A possible immunodominant epitope recognized by murine T lymphocytes immune to different myoglobins

(T lymphocyte receptors/antigenic determinant/antigen-stimulated proliferation)

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ABSTRACT   We find that a single region on the surface of different species of myoglobin appears to be immunodominant for T lymphocytes, even though the residues in that region vary sufficiently that the T cells immune to one myoglobin do not crossreact with other myoglobins bearing substitutions at that site. Immunization of B10.S mice with sperm whale myoglobin elicits T-lymphocyte populations capable of recognizing sperm whale myoglobin but not horse myoglobin, whereas the converse is true when these mice are immunized with horse myoglobin. Using a series of myoglobin variants, we tested the effect of changes in primary sequence on the T-lymphocyte proliferative response. We were able to divide the myoglobin variants into two groups, depending on whether they cross stimulate sperm whale immune or horse immune T lymphocytes. The patterns of cross stimulation of both populations of myoglobin immune T lymphocytes were explained by amino acid substitutions at position 109. However, because sperm whale and horse myoglobin differ at this residue (glutamate vs. aspartate, respectively), T lymphocytes immune to each myoglobin do not crossreact with the other myoglobin. Additional data suggest that this immunodominant epitope also includes other residues nearby on the surface of the native molecule. Mixing experiments showed that the specificity was that of T lymphocytes and not antigen-presenting cells. Monoclonal anti-I-A blocking studies showed that both myoglobins are presented in association with the same Ia antigen. Possible explanations for the apparent immunodominance of this antigenic epitope, consisting of residue 109 and nearby residues on the surface of both myoglobins, include a peculiar immunogenicity of the surface topography of this site or a preferred orientation of the molecule imposed by antigen-presenting cells when T cells first encounter the antigen. The latter explanation is related to but distinct from "determinant selection." T-cell recognition of conformation is discussed.

After immunization with small protein antigens, T lymphocytes will respond to rechallenge with the specific antigen by proliferating in culture. Epitopes responsible for stimulation of T lymphocytes have been identified for insulin (1), cytochrome c (2, 3), lysozyme (4), fibrinopeptide B (5), and angiotensin II (6). Previous studies from this laboratory have shown that the response to sperm whale myoglobin is under the dual control of two H-2-linked immune response genes (7). After immunization with native myoglobin, T-lymphocyte proliferative responses to two CNBr cleavage fragments of myoglobin have been mapped to two different I subregions, and the ability of each fragment to stimulate T lymphocytes depends on the immune response genes of the antigen-presenting cell (8, 9).

In the present study, we analyzed the immune response to sperm whale and horse myoglobins in the B10.S mouse strain. This strain is a high responder to both myoglobins, and the antibodies made in response to one myoglobin crossreact exten-

sively with the other myoglobin (10). However, the populations of T lymphocytes immune to one myoglobin are distinct and do not crossreact with the other myoglobin. By measuring T-cell proliferation in response to a series of species variants of myoglobin, we made the surprising observation that a single residue in both myoglobins—namely, residue 109—is immunodominant for T lymphocytes. Each T-cell population is specific for a different amino acid substituted at this position. In addition, presentation of both myoglobins involves the same Ia antigen on the antigen-presenting cell. The failure to cross stimulate is not due to presentation of each myoglobin in association with a different Ia antigen. Possible reasons for immunodominance of the same epitopic site in both noncrossreactive myoglobins are discussed.

MATERIALS AND METHODS

Animals. Strain B10.S/Sx mice were bred from breeding pairs generously provided by David Sachs (National Cancer Institute). Strains C57BL/10 (B10) and B10.D2/nSn were obtained from The Jackson Laboratory. Mice were used at 8–22 weeks of age and were of either sex.

Myoglobins. The various myoglobins were purified by the method of Hapner et al. (11). The sequences and individual preparations of the myoglobins have been described (cited in ref. 12). Pig and rabbit myoglobins were a gift of Morris Reichlin (Oklahoma Medical Research Foundation). All myoglobins were rechromatographed and found to be free of denatured material by optical spectroscopy before use.

