complete set of variants tested is summarized in Table I. Two of the T hybridomas, 22.1 and

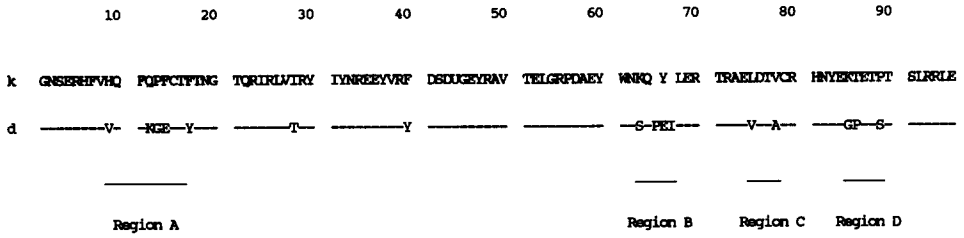

FIGURE 1. Amino acid sequences of the A^k_β and A^d_β β-1 domains (4). Mutant A^{k*}_β genes encoding *d*-allele residues at each of the 14 polymorphic positions, at multiple positions, or in polymorphic regions of the β1 domain have been constructed by site-directed mutagenesis (9). The boundaries and designation chosen for these polymorphic regions are indicated below the sequences.

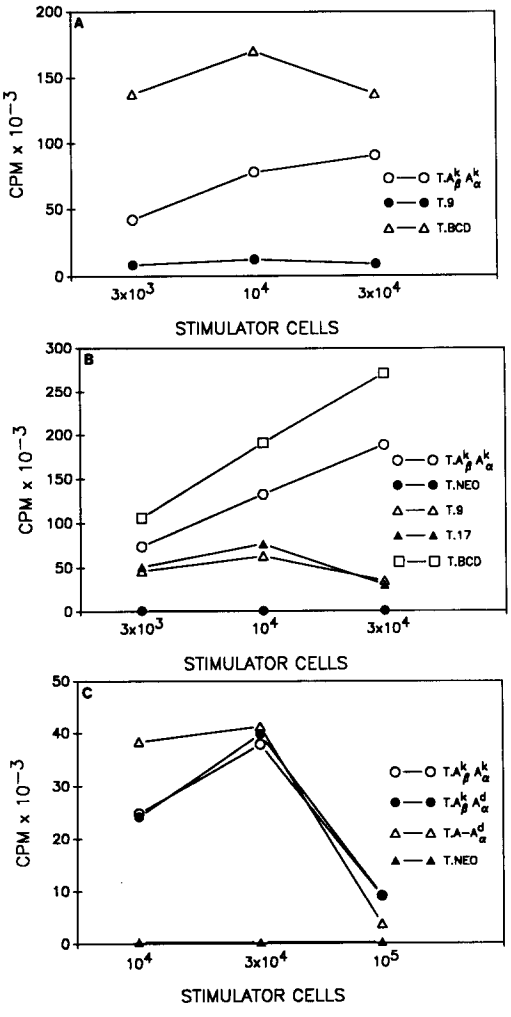

FIGURE 2. Representative activation responses of A^k alloreactive T hybridomas stimulated with transfected M12.C3 cells. (*A*) A^k-reactive T hybridoma 22.1 stimulated with M12.C3 cells expressing wild-type $A^k_\beta A^k_\alpha$ molecules (T.A^k_β A^k_α), $A^{k*}_\beta A^k_\alpha$ molecules containing *d*-allele residues at position 9 (T.9), or in regions "B", "C", and "D" (T.BCD) in the β chain. M12.C3 cells expressing $A^{k*}_\beta A^k_\alpha$ molecules containing *d* residues at either position 12, 13, 14, or 17 in the β chain elicited responses identical to mock-transfected, Ia^- cells (T.Neo) (data not shown). (*B*) A^k-reactive T hybridoma 59.16 stimulated with M12.C3 cells expressing wild-type A^k_β A^k_α molecules (T.A^k_β A^k_α), A^{k*}_β A^k_α molecules containing *d*-allele residues in the β1 regions "B", "C", and "D" (T.BCD), or at position 9 or 17, or mock-transfected, Ia^- cells (T.Neo). (*C*), A^k-reactive T hybridoma 98.11 stimulated with M12.C3 cells expressing wild-type $A^k_\beta A^k_\alpha$ molecules (T.A^k_β A^k_α), wild-type $A^k_\beta A^d_\alpha$ molecules (T.A^k_β A^d_α), A^{k*}_β A^d_α molecules containing *d* allele residues in the β1 region "A" (T.A-A^d_α), or mock-transfected, Ia^- cells (T.Neo).

