T CELL CLONES SPECIFIC FOR AN AMPHIPATHIC
α-HELICAL REGION OF SPERM WHALE MYOGLOBIN
SHOW DIFFERING FINE SPECIFICITIES FOR
SYNTHETIC PEPTIDES

A Multiview/Single Structure Interpretation of Immunodominance

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Characterization of immunodominant T cell sites has been effectively performed by a number of laboratories (reviewed in 1) using protein sequence variants, cleavage fragments, and synthetic peptides. Some studies (2) have been interpreted as supportive of the possibility of multiple conformations of peptide antigen and/or multiple antigen-binding sites on the Ia molecule. The possibility that distinct T cell specificities might reflect distinct recognition or views of a single peptide conformation associated with a single Ia site has received little attention, primarily because little could be inferred about the conformation of the antigenic peptide on the APC in most experimental systems studied.

We have previously described an immunodominant site in sperm whale myoglobin in a region encompassing glutamic acid 109, identified using myoglobin sequence variants (3), and have subsequently isolated T cell clones with the same reactivity pattern (4). In this paper we characterize two such clones using a panel of synthetic peptides. The clones showed different response patterns that are found to be totally consistent with a model of the distinct T cell specificities reflecting distinct "views" of an amphipathic α-helical conformation. Thus, when one considers the likely secondary structure of antigen existing in association with the complex structure of the Ia molecule, distinct T cell recognition specificities need not imply distinct structural forms of antigen or sites of antigen binding, but rather may reflect distinct views recognized by the T cell receptor.

Materials and Methods

Mice. B10.D2 and (B10.D2 × B10.BR)F₁ mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

T Cell Clones. T cell clone 9.27 was derived from B10.D2 mice as described (4). T cell clone 1.2 was derived independently from (B10.D2 × B10.BR)F₁ mice as described and has been referred to as $F_1(D2)1.2$ in previous studies (5). Both clones are specific for the glutamic acid 109 region of sperm whale myoglobin and are restricted to I-A^d (5).

Synthetic Peptides. Synthetic peptides of sperm whale myoglobin 102–118, 104–118, 106–118, 108–118, 109–118, 110–118 were synthesized by manual solid-phase peptide
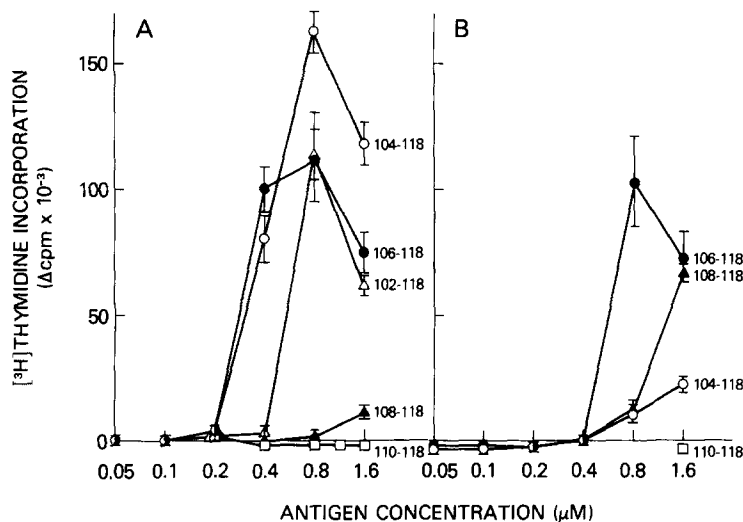
FIGURE 1. Proliferative responses of T cell clones 1.2 and 9.27 to synthetic myoglobin peptides corresponding to residues 102–118 (Lys-Tyr-Leu-Glu-Phe-Ile-Ser-Glu-Ala-Ile-Ile-His-Val-Leu-His-Ser-Arg) and portions thereof presented by H-2^d APCs. Assays were performed as described in the text. Background-subtracted geometric means are shown with SEM confidence intervals. (A) clone 1.2, (B) clone 9.27. The background thymidine incorporation without antigen was 2,298 cpm for clone 1.2 and 3,493 cpm for clone 9.27.

synthesis using a modification of the method of Corley et al. (6) and purified to homogeneity by gel filtration on BioGel P4 followed by reversed-phase HPLC. Concentration determination and composition confirmation were determined by amino acid analysis kindly performed by Robert Boykins (Food and Drug Administration).

T Cell Proliferation Assay. Assays were performed as described previously (4, 5).