Antibodies. The antisera and monoclonal antibodies used have been described (13). The anti-Ia^s alloantiserum was A.TL anti-A.TH (titer by cytotoxicity, 1:320). The anti-I-A^s monoclonal antibody was obtained from in vitro cultures of the MK-S4 hybridoma line generously provided by John Kappler and Philippa Marrack (14) (titer by cytotoxicity, 1:256). The anti-I-A^b monoclonal antibody 5224 was a culture supernatant generously provided by Keiko Ozato (15).

Immunization, Preparation of T Lymphocytes, and Cell Cultures. These procedures have been described in detail (16, 17). The only difference was that splenic adherent cells, rather than Kupffer cells, were used as the antigen-presenting cells. Cross-stimulating myoglobins consistently gave at least half the stimulation of the immunizing myoglobin or at least 10 times that of the medium control; nonstimulatory myoglobins generally gave less than one-quarter as great proliferation and often were not significantly above background radioactivity.

Preparation and Pretreatment of Splenic Adherent Cells. Splenic glass-adherent cells were prepared as described (18). In some experiments these cells were incubated overnight at 37°C with medium alone, 1:10 dilutions of control or monoclonal culture supernatants, or 1:80 dilutions of normal mouse serum or anti-Ia^s alloantiserum. They then were washed and

irradiated [1,200 rad (12 grays) from a [137] Cs source] before they were added to cultures with T lymphocytes.

RESULTS

T lymphocytes from B10.S mice immune to sperm whale myoglobin failed to proliferate in response to horse myoglobin and vice versa, even though this strain is a high responder to both myoglobins (Fig. 1). Immunization with each myoglobin elicited a population of T lymphocytes which were specific for the immunogen and did not respond to the other myoglobin. Because the splenic adherent cells used for antigen presentation were the same in both cases, we conclude that the macrophage population is capable of presenting either myoglobin to appropriately immunized T lymphocytes. Therefore, the observed specificity derives from the T-lymphocyte population expanded during immunization. (However, it is possible that selection of this population depends on differential handling of the two myoglobins by antigen-presenting cells.) We have used this defect in cross stimulation as a probe of the fine specificity of the T-lymphocyte receptor for myoglobin.

Using various B10 congeneic strains, we found that the proliferative responses to both sperm whale and horse myoglobins are controlled by *H-2*-linked immune response genes. The B10 (*H-2^b*) strain responded to neither myoglobin, whereas the B10.S (*H-2^s*) and B10.D2 (*H-2^d*) strains responded to both myoglobins. However, neither myoglobin was crossreactive in B10.S mice, and T lymphocytes from B10.D2 mice immune to sperm whale myoglobin did not crossreact with horse myoglobin *in vitro*. Because these two myoglobins share 87% of their sequences, failure to elicit crossreactivity between them suggests that T lymphocytes recognize a limited number of antigenic determinants, all of which are altered by the few amino acid substitutions by which these myoglobins differ.

In order to determine which residues were responsible for T-lymphocyte recognition, we immunized B10.S mice to horse
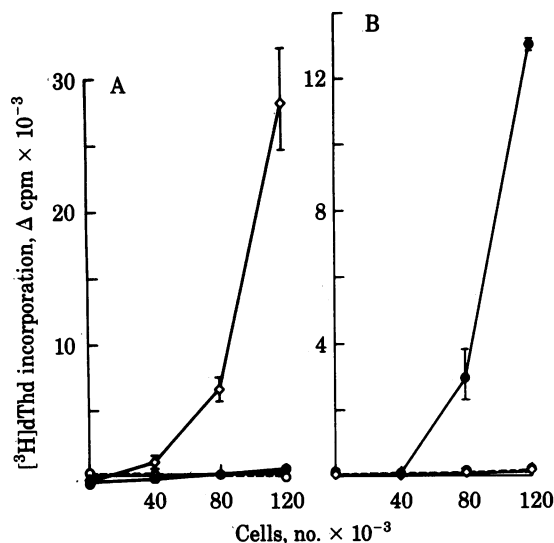

