DISTINCT RECOGNITION PHENOTYPES EXIST FOR T CELL CLONES SPECIFIC FOR SMALL PEPTIDE REGIONS OF PROTEINS

Implications for the Mechanisms Underlying Major Histocompatibility Complex-restricted Antigen Recognition and Clonal Deletion Models of Immune Response Gene Defects

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The extent of the expressed T helper cell repertoire is a result of many factors, including the quality of the initial gene pool of variable gene segments that comprise the receptor, the nature of the Ia molecules encoded by the major histocompatibility complex (MHC), and regulatory influences that either expand or delete its potential members. For T cell responses directed towards protein antigens, two extreme modes of response may be envisaged, one in which single T cell clones react at many sites along the molecule, the second in which very few sites of reactivity exist. Analysis of the specificity of the responses induced to several defined protein antigens (1), including those distantly related to self, has suggested that severe limitations exist in the choice of antigenic determinants. For example, T cells specific for the C-terminal peptide (amino acid residues 106–129) of hen egg-white lysozyme (HEL) have not been observed in H-2b mice (2, 3). Since H-2 congenic mice strains (B10.A and B10.D2) do respond to this region, the choice of reactivity is clearly dependent upon the MHC (4, 5).

It has been difficult to choose between the two major possibilities that may account for this limitation. Strong arguments (6) have been advanced in favor of MHC-determined gaps in the available peripheral repertoire, as well as for determinant selection mechanisms of antigen presentation necessary for T cell activation. It is possible that a specific interaction is necessary between a site(s) on the Ia molecule and another discrete, haplotype-specific site of perhaps three or four amino acids on the nominal antigen (7, 8). When regions on an antigen are encountered that seem unable to induce T cells in a particular MHC

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1 Abbreviations used in this paper: APC, antigen-presenting cells; HEL, BEL, REL, NEL, species-variant lysozymes isolated from egg whites of hen, bob-white quail, ring-necked pheasant, and guinea hen, respectively; MHC, major histocompatibility complex.
haplotype, these sites may be limiting (9). Alternatively, the repertoire may lack suitable T cells. Their absence or gaps have been largely attributed (10, 11) to Ia-influenced selection in the thymus and/or to negative selection required for maintenance of self-tolerance. Presumably, the self determinants purge only those T cells bearing the precisely complementary receptor: too extensive a purging risks consuming the entire potential repertoire.

If there exist limited sites of responsiveness to protein antigens because of self-tolerance (clonal deletion), one would expect the specificity of responses to single determinants to be very constrained, so that, in a particular haplotype, a fortuitous crossreaction with self antigen(s) occasionally could cause an apparent “hole” in the repertoire. However, if numerous T cell clones, each with its distinctive specificity, existed for a particular epitypic site on a foreign antigen, it is unlikely that all would be congruent with some structurally distinct self antigen(s) and/or self MHC, thereby allowing clonal deletion to cause complete unresponsiveness.

In this report, the heterogeneity of antigen-reactive T cell clones of two MHC haplotypes was examined using two sets of peptides 13–16 amino acids long. Clones of at least three discrete specificities could be isolated regularly within these short, circumscribed regions. Thus, there is considerable degeneracy in the recognition of limited peptide regions of protein molecules and it is unlikely that deletion from the repertoire of clones reactive to self antigens fully accounts for the limited reactivity to protein antigens.

Materials and Methods

Mice. C67BL/6 (B6), B10.A, and B10.A(5R) mice were obtained from The Jackson Laboratory, Bar Harbor, ME and bred at our facility. B10.A(4R) mice were obtained from Dr. J. Frelinger, University of Southern California. Mice, of either sex, were used at 3–6 mo of age.

T Cell Clones. Continuously growing T cell lines were generated from B6 and B10.A mice immunized in the footpads with an emulsion of complete Freund’s adjuvant containing 7 nmol of either the large cyanogen bromide fragment of HEL (amino acid residues 13–105) or the synthetic T11 peptide (residues 74–96). The methods described by Kimoto and Fathman (12) were used throughout for generation, maintenance, and cloning of the T cell lines. The clones were obtained by limiting dilution (0.3 cell per well) in the presence of antigen, irradiated syngeneic spleen cells, and growth factors, as described earlier (13).