Results and Discussion

Previous studies (4, 5) using sequence variants of native myoglobin had shown that the sites in native sperm whale myoglobin seen by these clones included glutamic acid 109 and possibly histidine 116. Available sequence variants do not enable higher resolution analysis by this approach. Thus, we synthesized the peptides in the nested series from 102–118 to 110–118. Fig. 1, A and B show peptide dose-response curves for clones 1.2 and 9.27. Both clones respond well to 102–118 and 106–118 (Fig. 1 and Table I). However, clone 1.2 responds much better to 104–118 than to 108–118, whereas the reverse is true for clone 9.27. The rank order of potency for clone 9.27 is not a simple function of peptide length, as 104–118 is less potent than the shorter peptides 106–118 and 108–118 even though it contains all of the sequence present in these latter peptides. The decrease in activity from peptide 106–118 to peptide 104–118 is then reversed when the peptide is further lengthened to 102–118. This result further indicates that activity is not simply a function of peptide length. Though not shown in this experiment, the potency of peptide 102–118 for stimulating clone 9.27 was consistently greater than or equal to that of peptide 106–118. Neither clone responded to 110–118. In subsequent experiments 109–118 was found to be inactive for both clones (Table I).

TABLE I

Proliferative Response of Clones 9.27 and 1.2 to Peptides
109–118 and 102–118

Clone	Peptide	Geometric mean [³H]thymidine incorporation (cpm) at an antigen concentration of:		
		0 μM	1.0 μM	3.2 μM
9.27	109–118		296 (1.15)*	257 (1.97)
	102–118		151,060 (1.03)	119,378 (1.09)
	No antigen	260 (1.27)		
1.2	109–118		570 (1.66)	238 (1.35)
	102–118		169,457 (1.03)	173,410 (1.03)
	No antigen	402 (1.52)		

Assays were performed as described in the text.
* Geometric means are shown with the SEM factor in parentheses.

Thus, the identification of the region around residue 109 as the immunodominant site was confirmed using synthetic peptides, and residues on both sides of 109 in the sequence appear to contribute to antigenicity. While these data show a consensus segment from 106–118 for stimulation of the clones, they also reveal a differential response pattern to longer and shorter peptides.

This segment folds into a highly amphipathic α helix in native myoglobin with the hydrophobic residues on one face and the hydrophilic on the opposite face (Fig. 2A). Refolding of this peptide into this conformation in the hydrophobic/ hydrophilic interface at the surface of the presenting cell should be energetically favored. The data we present here on the 102–118 region, along with our previously reported data (7a) on the 132–146 site of sperm whale myoglobin, led us to hypothesize (1) that the amphipathic helix may be a general feature common to many immunodominant T cell sites. Indeed, a sequence consistent with formation of an amphipathic helix is seen in the majority of T cell antigenic sites described to date. Thus, we suggest that such structures may frequently represent an integral part of the stimulation complex for the T cell receptor.

If in fact an α-helical antigen conformation is presented to and recognized by the T cell receptor, simple end effects and folding patterns that differentially affect one or the other T cell clone recognition regions would appear likely (Fig. 2B). For instance, clone 1.2, which is more sensitive to end effects at 108, that is to say, does not respond to peptide 108–118, may include this residue in its epitope (see Fig. 2B). In contrast, the N-terminal limit of the epitope recognized by clone 9.27 may be Glu 109 itself, so that adding on just one more residue at position 108, to mask the α-amino group of 109, is sufficient to stimulate the clone. Thus, these data are consistent with a model of multiple T cell specificities arising from multiple views of a single antigen conformation at a single Ia-binding site and do not require postulation of multiple conformations or binding sites.

Multiple distinct functional sites on each Ia molecule have been proposed (2, 8–13) to explain the results of antibody blocking and Ia mutant studies, as well as to account for findings of distinguishable T cell specificities for a given antigenic site. Among these, elegant studies by Allen et al. (2, 13) have focused on the hen egg lysozyme (HEL) system using molecular variations in antigen and Ia to probe the specificity of a panel of T cell hybridomas. Two T cell hybridomas specific for HEL 46-61 in association with I-A^k, but differing in fine specificity,
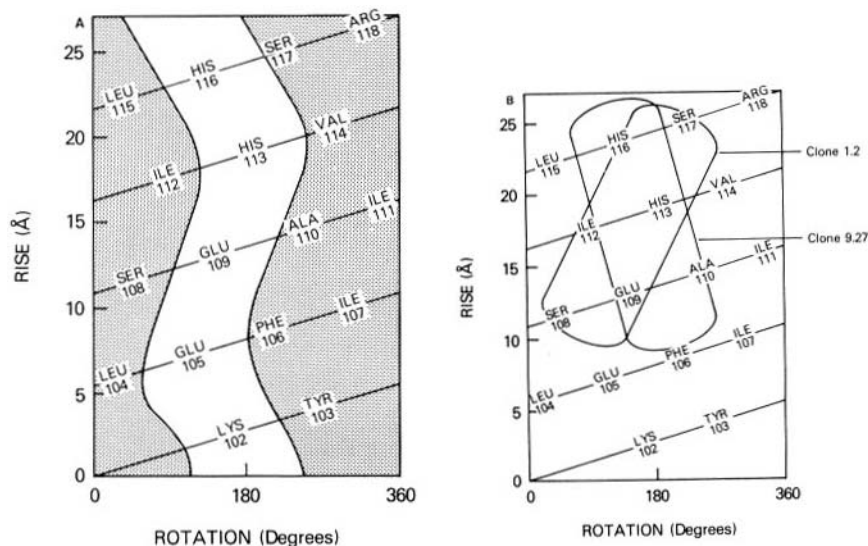
FIGURE 2.   Secondary structure presentation for the 102–108 region of sperm whale myo-globin. In these α-helical net displays the cylinder of the helix is "cut" longitudinally, opened, and folded flat (7). (A) The amphipathic character of this segment. Hydrophobic residues are shaded and tend to fall on one face of the helix while the hydrophilic residues are positioned on the opposite face. (B) Hypothetical T cell receptor recognition envelopes for clones 1.2 and 9.27 (see text).