FIG. 1. Proliferative response of B10.S T lymphocytes immune to sperm whale (*A*) or horse myoglobin (*B*). T lymphocytes from four sperm whale immune (*A*) or four horse immune (*B*) animals were purified by passage through two serial nylon wool columns and cultured at 2×10^5 cells per well in the presence of graded numbers of B10.S splenic adherent cells and either medium alone (○) or each myoglobin at 2.5 μM (◇, sperm whale; ●, horse). In both cases, proliferation depended totally on added splenic adherent cells. Control cultures without myoglobin or adherent cells gave 504 cpm for sperm whale immune T lymphocytes and 46 cpm for horse immune T lymphocytes. Error bars are SEM of triplicate cultures.

myoglobin and tested T-lymphocyte proliferation in response to various myoglobins (Table 1). If changes anywhere in the primary sequence could alter T-lymphocyte recognition, then we would expect crossreactivity to decrease as a function of evolutionary distance and the number of substitutions throughout the molecule. However, the responses to three whale myoglobins (sei, minke, and goosebeaked) were consistently as high as those to horse and beef myoglobins, suggesting that a small number of shared amino acid residues were essential for T-lymphocyte activation whereas most substitutions had no effect on the dominant epitope.

The 20 amino acid substitutions (among 153 residues) between horse and sperm whale myoglobin are shown in Table 1. The primary sequences of the other stimulating myoglobins tested are shown for these residues only. Most amino acid substitutions did not affect T-lymphocyte responses, and several conserved residues were found in nonstimulatory and stimulatory myoglobins alike. However, only one residue, aspartate-109, was conserved in all stimulatory myoglobins tested but in none of the nonstimulatory myoglobins. All of the nonstimulatory myoglobins contained glutamate at position 109. We conclude from this pattern of crossreactivity that aspartate-109 is a critical residue of the antigenic determinant recognized by T lymphocytes immune to horse myoglobin. Although not noted in the table, dog myoglobin, which stimulates horse immune T lymphocytes, differs from horse myoglobin at residues 113 and 116 (histidine to glutamine in both cases). Sei whale, minke whale, and goosebeaked whale myoglobins, which differ from horse myoglobin at residue 118, also stimulated these cells. Therefore, it is likely that residues 113, 116, and 118, although near residue 109, are not part of the antigenic site recognized by horse immune T lymphocytes.

The antigenic determinant of sperm whale myoglobin was mapped in a similar fashion. B10.S mice were immunized to sperm whale myoglobin and T-lymphocyte proliferation was tested with various myoglobins (Table 2). Sequence differences between the immunogen and horse myoglobin are shown in Table 2, as well as substitutions in the other myoglobins at these residues. Evolutionary distance does not seem to matter because rabbit myoglobin with 15 substitutions (among 20 residues shown) stimulated these T lymphocytes but minke whale myoglobin with 8 substitutions and goosebeaked whale myoglobin with 10 did not. Instead, changes at a single residue—glutamate-109—seemed to control the response. Substitutions at residue 109 are always associated with loss of crossreactivity whereas all the stimulatory myoglobins retained glutamate at 109.

Pig myoglobin was the only myoglobin that had glutamate at 109 but failed to stimulate sperm whale immune T lymphocytes. Sperm whale and pig myoglobins differ at 23 residues. Comparison of the primary sequences at these residues of all the myoglobins that have glutamate at 109 reveals three residues—lysine-87, histidine-113, and isoleucine-142—that may be responsible for the loss of T-lymphocyte stimulation. Analysis of the three-dimensional structure of myoglobin (19) shows that only the replacement of histidine by glutamine at 113 is close enough to glutamate 109 to be part of the same antigenic site (7.2 Å from glutamate carboxyl oxygen to histidine ring nitrogen). In contrast, the linear distances between glutamate-109 carboxyl oxygen and lysine-87 amino nitrogen or isoleucine-142 terminal carbon are 28.6 and 14.5 Å, respectively. Moreover, lysine-87 is on a different side of the molecule from glutamate-109, and isoleucine-142 is not exposed on the surface of native myoglobin. We conclude that glutamate-109 and probably histidine-113 are a critical pair of amino acids forming the antigenic determinant recognized by T lymphocytes from B10.S

Table 1. Proliferation of B10.S T cells immune to horse myoglobin

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Stimulating                          Residue number                                        Δ cpm
myoglobin                                                                Exp. I         Exp. II        Exp. III

                                      1 1 1 1 1 1
                          1 1 2 2 2 3 4 6 6 7 0 1 2 3 4 5
                      1 4 9 2 5 1 7 8 4 5 5 6 7 4 9 8 2 2 0 1            I              II             III

