# THE I-$A^b$ MUTANT B6.C-H-$2^{bm12}$ ALLOWS DEFINITION OF MULTIPLE T CELL EPITOPES ON I-A MOLECULES*

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The immune system of an organism is required to be capable of responding to a wide spectrum of antigens. T helper cells recognize these antigens in association with Ia molecules. Given that the number of different Ia molecules encoded in the genome is limited, the possible restriction specificities of the T cells would appear to be similarly limited. However, if T cells could use more than one restriction site per molecule, the immune response capability of the organism would be enlarged.

Through the use of monoclonal antibodies and monoclonal T cell lines, it has been possible to establish that an individual cloned T cell line, be it alloreactive or antigen-reactive, has specificity for one Ia molecule (1). Although it has not yet been possible to isolate and characterize the T cell receptor for antigen, studies on cloned T cell lines can provide indirect information about the receptor by analyzing the nature and variety of determinants with which the receptor interacts. These studies might also reveal information about the number of functionally important epitopes on the Ia molecules. Recently, there have been reports describing the definition of several serological epitopes on I-$A^k$ molecules (2, 3). If T cells also can recognize more than one epitope on an Ia molecule, then one would expect to be able to find individual T cell clones specific for each epitope.

Among the I-$A^b$-restricted alloreactive clones in our collection, we have identified one that is capable of recognizing cells of the strain B6.C-H-$2^{bm12}$, the I-$A^b$ mutant, whereas the other I-$A^b$ restricted clones cannot. Additionally, we have found that two clones that are specific for the $A^b_\alpha A^k_\beta$ hybrid molecule also can recognize bm12 cells, although they do not respond at all to the parent strain B6 cells. One of these two clones is an alloreactive A anti-(B6 × A)$F_1$ clone, whereas the other is a L-glutamic acid$^{60}$-L-alanine$^{30}$-L-tyrosine$^{10}$ (GAT)-reactive clone of (B6 × A)$F_1$ origin. The identification of these two distinct reactivity patterns suggests that there exist both on I-$A^b$ molecules and on hybrid $A^b_\alpha A^k_\beta$ molecules at least two distinct epitopes recognized by T cells. Additional data obtained by studying monoclonal anti-I-A antibody inhibition of stimulation of T cell clones demonstrate a lack of identity between serologically defined epitopes on I-A molecules and epitopes on I-A molecules recognized by cloned T cell lines.

## Materials and Methods

*Mice.* C57BL/6 (B6), A/J (A), B6.C-H-$2^{bm12}$ (bm12), (B6 × A)$F_1$, [B6 × B10.A(4R)]$F_1$, [bm12 × B10.A(4R)]$F_1$, BALB/c, and (B6 × BALB/c)$F_1$ mice were purchased from The

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Jackson Laboratory, Bar Harbor, ME, or bred from the stock in our animal facilities. Adult mice, aged 6–36 wk, were used in all experiments.

*Antigen and Culture Medium.* The random terpolymer L-glutamic acid$^{60}$-L-alanine$^{30}$-L-tyrosine$^{10}$ (GAT) (lot 9) was purchased from Miles-Yeda Ltd., Rehovot, Israel. Complete culture medium has been previously described (1).

*T Cell Clones.* The procedures and techniques for cloning murine alloreactive T cells (4) and antigen-reactive T cells (5) have been previously described.

*Monoclonal Antibodies.* The monoclonal antibodies used for blocking proliferation in these studies, 10-2.16 (6), H116.32 (7), and BP107 (8) have been previously described. Briefly, monoclonal antibody 10-2.16 reacts with specificity Ia.17 on the $A^k_\beta$ chain (1, 9). Monoclonal antibody H116.32 reacts with specificity Ia.19 on the $A^k_\alpha$ chain (1). Monoclonal antibody BP107 reacts with cells expressing I-$A^{b,d,p,q,u,j}$ (8).