were found (13, 14) to be differentially sensitive to two mutations in A^k. As in the case of the bm12 mutant (9, 10), these results could be interpreted equally well in terms of two disjoint sites on Ia or a single Ia site recognized differently by two T cells that are differentially sensitive to these substitutions. The recent finding that these mutations are only three residues apart (14; Allen, P., and D. McKean, personal communication) supports the latter interpretation. Given that Ia, a member of the Ig gene superfamily, shares homology and domain structure with Fab, it is not unreasonable to suppose that the several hypervariable regions may cluster to form a single combining site for antigen. Indeed, the fact that a single mutation at position 67 resulted in loss of stimulation of T cell hybridomas specific for at least three distinct peptides of HEL representing over half of the cases characterized (13) further supports a single site. In the other cases, antigen could still be in the same conformation at the same site on Ia but viewed by the T cell slightly differently in association with different adjacent Ia residues. The intriguing difference in rank order of potency of different antigenic peptides for two hybridomas (2) can be explained by the presence of a turn at residues 55–56, as in native HEL, such that additional residues introduce stimulatory information for one clone but only steric hinderance for the other. Other studies (15–17) have emphasized the importance of distinct T cell specificities for a common antigenic site but have not suggested structural interpretations. These cases are, however, also consistent with a multiview model of T cell recognition of antigen in a single conformation at a single site. Though all of the data, including our own, are also consistent with models proposing multiple antigen conformations and multiple Ia antigen–binding sites, we would argue that the

existing data do not necessitate postulating these more complex models. Thus, distinct T cell recognition specificities need not imply distinct structural forms of antigen or sites of antigen binding but rather may reflect distinct views recognized by the T cell receptor.

The finding of differing T cell clone fine specificities within an immunodominant site additionally suggests that immunodominance represents the focusing of a polyclonal response on a limited region of the antigen and is not a simple monoclonal T cell expansion. Other findings are also consistent with this interpretation (2, 15–17; Livingstone, A., J. Rothbard, and C. G. Fathman, personal communication).

Summary

The T cell response to sperm whale myoglobin in the H-2^d haplotype has been shown to be largely focused on a limited region around glutamic acid 109 recognized in association with I-A^d. T cell clones 9.27 and 1.2 have been previously (4, 5) shown to reflect this specificity and MHC restriction. In this study we have used a panel of synthetic peptides from the region 102–118 of myoglobin to characterize the specificities of these representative clones. The segment from 106–118 was found to represent a consensus region for recognition by both clones. However, we saw significant differences between clones in the hierarchy of responsiveness to peptides within the panel. In as much as the peptide and the I-A^d molecule remain constant, these differences derive from differences in how each T cell receptor interacts with the antigen. This peptide segment is an amphipathic α helix in native myoglobin, meaning that one side is hydrophobic and the other hydrophilic. It is one of the prototype cases that led us to find that amphipathic helices constitute the majority of immunodominant sites recognized by helper T cells (1). It is likely that the peptide will refold into an amphipathic helix stabilized by the interface at the surface of the presenting cell. When such secondary conformation is considered, these data are consistent with a model of multiple T cell specificities arising from multiple views of a single antigen conformation at a single Ia-binding site and do not require postulation of multiple conformations or binding sites.

Additionally, the finding of distinct specificities suggests that the immunodominance of this site depends not on the dominance of a single clone, but on the focusing of a polyclonal response on a single region of the molecule in association with I-A^d. The immunodominance of this particular region of the protein may thus depend on intrinsic features of the site, such as potential to form an amphipathic helix, as well as extrinsic factors such as binding properties of the I-A molecule.

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