 1. EQUINE            G D Q N G I E V T G K T V G[D]K N T N F            11,431 (1.15)  11,584 (1.07)  12,075 (1.17)
 2. BOVINE                A     V           N T  -          S            —              11,305 (1.07)  —
 3. SEI WHALE         V   L     A V D I K     N T  -R D N K              —              20,927 (1.21)  —
 4. MINKE WHALE       V   L     A V D I K     N T  -R E N K              10,222 (1.10)  13,604 (1.25)  7,089 (1.15)
 5. GOOSEBEAKED WHALE   E L H A L   I K       H T  -R D   K              11,827 (1.41)  10,373 (1.15)  9,488 (1.09)
 6. DOG                  I     L     K N      N T  -    D K              —              6,863 (1.09)   13,931 (1.07)
 7. CALIFORNIA SEA LION  L     L     K   K T[E]    D K                   3,816 (1.04)   683 (1.24)     2,489 (1.11)
 8. HARBOR SEAL          L     L     K S      N T[E]  E K                —              3,132 (1.33)   —
 9. KILLER WHALE         L     L D I K        N T A[E]R E N K            5,324 (1.08)   2,568 (1.18)   —
10. BOTTLENOSED DOLPHIN  L     L D   K        N T A[E]R E N K            2,433 (1.08)   2,184 (1.27)   —
11. DALL PORPOISE      E L     L D   K        N T  [E]R E N K            2,617 (1.06)   1,759 (1.11)   −2,535 (1.05)
12. SPERM WHALE       V E L H A V D I K S R V T A[E]R D N K Y            2,916 (1.16)   3,500 (1.12)   1,923 (1.15)
                                                    MEDIUM CONTROL      4,862          1,164          11,074
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In experiments I and III, 4 × 10^5 unpurified lymph node cells from three B10.S mice immunized with 100 μg of horse myoglobin were incubated with various concentrations of each myoglobin. In experiment II, lymph node cells from similarly immunized mice were purified by passage through a 0.15-g nylon wool column, and 4 × 10^5 cells were incubated with various concentrations of each myoglobin. All experiments were performed as dose–response curves over 2 orders of magnitude of antigen concentration. The results obtained at the optimal concentration, 2 μM in experiments I and II or 5 μM in experiment III, are shown. The complete table of sequence differences between horse and sperm whale myoglobin is given, as well as the amino acid differences from the horse sequence, at these residues only, for the other myoglobins tested. The single letter code for amino acids is used. Space or dash indicates same residue as in equine sequence. Results are expressed as geometric mean cpm of triplicate cultures; geometric SEM is shown in parentheses.

mice immune to sperm whale myoglobin.

The slightly lower response stimulated by harbor seal and California sea lion myoglobins, which differ from sperm whale, killer whale, and rabbit myoglobin by substitution of a basic lysine for a neutral asparagine at residue 132, suggests that this residue, which is only 4.3 Å from glutamate-109 on the surface of the molecule, may be involved in the same antigenic determinant recognized by T lymphocytes. The response to Dall porpoise myoglobin is also somewhat lower than that to sperm whale myoglobin, and this myoglobin, like the seal and sea lion proteins, differs from the highest stimulators at residue 74. If residue 74 is antigenic, it is presumably not part of the same determinant as residue 109 because it is on the opposite side of the molecule. It is possible that an additional population of T lymphocytes recognizing residue 74 and its environs increases the response to sperm whale, killer whale, and rabbit myoglobins, but the data do not allow us to be sure. In any case, because we expect that a heterogeneous population of immune T lymphocytes may respond to other minor determinants, these differences do not prevent us from identifying a major determinant around residue 109.