*Proliferation Assay.* The techniques used to assay the proliferative response have been described previously (5). Briefly, $1 \times 10^4$ alloreactive T cells are cultured with $1 \times 10^6$ irradiated stimulator spleen cells in 0.2 ml of culture medium in flat-bottomed microtiter plates. $1 \times 10^4$ antigen-reactive T cells are similarly stimulated with 200 μg/ml GAT (unless otherwise indicated) in the presence of $1 \times 10^6$ irradiated syngeneic spleen cells. Cultures were pulsed with 1 μCi of [$^3$H]thymidine at 24 h and harvested 16 h later. The results of the thymidine incorporation assay are expressed as the mean counts per minute of triplicate cultures. The standard deviations from replicate cultures were always within 10% of the mean value.

## Results

*Two Epitopes on I-$A^b$ Molecules Recognized by T Cell Clones.* Among the alloreactive T cell clones that we have obtained from long-term A/J anti-(B6 × A)$F_1$ mixed lymphocyte cultures, we have identified one clone specific for I-$A^b$, clone 11.16, which is capable of recognizing stimulator cells from the I-A mutant strain B6.C-H-$2^{bm12}$ as well as it does stimulator cells from strain B6 (Table I). That this clone recognizes an H-$2^b$ linked determinant is indicated by its stimulation by A.BY cells and by its failure to be stimulated by strain B10.A cells (data not shown). The observation that its stimulation can be blocked by monoclonal antibody 17/227, an Ia.15 reagent (7), maps the reactivity to the I-A locus (data not shown). Clone 11.4 and all other B6-reactive clones of A/J origin tested to date do not recognize bm12 stimulator cells. The bm12 mutation has been mapped to I-A both genetically (10, 11) and serologically

TABLE I

*Recognition of bm12 Stimulator Cells by an Alloreactive T Cell Clone*

| Stimulator cell | Haplotype | | | | Clone | |
|---|---|---|---|---|---|---|
| | | | | | 11.16 | 11.4 |
| | | | | | *cpm* | |
| | K | A | E | D | | |
| A | k | k | k | d | 365 | 68 |
| B6 | b | b | b | b | 12,996 | 10,908 |
| (B6 × A)$F_1$ | b/k | b/k | b/k | b/d | 15,564 | 7,435 |
| [B6 × B10.A(4R)]$F_1$ | b/k | b/k | b/b | b/b | 13,478 | 10,461 |
| bm12 | b | b* | b | b | 11,581 | 194 |
| [bm12 × B10.A(4R)]$F_1$ | b/k | b*/k | b/b | b/b | 10,580 | 462 |

Proliferation of $1 \times 10^4$ cells from alloreactive T cell clones measured as counts per minute of incorporated thymidine following a 72-h culture with a terminal 18-h pulse with 1 μCi of tritiated thymidine. Stimulator cells are $1 \times 10^6$ spleen cells from the strain indicated irradiated with 3,300 rad before co-culture. The haplotype of the stimulator cells have been included for ease of interpretation; bm12 has a mutation in the I-A subregion indicated by asterisks.

(12) and has been shown to result in an altered $A^b_\beta$ polypeptide (13). We interpret these data to mean that clone 11.16 recognizes an epitope on the I-$A^b$ molecule that is conserved on the I-$A^{bm12}$ molecule, whereas clone 11.4 recognizes an epitope which has been lost or altered on the I-$A^{bm12}$ molecule.

*One Epitope of the I-$A^{bm12}$ Molecule Resembles $A^b_\alpha A^k_\beta$, a Second Resembles I-$A^b$.* We recently reported (1) experiments that demonstrate that among our antigen-reactive and alloreactive T cell clones that are specific for hybrid I-$A^b$/I-$A^k$ molecules, we can discriminate those recognizing the $A^k_\alpha A^b_\beta$ molecule from those recognizing the $A^b_\alpha A^k_\beta$ molecule. This discrimination was based on blocking studies using monoclonal anti-I-$A^k$ antibodies and studies using [bm12 × B10.A(4R)]$F_1$ mice as compared with [B6 × B10.A(4R)]$F_1$ mice as sources of stimulator or antigen-presenting cells (APC). Thus, we identified two alloreactive clones, 11.17 and 11.19, which had specificity for the hybrid molecule $A^b_\alpha A^k_\beta$. To our surprise, one of the clones, 11.17, recognized bm12 cells as well as it did (B6 × A)$F_1$ cells, although it responded not at all to B6 cells (Table II). These data suggest that the altered I-$A^b$ molecule ($A^b_\alpha A^{bm12}_\beta$) expressed by the bm12 mouse now in some way resembles the $A^b_\alpha A^k_\beta$ hybrid molecule.