59.16, are activated by cells expressing the wild-type $A^k_\beta A^k_\alpha$ molecule (T.$A^k_\beta A^k_\alpha$), but not by cells expressing the wild-type hybrid $A^k_\beta A^d_\alpha$ molecule (T.$A^k_\beta A^d_\alpha$) (Fig. 2, *A* and *B*; Table I). In contrast, the third T hybridoma, 98.11, is activated equally well by cells expressing either wild-type $A^k_\beta A^k_\alpha$ or $A^k_\beta A^d_\alpha$ molecules (Fig. 2 *C*). None of the T hybridomas are activated by the Ia^-, mock-transfected M12.C3 cells (T.Neo) or by cells expressing an Ia molecule consisting of a A^{k*}_β polypeptide containing *d* allele sequence throughout the entire β1 domain associated with a wild-type A^d_α polypeptide (T.$A^{d/k}_\beta A^d_\alpha$). These results indicate that the antigen receptors on the T hybridomas 22.1 and 59.16 recognize some combination of *k* allele polymorphic residues in both the β1 domain and in the A^k_α polypeptide. In contrast, the antigen receptors on the T hybridoma 98.11 recognize *k* allele polymorphic residues in the β1 domain but do not discriminate A^k_α from A^d_α polymorphic residues.

When these three A^k-reactive T cell hybridomas were assayed for their response to the mutant class II molecules, the response of the T hybridomas 22.1 and 59.16 to the class II mutant that contains *d* allele residues in the entire COOH-terminal half of the β1 domain (T.BCD) was greater than the response to wild-type $A^k_\beta A^k_\alpha$ molecules. Since T.BCD cells do not express higher levels of class II molecules than wild-type T.$A^k_\beta A^k_\alpha$ cells (9), the observed response likely results from the TCRs on these two T hybridomas binding with higher affinity to the class II ligand complex on T.BCD cells than on T.$A^k_\beta A^k_\alpha$ cells (Fig. 2, *A* and *B*; Table I). Both T hybridomas also reacted to A^{k*}_β mutants containing single *d* allele residues at either position 28 or 40 (T.28, T.40). These results indirectly suggest that the antigen receptors on both T cell clones recognize β chain polymorphic residues in region "A" (residues 9–17). We cannot assess directly if the T.A $A^{k*}_\beta A^k_\alpha$ molecule will stimulate these two T hybridomas because the mutant A^{k*}_β polypeptide containing *d* allele residues in region "A" cannot be expressed in association with the A^k_α molecule (12). However, we were able to map the epitope recognized by these two T hybridomas by assaying cells expressing the wild-type A^k_α polypeptide with an A^{k*}_β polypeptide containing a single *d* allele substitution at either position 9, 12, 13, 14, or 17. As shown in Fig. 2, *A* and *B*, and Table I, T hybridoma 59.16 responds to mutants T.9 and T.17 but not to mutants T.12, T.13, or T.14, while T hybridoma 22.1 is not stimulated by any of these five single residue mutants. Together these analyses map the epitopes recognized by these T cell receptors to an overlapping set of polymorphic residues in region "A" of the β1 domain.