Previous studies have shown that proliferating T lymphocytes recognize protein antigens in association with Ia antigens of the antigen-presenting cell (1, 2, 9, 20). We tested whether immune T lymphocytes recognize horse and sperm whale myoglobin in association with the same or different Ia antigens. Strain B10.S splenic adherent cells were preincubated in the presence of control or anti-Ia^s alloantiserum or anti-I-A^s monoclonal antibody culture supernatants. They were then used to present sperm whale myoglobin to sperm whale immune T lymphocytes

Table 2. Proliferation of B10.S T cells immune to sperm whale myoglobin

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Stimulating                          Residue number                                            Δ cpm
myoglobin                                                        Exp. I        Exp. II       Exp. III      Exp. IV

                                      1 1 1 1 1 1
                          1 1 2 2 2 3 4 6 6 7 0 1 2 3 4 5
                      1 4 9 2 5 1 7 8 4 5 5 6 7 4 9 8 2 2 0 1    I             II            III           IV

 1. SPERM WHALE       V E L H A V D I K S R V T A[E]R D N K Y    14,047 (1.11) 18,593 (1.34) 5,871 (1.13)  2,348 (1.13)
 2. KILLER WHALE      G D   N G L         G K N   - E     F      14,183 (1.11) 16,745 (1.15) 5,571 (1.27)  —
 3. RABBIT            G D   N G L E V H T K N     -K   S N F      —             14,351 (1.29) —             —
 4. DALL PORPOISE     G     N G L   V     G K N G - E     F      —             7,654 (1.04)  2,885 (1.78)  —
 5. HARBOR SEAL       G D   N G L E V       K N G -K E K N F      —             9,592 (1.32)  2,609 (1.05)  2,945 (1.15)
 6. CALIFORNIA SEA LION G D N G L E V     G K K G -K   K N F      8,943 (1.09)  6,869 (1.24)  878 (1.51)    2,404 (1.32)
 7. SEI WHALE           D   N             G K N G D       F      —             1,205 (1.60)  —             —
 8. MINKE WHALE         D   N             G K N G D E     F      —             3,694 (1.16)  259 (1.15)    −339 (1.25)
 9. GOOSEBEAKED WHALE G         L E       G K H G D   T   F      —             —             492 (1.25)    −371 (1.08)
10. FINBACK WHALE       D   N             G K N G D       F      —             —             259 (1.24)    —
11. PIG               G D   N G   E V     G K N G -K   S N F      —             −448 (1.24)   —             —
12. DOG               G D I N G L E V     N K N G D K   K N F     —             —             204 (1.96)    −439 (1.08)
13. EQUINE            G D Q N G I E V T G K T V G[D]K N T N F     3,332 (1.06)  −72 (1.82)    93 (1.49)     −191 (1.07)
                                                MEDIUM CONTROL   1168          611           120           502
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In experiments I, II, and IV, lymph node cells from four B10.S mice primed with 100 μg of sperm whale myoglobin were passed through a 0.2-g nylon wool column and stimulated at 4 × 10^5 cells per well with various concentrations of sperm whale or other myoglobins. All experiments were performed as dose–response curves over 2 orders of magnitude of antigen concentration, and the results obtained at the optimal concentration, 2 μM, are shown. In experiment III, purified T lymphocytes from five B10.S mice were passed serially through 0.6- and 0.3-g nylon wool columns and 2 × 10^5 cells were mixed with 7.5 × 10^4 B10.S splenic adherent cells and stimulated in vitro with each myoglobin. The complete table of sequence differences between sperm whale and equine myoglobin is shown, as well as the amino acid differences from the sperm whale sequence, at these residues only, for the other myoglobins tested. Space or dash indicates same residue as in sperm whale sequence. Results are as in Table 1.

or to present horse myoglobin to horse specific T lymphocytes. The response to both myoglobins was blocked by anti-Ia^s alloantiserum (Fig. 2). Each response was blocked to a similar extent by anti-I-A^s monoclonal antibody. In a repeat experiment, this inhibition was shown to be specific for I-A^s because the response of B10.D2 T lymphocytes to sperm whale myoglobin was not inhibited by pretreatment of B10.D2 macrophages with the anti-I-A^s monoclonal reagent. In addition, anti-I-A^b monoclonal culture supernatants did not block the B10.S response to horse or sperm whale myoglobin. Thus, both sperm whale and horse immune T lymphocytes respond to the appropriate myoglobin in association with the same Ia antigen molecule on the antigen-presenting cell, although we cannot exclude the possibility that different determinants on this Ia molecule are used.