We have made a similar observation with $F_1$-restricted GAT-reactive clones of (B6 × A)$F_1$ origin. Both clone 12.5.a.1 and clone 12.5.a.31 use the $A^b_\alpha A^k_\beta$ hybrid molecule as the restriction element for antigen recognition, based on the pattern of blocking by

TABLE II

*Recognition of bm12 Stimulator Cells by an Alloreactive T Cell Clone*

| Stimulator cell | Haplotype | | | | Clone | |
|---|---|---|---|---|---|---|
| | | | | | 11.17 | 11.19 |
| | | | | | *cpm* | |
| | K | A | E | D | | |
| A | k | k | k | d | 249 | 48 |
| B6 | b | b | b | b | 200 | 60 |
| (B6 × A)$F_1$ | b/k | b/k | b/k | b/d | 10,832 | 4,326 |
| [B6 × B10.A(4R)]$F_1$ | b/k | b/k | b/b | b/b | 13,071 | 7,914 |
| bm12 | b | b* | b | b | 10,017 | 69 |
| [bm12 × B10.A(4R)]$F_1$ | b/k | b*/k | b/b | b/b | 12,079 | 8,357 |

See legend to Table I.

TABLE III

*Antibody Blocking of T Cell Proliferation*

| GAT-reactive clone: | 12.5.a.1 | | 12.5.a.31 | 14.14 |
|---|---|---|---|---|
| APC: | (B6A)$F_1$ | bm12 | (B6A)$F_1$ | B6 |
| Media control | 1,164 | 846 | 138 | 790 |
| + 200 μg GAT | 22,564 | 7,494 | 5,445 | 20,977 |
| Antibody | | | | |
| 10-2.16 ($A^k_\beta$) | 6,793 | 6,646 | 2,056 | 21,396 |
| H116.32 ($A^b_\alpha$) | 23,730 | 7,211 | 5,181 | 18,977 |
| BP 107 ($A^b_\beta$) | 21,513 | 1,482 | 5,410 | 11,764 |

Inhibition of T cell proliferation (see legend to Table I) by addition of 1 μg of purified antibody for the duration of the culture. The T cell clones are co-cultured with 1 × $10^6$ irradiated spleen cells as a source of APC from strains indicated under each T cell clone.

monoclonal antibodies, as illustrated by the data in Table III. Stimulation of both clones 12.5.a.1 and 12.5.a.31 was blocked by antibody 10-2.16 ($A^k_\beta$), but not blocked by antibody H116.32 ($A^k_\alpha$). In addition, neither clone was blocked by antibody BP107 (I-$A^b$) when GAT was presented on (B6 × A)$F_1$ APC. (We have provisionally assigned the reactivity of BP107 to the $A^b_\beta$ chain based on its pattern of blocking of these and other T cell clones [data not shown].) Unexpectedly, one of these clones, 12.5.a.1, responded to antigen when presented by bm12 spleen cells, although neither clone responded to antigen presented by B6 spleen cells. We then tested whether recognition of GAT as presented by bm12 APC to clone 12.5.a.1 could be blocked by antibodies in the same way as could presentation by (B6 × A)$F_1$ APC. As is illustrated in Table III, antibody 10-2.16 was unable to block stimulation of clone 12.5.a.1 in the presence of GAT and bm12 APC, but antibody BP107 was quite effective under these conditions. These blocking reactions are exactly the converse of the pattern obtained with (B6 × A)$F_1$ APC. These results suggest that the epitope recognized by this T cell clone and the epitopes recognized by these antibodies are not concordant. Our previous results have indicated that monoclonal antibody blocking studies can identify the particular Ia molecule that a T cell clone is recognizing. The current results suggest, however, that blocking with monoclonal antibodies cannot be used to identify specific epitopes on a given Ia molecule.