Specificity Assay. The specificity of clones was assessed by antigen-induced proliferation of T cells in the presence of irradiated spleen cells serving as antigen-presenting cells (APC). 1 x 10⁴ cloned T cells, purified over Ficoll-Hypaque, were cultured with 5 x 10⁵ irradiated spleen cells (3,000 rad) in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 2 mM glutamine, 1 mM pyruvate, 50 μM 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5% fetal bovine serum (lot 109617; Irvine Scientific, Santa Ana, CA) at 37°C in an atmosphere of 5% CO₂ in air. The antigens were included at the optimal concentration of 7 μM except when the concentration was varied to study the dose dependence of the response. Proliferation of T cells as recognized by DNA synthesis was assessed after the addition of 1 μCi [³H]-thymidine (Amersham Corp., Arlington Heights, IL) during the last 18 h of the 3 d culture period. Results are expressed as the mean ± standard error of triplicate cultures.

Antigens. Lysozymes from the egg whites of hen (HEL), bob white quail (BEL), ring-necked pheasant (REL), and guinea hen (NEL), and the major cyanogen bromide cleavage fragment (L2) of HEL were purified as described (2, 3). The peptides shown in Table I were synthesized by an improved solid phase technique (14–16). Manual stepwise synthesis was carried out with the carboxyl end of the peptide chain anchored through an ester
Antigen-Specific T Cell Repertoire

Table I

Amino Acid Sequences of the T11 Region (Amino Acids Residues 74–96) of Species-variant Lysozymes and Synthetic Peptides

<table>
<thead>
<tr>
<th>Lysozyme</th>
<th>Synthetic peptide</th>
<th>75</th>
<th>80</th>
<th>85</th>
<th>90</th>
<th>95</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEL</td>
<td>T11(H) (81–96)</td>
<td>N L C N I P C S A L L S S D I T A S V N C A K</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T11(H) (74–86)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REL (10)*</td>
<td>T11(R)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEL (4)</td>
<td>T11(N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEL (12)</td>
<td>T11(N')</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Numbers in parentheses show the total amino acid substitutions between the HEL and the species-variant lysozyme sequence.

Results

Recognition of the T11(H) Peptide by B6 T Clones Is Associated With Three Distinct Recognition Patterns. Our earlier study (13) of the fine specificity analysis of T cell clones induced by the large cyanogen bromide fragment (L2, residues 13–105) of HEL had shown that all the clones isolated were specific for the largest tryptic peptide fragment, T11 residues (74–96). Moreover, the clones could be subgrouped into three specificity phenotypes based on reactivities to only two species-variant lysozymes, BEL and NEL. To absolutely confirm that the clones were reactive only with T11 and not with a contaminating impurity, we prepared a synthetic peptide corresponding to the HEL sequence [T11(H), Table I] and tested it for the ability to stimulate the clones. Table II shows the proliferation response of representative clones with synthetic peptide T11(H) and four species-variant lysozymes. All clones were specifically stimulated by the synthetic T11(H) and could be subgrouped into three distinct phenotypes depending on their reactivity to BEL and NEL.

Based on the known sequences of these variant lysozymes in the T11 region (Table I), it could be reasoned that the Ser → Thr substitution at position 91 was sufficient to completely abrogate the reactivity of group III clones, but not of group I or II clones. Since NEL has three substitutions (91, 92, and 84), including the same substitution as BEL at residue 91, we expected that group III clones would also fail to recognize NEL. On the other hand, the lack of reactivity to NEL for group II, compared with group III clones, could be attributed either to a substitution at residue 84 or to the additional substitution at residue 92. All clones were found to react to REL despite the drastic Asn17 →
His substitution. Group I clones were quite capable of recognizing both BEL and NEL.

Since the differences in the reactivities of BEL and NEL could not be attributed to the APC, known to be essential for antigen recognition by T cells, the reactivity differences were clearly dependent upon the T cell clones. Moreover, since substitutions at only two sites (91 and 92 or 91 and 84) were sufficient to separate the clonal specificities into distinct phenotypes, we suggest that there is still greater diversity in the B6 T cell repertoire specific for the T11(H) peptide.

However, two complications arise from this interpretation of specificity patterns. First, the influence of substitutions affecting recognition, even among closely related antigens, does not necessarily relate to the determinant region itself. This was shown by mapping the sites responsible for the heterocliticity and the lack of reactivity of REL to regions clearly independent of the determinant region (17). Second, amino acid substitutions can have subtle effects on peptide secondary structure. That such effects of amino acids which are not directly involved in recognition can have a profound influence on T cell reactivity has been suggested (18) by the correlation of T cell reactivity with the conformational predictions of the secondary structure of the C-terminal peptide of pigeon cytochrome c.