DISCUSSION

We have found that sperm whale and horse myoglobin do not crossreact at the T-lymphocyte level, although they do crossreact extensively at the antibody level both for antisera and monoclonal antibodies (10, 12, 21). This failure to crossreact occurs in strain B10.S despite the fact that *H-2*^{s} is a high-responder haplotype for both myoglobins. We have examined the cellular and biochemical bases for the apparent inability of either myoglobin to stimulate a population of T lymphocytes immune to the other myoglobin. B10.S antigen-presenting cells are capable of presenting either myoglobin, and both myoglobins are presented in association with the same I-A^s antigen. However, the population of T lymphocytes immune to sperm whale or horse myoglobin recognizes the immunizing myoglo-
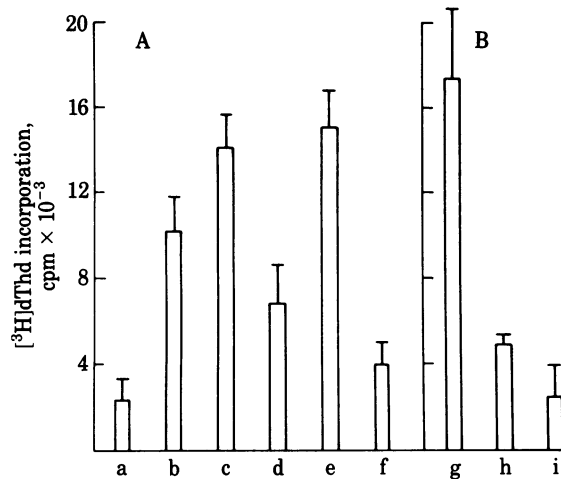
bin specifically and does not crossreact with the other myoglobin. Therefore, this specificity is carried by T lymphocytes.

In order to map the antigenic determinants recognized by myoglobin-specific T lymphocytes, we immunized with sperm whale or horse myoglobin and measured T-lymphocyte proliferation in response to a series of myoglobins. We found that some myoglobins that were far apart in evolutionary distance cross stimulate in spite of substitutions at numerous amino acid residues, whereas more closely related myoglobins fail to stimulate. This suggests that a limited number of amino acids are immunodominant; changes at these positions interfere with antigen recognition and changes at most other residues have no effect. In analyzing sequence differences between stimulatory and nonstimulatory myoglobins, we assume that substitutions that correspond to contact residues within the antigenic site would greatly alter antigenicity, whereas substitutions at residues far from the antigenic site would have little effect on overall structure or antigenicity. This analysis led to the following conclusions. Immunization with sperm whale or horse myoglobin elicits different populations of T lymphocytes with distinct and nonoverlapping specificity. Both populations of T lymphocytes recognize the same antigenic site centering on residue 109 (and presumably involving other residues as well); but sperm whale immune T lymphocytes are specific for glutamate-109 whereas horse immune T lymphocytes are specific for aspartate-109. The lack of cross stimulation is due to the striking ability of both sets of T lymphocytes (sperm whale and horse immune) to distinguish between the similar amino acid residues aspartate and glutamate at this position.

We suggest two possible explanations for the immunodominance of this residue in the T-lymphocyte response to both myoglobins. First, the surface topography of this site around residue 109 may be highly immunogenic for T lymphocytes. Second, previous studies suggest a possible role of macrophages as well, because the specificity of myoglobin immune F_1 T lymphocytes for different fragments of myoglobin, when cultured with macrophages from either parent, is determined by the H-2 phenotype of the antigen-presenting cell (9). Therefore, one explanation for the immunodominance of the epitope centered on residue 109 is that macrophages preferentially present this epitope on either myoglobin, regardless of which amino acid is present. The T-lymphocyte population then gives a determinant-specific response depending on whether the challenge antigen matches the immunizing antigen at this site. We should emphasize that this mechanism is different from "determinant selection" as originally defined for insulin (1), which involved selection of different epitopes by different macrophages. In our hypothesis, the same antigen-presenting cells present the same epitopic region of both myoglobins, but the T-lymphocyte receptor carries the specificity for whichever amino acid is at this position. (A different, and we believe less likely, explanation of these data is that macrophage processing may be controlled by residue 109, resulting in the presentation of completely different determinants or fragments of sperm whale or horse myoglobin.)