*Different Antigen Presenting Capabilities of (B6 × A)$F_1$ and bm12 APC.* While analyzing the unexpected response of GAT-reactive clone 12.5.a.1 to GAT when presented by bm12 spleen cells, we noticed that the level of response to the usual concentration of GAT when presented by bm12 APC was less than optimal. Thus, we examined the proliferative response of this clone to varying antigen concentration in the presence of several different sources of APC. As shown by the data presented in Fig. 1 A, clone 12.5.a.1 responded optimally to GAT at concentrations of 100 μg/ml and above when presented by (B6 × A)$F_1$ or by [bm12 × B10.A(4R)]$F_1$ cells, whereas the optimal response to GAT presented by bm12 cells was not achieved until the antigen concentration was increased to 1 mg/ml. The data presented in Fig. 1 B show that

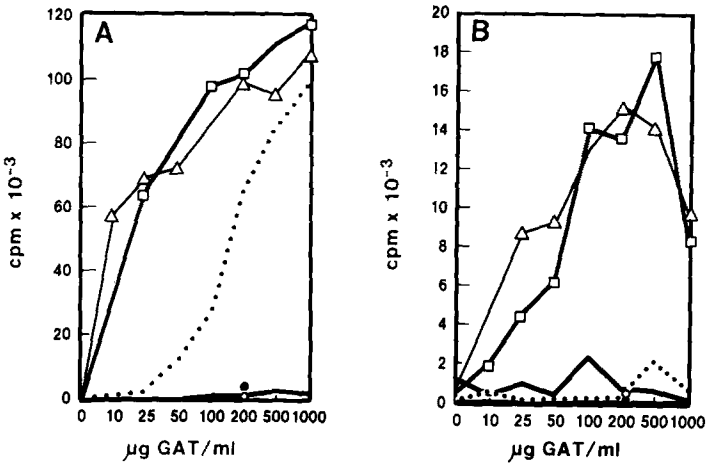


FIG. 1. Strain of antigen presenting cells versus antigen concentration. Proliferation of $10^4$ cloned T cell (as described in Legend to Table I) was measured with increasing concentration of GAT and $10^6$ irradiated spleen cells from (B6 × A)$F_1$ (□); [bm12 × B10.A(4R)]$F_1$ (△); bm12 (···); (BALB/c × B6)$F_1$ (——); BALB/c (●); or B6 (○). Panel A: clone 12.5.a.1; panel B: clone 12.5.a.31.

clone 12.5.a.31 achieved the optimal response to GAT again at 100–200 μg/ml when presented by (B6 × A)$F_1$ or by (bm12 × B10.A(4R))$F_1$ cells, but did not respond to GAT presented by bm12 cells at any concentration. This observed difference in the ability of bm12 spleen cells to present GAT as compared with (B6 × A)$F_1$ spleen cells may be a function of the lower cell surface density of I-A molecules on bm12 cells (see Discussion). We also tested the ability of spleen cells from BALB/c and (B6 × BALB/c)$F_1$ animals to present antigen to GAT-reactive clones 12.5.a.1 and 12.5.a.31. This was done because of the remote possibility that the bm12 mutation resulted from gene conversion in the original (B6 × BALB/c)$F_1$ mutant animal (see Discussion). According to this hypothesis, the apparent "k-ness" of the $A_\beta^{bm12}$ chain might be the result of the exchange of a segment of $A_\beta^d$ for $A_\beta^b$, and the hybrid molecule, $A_\alpha^b A_\beta^d$, expressed on (B6 × BALB/c)$F_1$ cells, might also appear "k-like". However, neither clone 12.5.a.1 nor clone 12.5.a.31 responded to antigen in the presence of BALB/c or (B6 × BALB/c)$F_1$ APC (Fig. 1 A and B).