Differences in the Reactivity of Clones Are Related to Sequence Differences Within the T11 Region. To assess the above possibilities directly, we synthesized peptides corresponding to region 74–96 of NEL [T11(N)] and a peptide [T11(N')] corresponding to the NEL sequence at residues 91, 92 but to HEL at 84 (Table I). Table III shows the recognition of these peptides by the T cell clones. As expected, group III clones did not respond to either T11(N) or T11(N'), as both peptides have the BEL substitution at residue 91. Significantly, group II clones did react to T11(N') but not to T11(N). Since these two peptides differ in only a single substitution, it was clear that residue 84 contributed to the specificity of group II clones. As expected, group I clones reacted to both peptides, although with somewhat variable reactivity to the T11(N) analog. These results confirmed...
that the differences in reactivity of the clones could be attributed to substitutions within the T11 region itself.

All Three B6 T Cell Specificity Phenotypes Are Contained Within the Peptide 81–96. Results with substituted synthetic T11 peptides, made it clear that both residues 84 and 91 could independently affect the recognition of group II and III clones. This raised the possibility that the clones were specific for distinct regions within the 23 amino acid T11 peptide. For example, group II and III clones could be specific for the N- and C-terminal regions of the T11 peptide, respectively, and thus could be differentially affected by substitutions at residues 84 or 91. To define the determinant regions with greater precision, we synthesized additional peptides of different lengths. The C termini were arbitrarily chosen to be either the same as that of T11 (residue 96) or residue 86 in the middle of the T11 region (Table I). Table IV shows that all three groups of clones recognized the 16 amino acid peptide 81–96. The next shorter peptide tested, 83–96, failed to stimulate all the clones assayed. Peptide 74–86 was also not recognized by any of the clones.

It was concluded that despite the differential effect of substitutions, all the
specificity phenotypes were contained within the same peptide, 81–96. Experiments to narrow the determinant region using synthetic peptides with varying C termini are currently in progress. The determinant region may be entirely contained within amino acid residues 81–93, as suggested by the fact that carboxymethylation of the three cysteine residues at positions 76, 80, and 94 did not affect recognition (Table I). This has been confirmed with the carboxymethylated peptide 81–96 (data not shown).

**Degeneracy in the Recognition of Peptide 74–86 by B10.A T Cell Clones.** The Ia molecule, expressed by the APC, is intimately involved in antigen recognition by T cells (19, 20). Moreover, elegant studies by Hansburg et al. (21) strongly suggest that distinct antigenic residues can independently affect interactions with the T cell receptor and Ia molecule. We wished to determine if the degeneracy evident in the recognition of T11 was a special case in that all of these clones recognized T11 in the context of the I-A<sup>B</sup> molecule expressed by B6 APC (3 and data not shown). To extend this analysis to another strain, we immunized B10.A mice with the T11 peptide and generated a continuous T cell line, designated AOIT. This line was cloned by limiting dilution and the clones assayed for specificity for the peptides. Table V shows the results for three representative clones. Significantly, these clones were specific for a region circumscribed by residues 74–86, and were unresponsive to peptide 81–96, which had accounted for all the reactivity of the B6 T cell clones.

To assess the influence of amino acid substitutions within peptide 74–86, we compared the reactivities of the variant T11 peptides containing substitutions both within this region (residues 77 and 84) and outside this region (residues 91/92). Again (Fig. 1), it was possible to clearly distinguish three distinct specificity phenotypes within the clones, based on the sensitivity to these substitutions. The very similar antigen dose responses of T11(H) and peptide 74–86 show that, despite the fact that these clones were induced by T11(H), clones could be found that recognize determinants contained entirely within the 13 amino acid peptide 74–86. The clones exhibited quite different reactivities to the variant T11 peptides. Clone AOIT.2.1 was completely unreactive to peptide T11(N) while the other two peptides, T11(R) and T11(N'), gave excellent responses. This suggested that the substitution of Leu<sup>84</sup> → Gln was important for recognition. The Asn<sup>77</sup> → His substitution in T11(R) had only a marginal effect on reactivity. That residue 77 could affect recognition of this region was seen in the response of clone AOIT.3.5. This clone did not recognize either T11(R) or T11(N). T11(N') gave superimposable responses in comparison with homologous