Recent studies with antimyoglobin hybridoma antibodies prepared from A.SW animals have mapped the antigenic determinants of myoglobin for several clones of B lymphocytes in an *H-2*^{s} strain (12). Analysis of the native three-dimensional structure of myoglobin assigns three B-lymphocyte sites to what are arbitrarily called the front and left surfaces of the molecule. Residue 109 of the predominant T-lymphocyte determinant is located on the back surface (Fig. 3). Adjacent antigenic sites could permit simultaneous binding of both cell types to myoglobin without steric hindrance. This could be the structural basis of antigen bridging between helper T lymphocytes and B lym-
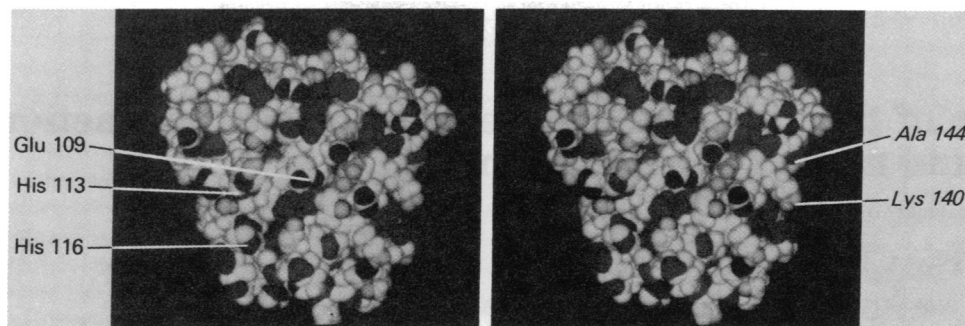

FIG. 2. Proliferative response of B10.S T lymphocytes immune to sperm whale (*A*) or horse (*B*) myoglobin after overnight preincubation of antigen-presenting cells with anti-Ia^s alloantiserum or monoclonal anti-I-A^s antibody from culture supernatants in the absence of complement. As controls, we used normal mouse serum (NMS) or control culture supernatant from the parent myeloma cell line, NS-1. T lymphocytes from five sperm whale immune or four horse immune B10.S mice were purified by serial passage through two nylon wool columns (0.6 g and 0.3 g) and cultured at 2×10^5 cells per well in the presence of 8×10^4 B10.S splenic adherent cells with 2.5 μM myoglobin. Control cultures without myoglobin or splenic adherent cells gave 371 cpm for sperm whale immune and 33 cpm for horse immune T lymphocytes. T lymphocytes with antigen in the absence of splenic adherent cells gave less than background values. *P* values by a two-tailed Student's test: for sperm whale myoglobin, NS-1 vs. anti-I-A^s, *P* < 0.05, and NMS vs. anti-Ia^s, *P* < 0.005; for equine myoglobin, NS-1 vs. anti-I-A^s, *P* < 0.005. Bars: a, medium control; b–f, sperm whale myoglobin; g–i, horse myoglobin. The presenting cells were precultured with NS-1 supernatant (c and g), anti-I-A^s supernatant (d and h), normal mouse serum (e), or anti-Ia^s serum (f and i).

FIG. 3. Stereo views of a computer-generated space-filling model of the back surface of native sperm whale myoglobin (22), based on the co-ordinates of Takano (19). Residue 109 (and probably residue 113), indicated at the left, is part of the immunodominant epitope for T lymphocytes described in this paper. Residues 140 and 144, indicated at the right, are recognized by different monoclonal antimyoglobin antibodies (12). Other residues recognized by three monoclonal antibodies are farther around to the right in this view and out of sight. The carboxyl oxygens are shaded darkest, followed by the heme and aromatic carbons, aliphatic side-chain carbons, noncarboxylic oxygens, primary amino groups, and finally other nitrogens. The backbone and side chains of nonaliphatic residues, except for functional groups, are shown in white.

phocytes. Another well-described example of T and B lympho-cytes binding to distinct epitopes of an antigen is provided by lysozyme, in which helper T lymphocytes respond to the L II fragment whereas most antibodies bind to the N-C fragment (23).