In contrast, T hybridoma 98.11, which fails to discriminate A^d_α from A^k_α polypeptides, responds to the cell line expressing the $A^{k*}_\beta A^d_\alpha$ molecule containing *d* allele residues in region "A" (T.A-A^d_α) (Fig. 2 *C*). When the β chain mutants with *d* substitutions in either region "B", "C", "D", or combinations of these polymorphic regions were assayed, none stimulated the 98.11 T cells. Thus, although the epitopes recognized by T hybridomas 59.16 and 22.1 map to a limited number of *k* allele polymorphic residues (12, 13, 14 or 9, 12, 13, 14, 17, respectively), the T hybridoma 98.11 appears to recognize a complex epitope determined by *k* allele polymorphic residues in regions "B", "C", and "D".

Gain of d Allele Specificities. Although TCR epitopes can be mapped by measuring the inability of certain β chain mutants to initiate T cell activation, results from such analyses must be interpreted with caution. The introduction of certain amino acids into the class II polypeptides may result in an alteration of the conformation

TABLE I

Identification of Alloreactive TCR Binding Sites on A^d and A^k β1 Domains

			Wild-type Ia			Chain mutants						
Hybridoma	Epitope	T. Neo	T.$A^k_\beta A^k_\alpha$	T.$A^k_\beta A^d_\alpha$	T.$A^{d/k}_\beta A^d_\alpha$	T.ABC-A^d_α	T.ACD-A^d_α	T.BCD-A^d_α	T.BCD	T.AB-A^d_α	T.AC-A^d_α	T.AD-A^d_α
A 59.16	A	–	+ +	–	–	–	–	–	+ +	–	–	–
22.1	A	–	+ +	–	–	NA	NA	NA	+ +	NA	NA	NA
98.11	BCD	–	+ +	+ +	–	–	–	–	–	–	–	–
B DG11	B	–	–	–	+ +	+ +	–	+ +	–	+ +	–	–
IC4	AD	–	–	–	+ +	–	+ +	–	–	–	–	+ +
D1G10	?	–	–	–	+ +	–	–	–	–	–	–	–

		Chain mutants									
Hybridoma	Epitope	T.A-A^d_α	T.B	T.B-A^d_α	T.C	T.D	T.D-A^d_α	T.63	T.65-67	T.28	T.40
A 59.16	A	–	+ +	NA	+ +	+ +	NA	+ +	+ +	+ +	+ +
22.1	A	–	+ +	NA	+ +	+	NA	NT	NT	+ +	+ +
98.11	BCD	+ +	–	–	–	–	–	NT	NT	NT	NT
B DG11	65-67	–	+ +	+ +	–	–	–	–	+	–	–
IC4	AD	–	–	–	–	–	–	NT	NT	–	–
D1G10	?	–	–	–	–	–	–	NT	NT	–	–

		β Chain mutants				
Hybridoma	Epitope	T.9	T.12	T.13	T.14	T.17
A 59.16	12,13,14	+	–	–	–	+
22.1	A	–	–	–	–	–

Results are a summary of data obtained from measuring T hybridoma IL-2 responses stimulated by M12.C3 cells transfected with wild-type or mutant class II α and β genes. T.$A^{d/k}_\beta A^d_\alpha$ = wild-type A^d_α associated with polypeptide containing *d* residues throughout the β1 domain. Other mutants are described in Fig. 1. Response levels: –, <10%; +/–, 10–50%; +, 50–75% or + +, >75% of the response obtained with comparable numbers of (*A*) wild-type T.$A^k_\beta A^k_\alpha$ stimulator cells or (*B*) T.$A^{d/k}_\beta A^d_\alpha$ stimulator cells. In each assay, the range in the numbers of stimulator cells varied over 1 to 2 logs (as shown in Fig. 2). All analyses were performed a minimum of three times. NA, not applicable (hybridoma does not react with $A^k_\beta A^d_\alpha$). NT, not tested. The Ia mutants T.12, T.13, and T.14 have been shown to stimulate other T cell hybridomas (data not shown).

of spatially distant residues. Such conformational alterations are difficult to detect and could result in the misidentification of the polymorphic residues that comprise the TCR contact points. An alternative to measuring the loss of allospecificities in the panel of mutant A^k_β* stimulator cells is to assay for the gain of new *d* allospecificities after the introduction of a limited number of *d* allele residues into the A^k_β polypeptide sequence. It is more likely that the gain of an A^d-specific epitope will be due directly to the structural alterations introduced by the *d* allele polymorphic residues.