## Discussion

*The Origin of the bm12 Mutation.* The experiments we have described in this report have used the B6.C-H-$2^{bm12}$ mouse, and therefore the interpretation of these experiments depends upon what is known about the bm12 mutation. Both serologically and genetically, the mutation has been mapped to the I-A region of the *b* haplotype (10–12) and the biochemical data suggest that the locus of the mutation is in fact the $A_\beta^b$ gene (13, 14). There are no data conflicting with this conclusion. The mutation arose spontaneously and was initially detected by skin grafting in a (C57BL/6 × BALB/c)$F_1$ mouse in a screening program for measuring the mutation rates of histocompatibility loci (15). The original mutant mouse rejected B6 skin, but accepted both BALB/c and B6.C-H-$2^d$ grafts and transmitted this mutant phenotype to half its progeny. The mouse itself had parents that were phenotypically normal by skin grafting. The most probable interpretation is that this mouse resulted from the union of a normal gamete from the BALB/c parent with a gamete carrying a new mutation from the B6 parent. The mutant mouse was then backcrossed to the B6 strain to initiate the formation of the congenic line B6.C-H-$2^{bm12}$, which should differ from the parent B6 strain only at the mutant site in I-$A^b$.

Our observation that two clones whose restriction specificity was known to be the $A_\alpha^b A_\beta^k$ hybrid molecule were also able to recognize $A^{bm12}$ ($A_\alpha^b A_\beta^{bm12}$) molecules might lead one to suggest that the $A_\beta^{bm12}$ chain had acquired k-ness. One could imagine that this could have resulted from a gene conversion event, during which a small segment of the $A_\beta^b$ gene was replaced with $A_\beta^d$ information. However, the expression of the mutant phenotype in the original $F_1$ animal and its subsequent transmission of this phenotype argues strongly against this hypothesis. Nonetheless, because the experiment could be easily done, we did test whether either of the $F_1$-specific clones that could recognize bm12 cells would recognize (B6 × BALB/c)$F_1$ spleen cells, asking whether $A^b/A^d$ hybrid molecules might resemble $A_\alpha^b A_\beta^k$ molecules in the way $A^{bm12}$ molecules apparently do for these clones. The data in Fig. 1 show that neither $F_1$-restricted GAT clone responded to antigen presented by (B6 × BALB/c)$F_1$ spleen cells to any greater extent than they did to either B6 or BALB/c APC, nor did the alloreactive clone 11.17 respond to stimulation by (B6 × BALB/c)$F_1$ spleen cells (data not shown).

*There Must Exist at Least Two T Cell Epitopes on I-A Molecules.* The observation that two cloned T cell lines with specificity for the I-$A^b$ molecule show differential reactivity to the I-$A^{bm12}$ molecule suggests that these two cell lines possess different receptors for the I-$A^b$ alloantigen. The data indicate that the determinant on I-$A^b$ with which the clone 11.4 receptor interacts is not expressed on the I-$A^{bm12}$ molecule, whereas the determinant recognized by clone 11.16 has been retained. In parallel, the serological data indicate that some determinants are retained and others missing on the I-$A^{bm12}$ molecule (16, 17). It seems reasonable to conclude that these two T cell clones are recognizing distinct epitopes on the I-$A^b$ molecule. An alternative hypothesis is that there are two distinct I-$A^b$ molecules, one of which is altered in the bm12 mutant. In this case, each of these two clones would be specific for one of the two molecules. To date there are no data that specifically indicate the existence of multiple I-$A^b$ molecules. And, in fact, the peptide mapping experiments demonstrating that the $A_\beta$ polypeptide is altered in the bm12 mouse suggest that there is only one species of $A_\beta$ polypeptide expressed (13, 14). Furthermore, recent experiments analyzing the structure and organization of the $A_\beta$ gene(s) at the DNA level suggest very strongly that there is only one $A_\beta$ gene per haploid genome.[1]