### Table V

**Peptide 74–86 Contains the Determinant(s) Recognized by B10.A T Cell Clones**

<table>
<thead>
<tr>
<th>Clone</th>
<th>[&lt;sup&gt;3&lt;/sup&gt;H]Thymidine incorporation (Δcpm × 10&lt;sup&gt;4&lt;/sup&gt;/culture*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td>AOIT.2.1</td>
<td>(2.2 ± 0.0)</td>
</tr>
<tr>
<td>AOIT.3.4</td>
<td>(5.8 ± 2.2)</td>
</tr>
<tr>
<td>AOIT.3.5</td>
<td>(5.6 ± 2.8)</td>
</tr>
</tbody>
</table>

*The peptides were assayed at 7 μM concentration in a proliferation assay described in footnote to Table II.
Three distinct recognition patterns are evident among B10.A T cell clones specific for determinants contained within peptide 74-86. 1 x 10⁴ Ficoll-Hypaque-passed T cells were cultured with 5 x 10⁴ 3,000-rad-irradiated syngeneic B10.A strain spleen cells in the presence of varying concentrations of synthetic peptides. The abbreviations and the amino acid sequences of the peptides are given in Table I. The incorporation of 1 μCi [³H]thymidine was assessed during the last 18 h of the 3 d culture.

antigen T11(H). Thus, reactivity of this clone could be abolished by substitutions at either residue 77 or residue 84, whereas substitutions at residues 91/92, outside the determinant region (74–86), were without effect. Analogous to group I B6 T clones, clone AOIT.3.4 did not critically distinguish any of the variant peptides. The finding of comparable dose response curves to positively recognized peptides argues against the possibility that differences in reactivity to substitutions are related to affinity differences among the clones for the determinant region. We concluded that the degeneracy of T cell recognition observed with peptide 81–96/IAb was also characteristic of B10.A strain T cells recognizing peptide 74–86/IAk.

All Three B10.A Specificity Phenotypes Are Restricted by the I-Ak Molecule. Unlike the B6 strain APC, which express only the I-Ak molecule, restricting antigen recognition by all T cells, the B10.A strain APC express both the I-Ak and I-Ek molecules. It was possible that the peptide specificities of clones differed because some T cell clones were restricted by the I-Ak and others by the I-Ek molecule. To test which of the two Ia molecules was restricting recognition, APC from MHC-congenic, I region-recombinant mice were used to stimulate a T11(H)-specific response by these clones. Table VI shows that both the syngeneic B10.A and B10.A(4R) but not B10.A(5R) APC could elicit a response from all the clones. Thus, the same I-Ak molecule restricted antigen recognition by all the degenerate specificity phenotypes. We concluded that the recognition of two different determinants within the T11 peptide was degenerate for both I-Ab-
TABLE VI

<table>
<thead>
<tr>
<th>APC strain</th>
<th>I-A</th>
<th>I-E</th>
<th>[³H]Thymidine incorporation (Δcpm × 10⁻³)/culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AOI.T 2.1 Medium</td>
</tr>
<tr>
<td>B10.A</td>
<td>k</td>
<td>k</td>
<td>0.2</td>
</tr>
<tr>
<td>B10.A(4R)</td>
<td>k</td>
<td>--</td>
<td>2.8</td>
</tr>
<tr>
<td>B10.A(5R)</td>
<td>b</td>
<td>b/k</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* The T11(H) peptide was used at 7 μM concentration in a proliferation assay using APC from the strains listed.

and I-A<sup>k</sup>-restricted T cells, indicating considerable diversity in the T cell repertoires of both B6 and B10.A haplotypes.

Discussion

That T cell clones of several distinct specificities could be readily demonstrated within both the I-A<sup>b</sup>- and I-A<sup>k</sup>-restricted recognition of small peptides suggests that great diversity exists in the T cell repertoire. Since arbitrary substitutions at only two amino acid residues yielded three distinct subsets for both the 13 and 16 amino acid determinant/la combinations, the total number of distinct clonal specificities should be considerably larger. Furthermore, since the total peptide length sufficient for T cell activation was close to the reported minimum of 7–10 amino acid residues (21–23), the clonal diversity within the response could represent that to single determinants.

T cell clones specific for limited determinant regions of other antigens, such as pigeon cytochrome c (24), insulin (25), ovalbumin (26), and the small hapten, L-tyrosine-p-azobenzene arsonate (27), have been distinguished by several criteria, including reactivity with clonotypic antibodies, differences in the size of peptides recognized, and crossreactivity to closely related antigenic analogs. Thus, the T cell diversity evident in the recognition of what may be single determinants is a general property of T cell response to limited antigenic determinants rather than a peculiar feature of the T11 peptide.