When three different A^d reactive T hybridomas were tested with the panel of mutants, each of the T cells was activated by the stimulator cells expressing wild-type A^d_α and an A^k_β* molecule containing *d* allele residues throughout the β1 domain ($T.A^{d/k}_\beta$ A^d_α) (Fig. 3; Table I). Cells expressing Neo, wild-type A^k_β A^k_α, or wild-type A^k_β A^d_α molecules failed to stimulate these T cells. These results suggest that the antigen receptors on these T hybridomas recognize *d* allele polymorphic residues in the β1 domain. Since the A^d_β polypeptide fails to associate with the A^k_α polypeptide (7), possible contributions of the α polymorphic residues to the T cell epitopes were assessed from mutant A^k_β* polypeptides that can associate with A^k_α.

When the three T hybridomas were assayed with the β chain mutants, each exhibited a distinct reactivity pattern. As shown in Fig. 3 *A*, clone DG11 is stimulated by class II molecules comprised of a wild-type A^k_α (or A^d_α) polypeptide and an A^k_β* polypeptide containing three *d* allele residues at positions 65–67 (T.65-67 or T.65-67-A^d_α). As summarized in Table I, the DG11 cells are also stimulated by any other β chain mutant into which these three *d* residues have been substituted (T.B, T.B-A^d_α, T.ABC-A^d_α, T.BCD, T.BCD-A^d_α, T.AB-A^d_α). Residues 65–67 have been identified

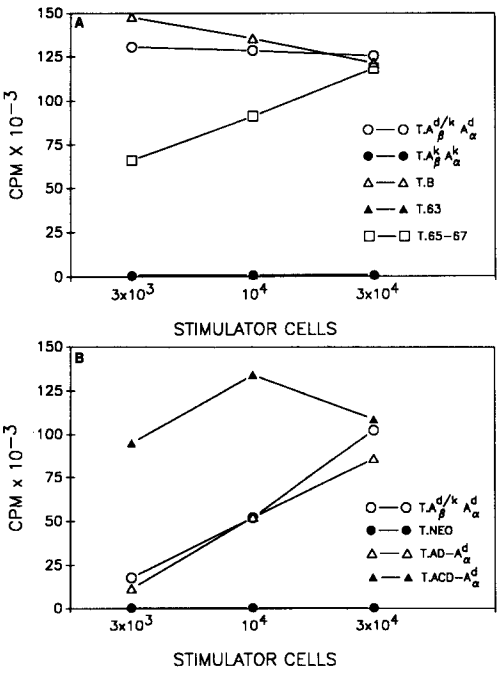

FIGURE 3. Representative activation responses of A^d alloreactive T hybridomas stimulated with transfected M12.C3 cells. (*A*) A^d-reactive T hybridoma DG11 stimulated with M12.C3 cells expressing wild-type $A^k_\beta A^k_\alpha$ molecules ($T.A^k_\beta$ A^k_α), wild-type A^d_α polypeptides associated with A^k_β* polypeptides containing *d*-allele residues either throughout the β1 domain ($T.A^{d/k}_\beta A^d_\alpha$), or wild-type A^k_α polypeptides associated with A^k_β* polypeptides containing *d* residues in either region "B" (T.B), at position 63, (T.63) or at position 65, 66, and 67 (T.65-67). M12.C3 cells transfected only with pRSV-neo (T.neo) elicited a response identical to that obtained with $T.A^k_\beta$ A^k_α cells. (*B*) A^d-reactive T hybridoma IC4.4 stimulated with M12.C3 cells expressing wild-type A^d_α polypeptide associated with A^k_β* polypeptides containing *d* allele residues throughout the β1 domain ($T.A^{d/k}_\beta$ A^d_α), in regions "A" and "D" (T.AD-A^d_α) or in regions "A", "C", and "D" (T.ACD-A^d_α). Mock-transfected, Ia⁻ M12.C3 cells (T.Neo) elicited a response comparable to cells expressing a wild-type A^d_α polypeptide associated with a A^k_β* polypeptide containing *d* allele residues in either region "A" (T.A-A^d_α) or "D" (T.D-A^d_α) (data not shown).