We interpret the experiments on the I-$A^b$/I-$A^k$-reactive clones in a similar manner. We had assigned the specificity of clones 11.17 and 11.19 to the $A^b_\alpha A^k_\beta$ molecule based on the pattern of inhibition of stimulation with monoclonal antibodies and on recognition of [bm12 × B10.A(4R)]$F_1$ spleen cells (1). The observation that clone 11.17 recognizes bm12, whereas 11.19 does not, again suggests that two clones with apparent specificity for the same molecule, in this case $A^b_\alpha A^k_\beta$, possess different receptors for this molecule, implying that they recognize distinct epitopes on this molecule. Exactly the same argument applies to the GAT-reactive clones 12.5.a.1 and 12.5.a.31.

*The Effect of Antigen Dose on the Presentation of GAT by bm12 Spleen Cells.* In serological and biochemical studies of the expression of Ia molecules by bm12 spleen cells, it has been observed that these cells express fewer Ia molecules per cell than do the parent B6 cells (16). We routinely observed that GAT-reactive clone 12.5.a.1 responded less well to presentation of antigen by bm12 cells than it did to presentation of antigen by (B6 × A)$F_1$ or [bm12 × B10.A(4R)]$F_1$ cells. This may be due to a less than optimal density of Ia molecules on the bm12 spleen cells. If, as Cohen and Eisen have suggested (18), the complex of antigen and restricting element, be it Ia or H-2K or D, is a strictly concentration-dependent phenomenon and not a stereo-specific interaction, then one might expect to override a deficiency in the concentration of one component by increasing the concentration of the other component. In fact, we found that increasing the concentration of GAT to 1 mg/ml from the standard assay concentration of 200 μg/ml did boost the response of clone 12.5.a.1 cells to GAT presented by bm12 cells to the level of response achieved in the presence of (B6 × A)$F_1$ cells. This result is consistent with the concentration dependence hypothesis, but in no way proves it. Similarly, other investigators have reported (19–21) that T cell proliferation to antigen is dependent on the quantity of Ia on the surface of antigen presenting cells. The observation that the alloreactive clones that recognize bm12 are stimulated to the same extent by bm12 as by the nonmutant spleen cells suggests that for alloreactivity, the surface density of I-A on bm12 cells must be sufficient. In the

[1] Robinson, R. R., R. N. Germain, D. J. McKean, M. Mescher, and J. G. Seidman. Extensive polymorphism surrounding the single murine Ia $A_\beta$ chain gene. Manuscript submitted for publication.

alloreactive response there is no requirement for complex formation between Ia molecules and any other antigen. There are also other possible explanations for the decreased efficiency of antigen presentation by bm12 spleen cells. The T cell receptor of clone 12.5.a.1 may have a lower affinity for the $A^b_\alpha A^{bm12}_\beta$-GAT complex as compared with the $A^b_\alpha A^k_\beta$-GAT complex. Alternatively, the bm12 spleen cells may not "process" GAT as efficiently as the nonmutant spleen cells. Given the origin of the bm12 strain, however, it seems unlikely that there would be a difference in antigen-presenting ability between the bm12 and parent cells, unless I-A molecules themselves are directly involved in the processing steps. Finally, one could propose that GAT does not complex as efficiently with $A^b_\alpha A^{bm12}_\beta$ molecules as it does with $A^b_\alpha A^k_\beta$ molecules on the surface of the APC. Such hypothetical complexes have not been amenable to isolation, so this hypothesis is not currently testable.