We have observed that B10.S T lymphocytes immune to different myoglobins recognize a determinant involving residue 109, and nearby residues such as residue 113. In other studies we have observed that B10.D2 T lymphocytes immune to sperm whale myoglobin also recognize a determinant centering on res-idue 109 (and possibly including residue 116). Thus, we can define three predominant T-lymphocyte determinants, each centering on the immunodominant residue 109 and involving nearby residues on the surface of the myoglobin molecule. We term this region of overlapping T-lymphocyte determinants an "immunodominant epitope." We do not know how critical the native tertiary conformation is to this T-lymphocyte response, although all the residues we have described appear on the sur-face of the native structure (Fig. 3). In fact, residues 109, 113, and 116 are brought together on the surface of myoglobin by successive turns of the α helix. Therefore, at least the secondary structure (α helix) may be necessary to maintain this relation-ship. If residue 132 is also involved, then this epitope would be conformation dependent, and the T lymphocytes would not be recognizing a linear sequence. We have looked for further evi-dence of topographic determinants consisting of residues that are nearby in the native three-dimensional structure but far apart in the primary sequence, but so far we have found these topographic determinants only for antibodies (12). However, we believe that there may also exist some T cells that are specific for topographic determinants.

1.   Rosenthal, A. S. (1978) *Immunol. Rev.* **40**, 136–152.
2.   Solinger, A. M., Ultee, M. E., Margoliash, E. & Schwartz, R. H. (1979) *J. Exp. Med.* **150**, 830–848.
3.   Corradin, G. & Chiller, J. M. (1979) *J. Exp. Med.* **149**, 436–447.
4.   Maizels, R. M., Clarke, J. A., Harvey, M. A., Miller, A. & Ser-carz, E. E. (1980) *Eur. J. Immunol.* **10**, 509–515.
5.   Thomas, D. W., Hsieh, K. H., Schauster, J. L., Mudd, M. S. & Wilner, G. D. (1980) *J. Exp. Med.* **152**, 620–632.
6.   Thomas, D. W., Hsieh, K. H., Schauster, J. L. & Wilner, G. D. (1981) *J. Exp. Med.* **153**, 583–594.
7.   Berzofsky, J. A. (1978) *J. Immunol.* **120**, 360–369.
8.   Berzofsky, J. A., Richman, L. K. & Killion, D. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4046–4050.
9.   Richman, L. K., Strober, W. & Berzofsky, J. A. (1980) *J. Immunol.* **124**, 619–625.
10. Berzofsky, J. A., Buckenmeyer, G. K. & Hicks, G. (1982) *J. Immunol.* **128**, 737–741.
11. Hapner, K. D., Bradshaw, R. A., Hartzell, C. R. & Gurd, F. R. N. (1968) *J. Biol. Chem.* **243**, 683–689.
12. Berzofsky, J. A., Buckenmeyer, G. K., Hicks, G., Gurd, F. R. N., Feldmann, R. J. & Minna, J. (1982) *J. Biol. Chem.* **257**, 3189–3198.
13. Berzofsky, J. A. & Richman, L. K. (1981) *J. Immunol.* **126**, 1898–1904.
14. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. (1981) *J. Exp. Med.* **153**, 1198–1214.
15. Ozato, K. & Sachs, D. H. (1981) *J. Immunol.* **126**, 317–321.
16. Corradin, G., Etlinger, H. M. & Chiller, J. M. (1977) *J. Immunol.* **119**, 1048–1053.
17. Richman, L. K., Klingenstein, R. J., Richman, J. A., Strober, W. & Berzofsky, J. A. (1979) *J. Immunol.* **123**, 2602–2609.
18. Kohno, Y. & Berzofsky, J. A. (1982) *J. Immunol.* **128**, 2458–2464.
19. Takano, T. (1977) *J. Mol. Biol.* **110**, 537–568.
20. Berzofsky, J. A. (1980) in *Biological Regulation and Develop-ment*, ed. Goldberger, R. F. (Plenum, New York), Vol. 2, pp. 467–594.
21. Berzofsky, J. A., Hicks, G., Fedorko, J. & Minna, J. (1980) *J. Biol. Chem.* **255**, 11188–11191.
22. Feldmann, R. J., Bing, D. H., Furie, B. C. & Furie, B. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5409–5412.
23. Adorini, L., Harvey, M. A., Miller, A. & Sercarz, E. E. (1979) *J. Exp. Med.* **150**, 293–306.