The concept of an antigenic determinant, so central to the understanding of B cell diversity, has remained somewhat obscure for T cell recognition. It is well known that, within the B cell repertoire, it is quite possible to generate multiple antibodies with distinct specificity phenotypes to protein antigens (1). Largely due to limitations inherent in the interpretation of complex cellular assays for T cell specificity analysis in heterogeneous populations, and to the difficulties involved in the precise delineation of determinant regions (3), it has not been possible to extend the principle of degeneracy of immune recognition to T cells. Our doing so was possible only because of the availability of procedures for isolating T cell clones (12) and the availability of defined synthetic peptides (16).

Antigen recognition by T cells has been intensively studied through the influence of MHC-linked immune response (Ir) genes (6). It has been clear for sometime that the influence of MHC-encoded Ia molecules is a fundamental property of T cell recognition of all antigens (28). The fact that, for some
antigens, the same Ia molecules affect the entire response status of MHC-congenic pairs of mice has been widely attributed to a defect in the recognition process itself. Evidence that Ir-regulated responses are directed to limited antigenic regions provides a rationale for the existence of these defects (29). This is the basis for the debate regarding whether these failures of recognition are due to defects in the presentation of antigens (determinant selection) or gaps in the preexisting T cell specificity repertoire (holes in the repertoire). An important assumption for the latter is that the failure to identify antigen-specific T cells in the nonresponder strain is not attributable to regulatory influences, such as suppression, on the expression of the repertoire. Since considerable difficulties are inherent in the demonstration of specific suppression, owing to the complexity of the regulatory pathways, it cannot be formally excluded that suppression underlies all Ir gene defects. However, the failure to find definitive evidence for suppression in many instances favors the view that the Ir gene question is a problem of recognition (30).

Several recent studies (19, 20) have strongly suggested that T cells recognize a complex determinant composed of parts of both the nominal antigen and the Ia molecule, through the use of a clonally distributed single receptor. Studies suggesting elements of specificity within physical antigen/Ia interactions have been reported (31, 32). In particular, analysis of T cell recognition of different cytochrome peptides suggests (18) a circumscribed mode of presentation of the \( \alpha \)-helical conformation of the determinant in the context of the Ia molecule. Thus, substitutions at Lys\(^{99} \) (within the epitope) result in the induction of nonoverlapping sets of T cell clones (18). Changes closer to the C terminus, particularly those involving Ala\(^{103} \) (within the agretope), affect only the antigen/Ia interaction (18). Based on this view of peptide/Ia interactions, it has been argued (9) that instances of nonresponsiveness could be due to either a failure of an antigen/Ia interaction, such as could occur for pigeon cytochrome c in the B10.A(5R) strain, or an elimination during development of the relevant clones specific for the self mouse cytochrome c (clonal deletion model).

Our finding that amino acid substitutions within the determinant region strongly affect recognition of some but not all clones argues against a restricted repertoire of T cell recognition. Neither of the residues that varied within the T11 peptide could be exclusively involved in any critical function, such as a single epitope or agretope region defined by Lys\(^{99} \) and Ala\(^{103} \), respectively, of the cytochrome determinant. Although it is possible that substitutions at some other residue(s) within the two determinant regions identified here may affect recognition by all clones, this would not change the conclusion that recognition of a given determinant/Ia complex can be degenerate. This degeneracy does not appear to be related to affinity for the determinant, since comparable dose response curves were obtained with each of the variant peptides recognized. Also, all of the clonal specificity phenotypes were isolated from independently derived T cell lines (13 and unpublished results), showing that the different clonotypes do not represent rare clones.

Using the terminology introduced by Schwartz (9), it is not possible at present to distinguish whether our results reflect the recognition of different epitopes, which are unique perspectives of a given peptide bearing a single agretope, or if
they represent a limited perspective of a given peptide bearing many agretopes. The evidence that residues 81 and 82 are essential to the reactivity of the B6 clones makes it a tempting hypothesis that these residues are part of a single agretope within T11. Alternatively, it is possible that deletion of these two residues causes extensive disruption of the overall conformation of peptide 81–96, causing loss of reactivity. It must be emphasized here that physical interpretations of recognition of peptides are complicated by our ignorance of the conformations possible in solution or at the cell surface where recognition is actually thought to occur. Both possibilities are consistent with results demonstrating that multiple restriction sites exist on Ia molecules. Our results clearly show that a given peptide/Ia complex does not constrain T cells to recognize a unique epitope. There is selection from within T11 of different peptide determinants for response: amino acid residues 74–86 in the context of I-Ak molecules and residues 81–96 in the context of the I-Ab molecules. The basis for this Ia-dependent selectivity in the choice of determinant region remains to be fully explored.