in previous experiments as being directly responsible for determining the loss of A^k_β-specific serologic epitopes and the gain of A^d_β-specific serologic epitopes (9). Thus, the DG11 antigen receptor recognizes an epitope composed of, in part, the same polymorphic residues that comprise the immunodominant β chain serologic epitope. In addition, both the antibodies that react with the epitope comprised of residues 65–67 and the DG11 TCR recognize the T.65-67, T.B, and T.BCD A^k_β* polypeptides associated with either the A^k_α or the A^d_α polypeptide (Table I). These results demonstrate that neither these serologic epitopes nor the DG11 TCR epitope is affected by the substitution of *k* or *d* α chain polymorphic residues.

The hybridoma IC4 is reactive to Ia molecules composed of a wild-type A^d_α polypeptide and a A^k_β* polypeptide containing *d* allele residues in both regions "A" and "D" (T.AD-A^d_α, T.ACD-A^d_α) (Fig. 3, *B*; Table I). Since neither T.A A^k_β* A^d_α molecules nor T.D A^k_β* A^d_α molecules activate IC4, the IC4 antigen receptor appears to recognize a complex epitope composed of polymorphic residues in these two regions that are widely separated in the primary sequence. As will be discussed later, these polymorphic residues are also predicted to be widely separated in the molecule's three-dimensional structure (1).

Although the panel of class II mutants has enabled us to map the β chain location of the epitopes recognized by receptors on five of the six T hybridomas, we have been unsuccessful at identifying any mutant A^k_β* molecule, other than T.$A^{d/k}_\beta$ A^d_α that will stimulate the T hybridoma D1G10 (Table I). The antigen receptor on this T hybridoma may recognize a complex epitope containing a large number of *d* allele polymorphic residues in the β1 domain (as expressed by T.$A^{d/k}_\beta$ A^d_α or combinations of *d* allele residues not represented in our panel of β chain mutants. For example, an epitope composed of *d* residues at both positions 40 and 63 would not be identified in this analysis. A serologic epitope containing these two residues has been identified previously in the analysis of the A^d_β-reactive mAb Y-212 (9).

Discussion

Brown et al. (1) have recently proposed a hypothetical model of the three-dimensional conformation of the class II α1β1 domain based on the crystal structure of the HLA A2 molecule. In this model, the polymorphic positions that we have identified as contributing to the TCR binding sites are predicted to be located in distinct areas of the class II molecule. The polymorphic residues in region "B", which are recognized by the A^d-reactive T hybridoma, DG11, as well as by several A^d- or A^k-reactive mAbs, are predicted to be located in a turn between two α-helices that comprise two sides of the putative antigen binding site. The side chains of these polymorphic residues should be exposed to the aqueous environment on the exterior of the class II molecule. Secondary structure predications suggest that region "D" is located in a turn at the end of an α-helix (comprised, in part, of "C" region residues) that forms one side of the putative antigen binding site. Thus, the 98.11 TCR epitope, which maps to regions B, C, and D, is predicted to be located on one side wall of the putative antigen binding site. In contrast, region "A", which has been identified as comprising part of the TCR epitope for three of the six T hybridomas assayed, is predicted to be located in a β-pleated sheet that forms the bottom of the putative antigen binding site. Together these results indicate that the TCR epitopes map to a number of residues that may be located in distinct regions in the β1 domain.