*The Inhibition by Certain anti I-A Antibodies of T Cell Recognition of Antigen Is a Steric Effect and Is Not Mediated through Direct Competition for the Same Epitope.* The data presented in Table III clearly show that the epitopes recognized by certain monoclonal antibodies on Ia molecules are not the same epitopes or restriction sites used by these antigen-reactive T cell clones. The ability of monoclonal antibodies to inhibit the recognition of GAT by clone 12.5.a.1, when presented by APC of (B6 × A)$F_1$ mice, is clearly different from the inhibition seen when clone 12.5.a.1 is presented GAT by APC of strain bm12. Thus, the antibody BP107, which has no inhibitory effect on GAT presentation to clone 12.5.a.1 by (B6 × A)$F_1$ APC, totally inhibits antigen presentation to clone 12.5.a.1 by bm12 APC. Conversely, whereas antibody 10-2.16 inhibits the presentation of GAT by (B6 × A)$F_1$ APC to clone 12.5.a.1, this antibody has no inhibitory effects on antigen presentation by bm12 cells. These results indicate two important findings. The first is that the antibody BP107, which may recognize an epitope on the beta chain of $I\text{-}A^b$ molecules, retains its reactivity for bm12, suggesting that this epitope has not been changed by the mutational event. However, the mutational event has changed the ability of bm12 cells to present antigen to clone 12.5.a.1. Thus, it is quite clear that the mutation that has altered the antigen presentation/restriction site has not altered the epitope recognized by this antibody; yet this antibody is capable of inhibiting the presentation of GAT to clone 12.5.a.1 by bm12 cells. Second, that antibody 10-2.16 blocks the ability of (B6 × A)$F_1$ APC to present GAT to clone 12.5.a.1, while not affecting the antigen presentation capabilities of bm12 cells, suggests that the antigen-presenting site on bm12 cannot be identical to this serologically defined epitope. However, this antibody blocks GAT presentation by (B6 × A)$_1$ cells, suggesting again that the restriction site for antigen presentation is not identical to the site recognized as an epitope by the monoclonal anti I-A antibody.

These data provide strong evidence for the existence of multiple functional restriction sites on a given molecular Ia complex. Although this functional study does not reveal the physical nature of a restriction site, it suggests that the number of potential functional Ia restriction sites is much greater than the number of Ia molecules encoded in the genome. Thus, the immune response of an animal to any particular antigen can potentially be enhanced through the use of multiple restriction sites on a given Ia molecule, as well as through the use of novel Ia molecules generated by combinatorial association of $\alpha$ and $\beta$ chains in heterozygous individuals.

## Summary

The experiments presented in this study demonstrate that there exist at least two functional epitopes on an I-A molecule that can be recognized by T cell clones. By comparing the abilities of spleen cells from C57BL/6 mice and the congenic I-A mutant line B6.C-H-$2^{bm12}$ to stimulate alloreactive T cell clones specific for the I-$A^b$ molecule, we have discriminated two sets of clones, those recognizing the I-$A^b$ and I-$A^{bm12}$ molecule equally well and those able to recognize only the I-$A^b$ molecule. These results imply that the two sets of clones have different receptors for I-A and that they therefore recognize separate epitopes on the I-A molecule. We have similarly been able to separate T cell clones, both alloreactive and L-glutamic $acid^{60}$-L-$alanine^{30}$-L-$tyrosine^{10}$-reactive, specific for the $A^b_\alpha A^k_\beta$ hybrid molecule into two groups based on their ability to recognize bm12 spleen cells. Although the recognition of bm12 spleen cells by these clones was unexpected since none of them responds to B6 spleen cells, these data again allow us to conclude that these groups of clones have different receptors for the same I-A molecule and therefore that they recognize distinct epitopes on the molecule. Additional studies, in which monoclonal anti-I-A antibodies were used to block the stimulation of T cells by stimulator or antigen-presenting cells, have demonstrated that this blockade can be a steric effect and therefore is not necessarily indicative of direct competition between the antibody and the T cell for the same site on an I-A molecule.

Although this study does not reveal the physical nature of an I region-controlled "antigen-restriction site," we can suggest that increasing the number of possible functional Ia restriction sites either through combinatorial association of alpha and beta chains or by using more than one site per molecule will increase the number of configurations the ternary complex of Ia, antigen and T cell receptor(s) can form.

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