Regardless of the still obscure mechanisms affecting the choice of T cell determinants and the influence of amino acid substitutions on T cell recognition of peptides, the concept that the recognition of a given determinant/Ia combination is degenerate has profound implications for clonal deletion models of Ir gene defects. These models attribute the absence of T cell clones specific for a foreign antigen to their elimination during development of the T cell repertoire. Since this absence occurs uniquely in the nonresponder strains, it has been suggested that this elimination of receptor specificities reflects a fortuitous crossreaction with some self antigen and/or self MHC. We argue that the diversity in the recognition of single peptide regions makes it very unlikely that, for any two different determinants (for example, the self and foreign antigens), the entire range of possible specificities would completely overlap and cause tolerance in every potential clone recognizing this region. For example, even a conserved substitution such as Ser → Thr at position 91 completely eliminated responsiveness of B6 clone 2.6, without effect on clones 2.12 and 1.4 (Table II). It thus would be predicted that the response status of B6 mice to the Ser91-containing T11(H) peptide would not be affected by inducing neonatal tolerance to the Thr91-containing T11(B) peptide because clones (groups I and II) that remain unaffected by this substitution would not be eliminated by the toleragen. This reasoning does not, of course, imply that clonal deletion cannot occur, only that fortuitous deletion of clones is an unlikely explanation for Ir gene defects. In studies to be reported elsewhere, we have shown that profound unresponsiveness induced to minimal peptide determinants in neonatal mice is most consistent with clonal deletion mechanisms (A. Oki, G. Gammon, N. Shastri, and E. Sercarz, manuscript in preparation).

The only Ir gene models affected by the wide range of specificities induced by single determinants are those based on deletion of clones upon direct engagement of the T cell receptors at an appropriate stage of repertoire development. Mechanisms based on specific suppression due to nonoverlapping suppressor determinants acting via an antigen bridge are compatible with a diversity of clonotypes recognizing other determinants on the same molecule. Such suppres-
sion has been demonstrated for lysozyme in both the genetic nonresponder C57BL/10 (36) and specifically tolerized responder B10.A strains (37). More recently (38), a nonoverlapping suppressor determinant has been demonstrated mediating unresponsiveness to heterologous insulins. Similarly compatible are determinant selection models requiring only that the APC be capable of presenting the relevant antigen (9, 32). Absence of this ability would lead to an apparent absence of all possible specificities. The present analysis does not distinguish between these and other possibilities.

However, a possible argument in favor of clonal deletion models remains. It is conceivable that all the observed T cell fine-specificity phenotypes to single determinants are derived as somatic hypermutations of a single set of receptor genes, thereby reducing the number of originally present receptor specificities required to be eliminated in the repertoire to cause unresponsiveness. Deletion of this “parental set” during developmentally induced tolerance would appear as elimination of a subsequently derivable set of receptor specificities. For this to be true, it would be predicted that all the clones specific for a given peptide/Ia complex, irrespective of their distinct specificity phenotype, would use the same set of rearranged V/D/J genes encoding the two subunits of the receptor molecule. Genes encoding the receptor subunits of these T cell clones are currently under investigation. However, the differences observed in the use of at least the Vβ subunit of the T cell receptors of some of the clones specific for cytochrome c and lysozyme peptide 81–96 suggest that this is not very likely (39).

Summary

Using synthetic peptides as antigens, it was found that T cell clones of a given haplotype specific for 13–16 amino acid peptides could be clearly distinguished by the varied influence of amino acid substitutions on recognition. This was true for different antigenic determinants within peptides 81–96 and 74–86 of hen egg-white lysozyme, recognized in the context of the I-Ak and I-Ak molecules, respectively. Considerable complexity was demonstrated in the induced T cell repertoire specific for apparently single determinants, which implies that diversity of T cell recognition approaches that for B cells. The implications of the degeneracy of T cell recognition are discussed in the context of mechanisms through which Ia molecules restrict recognition and theories of Ir gene defects.

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