Results from a number of different studies have indicated that TCRs bind to regions on class II molecules that are distinct from the regions bound by mAbs (13–15). The results presented here demonstrate that at least four of the six T hybridomas tested recognize regions on the β chain that are distinct from the serologically immunodominant region that maps to residues 63–67. However, the antigen receptor on one T hybridoma, DG11, appears to recognize the polymorphic residues 65–67. Although we cannot determine from these analyses if the same nonpolymorphic residues comprise both TCR and serologic epitopes, it is apparent that both types of molecules are capable of binding to similar regions on the class II molecule.

It has been suggested previously that, unlike serologic epitopes, the ligand recognized by most Th cells requires contributions of polymorphic residues in both the α and β chains (8). Results obtained in this study are consistent with this observation in that four of the six alloreactive T hybridomas tested recognize epitopes determined by polymorphic residues in both polypeptides. Antigen-reactive TCR combining sites may interact with residues from both polypeptides because it is these residues in the $\alpha 1 \beta 1$ domain that comprise or are adjacent to the putative antigen binding site. It is unlikely that the location of serologic epitopes on class II molecules is similarly constrained. It is interesting to note that the DG11 TCR binding site, which is comprised of the same $\beta 1$ domain polymorphic residues shown previously to comprise part of the immunodominant serologic epitope, is similar to the β chain–specific serologic epitopes in that the TCR reacts with class II molecules consisting of either A^d_α or A^k_α polypeptides. Although the similarity in the Ab and TCR epitopes may be fortuitous, it may reflect a fundamental difference between the nature of some allospecific T cell determinants and determinants that are comprised of processed antigen and self class II molecules.

The data presented in this report clearly identify the polymorphic residues responsible for the activation of several A^k- and A^d-specific T hybridomas. These residues may be the contact points that mediate TCR binding. However, it has been hypothesized that the ligand recognized by alloreactive TCRs is a composite of foreign class II residues and residues of a processed peptide located in the class II antigen binding site (16, 17). If this hypothesis is correct, we may have identified residues that are actually contact points for a processed polypeptide, which may be the principal ligand bound by the alloreactive T cell hybridoma receptors. However, both types of ligands could be represented in this analysis. For example, TCRs that map to region "A" may recognize ligands that are composed of some combination of residues from a processed peptide and the class II molecule, while TCRs that map to polymorphic residues that also comprise the serologically immunodominant site (region "B") may recognize ligands made up of only class II residues. Direct experimental evidence for either type of allo-reactive T cell ligand is lacking. The panel of Ia mutants and T hybridomas described in this report provides a defined model system with which to experimentally evaluate this problem.

Summary

In an effort to characterize the ligand that is bound by T helper lymphocyte antigen receptors, we have begun to identify class II polymorphic residues that comprise part of the allospecific TCR binding sites. Site-directed mutagenesis was used to construct mutant A^k_β (A^{k*}_β) genes that encode polypeptides into which single or

multiple residues of the A^d_β polypeptide have been substituted in the β1 domain. A panel of cloned cell lines expressing the mutant A^{k*}_β A^k_α or A^{k*}_β A^d_α molecules was analyzed for the ability to stimulate A^k or A^d alloreactive T cell hybridomas. Substitution of *d* for *k* residues at specific positions in the β1 domain resulted not only in the loss of epitopes recognized by A^k-reactive T cells but, more importantly, in the gain of epitopes recognized by A^d-reactive T cells. Some of the polymorphic residues identified as contributing to the T cell epitopes are the same residues that contribute to the serologically immunodominant epitope. Other T cell epitopes map to positions predicted to be located either in an α-helix forming one side, or in a β-pleated sheet forming the bottom of the putative antigen binding site. Thus, unlike serologic epitopes, TCR epitopes can be determined by $A\beta$ polymorphic residues in many different regions of the β1 domain and frequently depend upon contributions of A_α polymorphic residues.

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