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The murine *bm12* gene conversion provides evidence that T cells recognize predominantly Ia conformation

(immune response genes/allogeneic recognition/major histocompatibility complex)

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ABSTRACT **The structure of the highly polymorphic Ia dimer is the genetically determined factor that controls the immune response to foreign antigens, albeit the mechanism remains unresolved. However, it is clear that, in diverse immune responses, effector T lymphocytes require recognition of self-Ia and foreign antigenic determinants on the surface of an antigen-presenting cell or an antibody-secreting B cell. Furthermore, a single Ia molecule has been found to possess several independently acting functional domains. In this report T-cell recognition of Ia was limited to a single, defined structure by using the Ia mutant mouse strain B6.C-*H-2*^{bm12} (bm12). The Ia determinant being recognized is the site of the mutation that represents a difference in three of five amino acid residues in a hypervariable region of its β chain. This mutation has been proposed to have resulted from a gene conversion-like event and is known to have functional importance. Recognition of the *bm12* mutation site was studied here in *in vitro* cultures of T cells generated against Ia^{bm12} antigens. The specificity of these alloreactive T cells was tested by using stimulator cells expressing various Ia alloantigens of known structure. Our findings provide direct genetic evidence that T cells recognize predominantly conformational determinants on Ia molecules and not their primary structure. The implications of these findings on our understanding of the genetic control of the immune response and the potential to modulate these responses in an antigen-specific way are discussed.**

The class II major histocompatibility antigens, the Ia (I region-associated) antigens, are highly polymorphic cell-surface glycoproteins expressed on B lymphocytes and a subpopulation of macrophages as heterodimers consisting of an α and β polypeptide chain (cf. ref. 1). Obligate recognition by T lymphocytes of surface Ia on B cells and macrophages regulates (restricts) the interaction of these three cell types in immune responses. For example, in immune responses to foreign protein antigens, activation of helper T cells requires the concomitant recognition of Ia and nominal antigen on the surface of the antigen-presenting cell (e.g., macrophage) (2). And activation of B cells to secrete specific antibody requires help from T cells recognizing B-cell surface Ia (3, 4). Therefore, the interaction of T cells, B cells, and antigen-presenting cells is said to be Ia-restricted. Allogeneic responses to Ia provide a model system to study recognition of Ia, since allogeneic Ia is thought to mimic self-Ia plus antigen (5). Furthermore, recent studies indicate that both alloreactive and antigen-specific T cells use a common pool of receptors (6).

The *I-A* mutant mouse strain B6.C-*H-2*^{bm12} (bm12) has been used to probe several structure–function correlates of immune recognition of Ia molecules. The structural difference between B6 and bm12 Ia is confined to three amino acid differences in a segment of five amino acids (7–9). And the site of the mutation is located in a hypervariable region of the outer extracellular domain of the I subregion A_β polypeptide chain. It has been proposed that the *bm12* mutation resulted from a gene conversion event, whereby a minimum of 14 nucleotides of the A^b_β gene were replaced by homologous information from the E^b_β gene (8, 9). Functional comparisons of B6 and bm12 mice have defined differences in both allogeneic and antigen-specific T-cell responses. For example, B6 and bm12 mice reject each other's skin grafts (10) and generate cytotoxic (11) and proliferative (10) *in vitro* T-cell responses. Differences in the immune responses of B6 and bm12 mice have been reported with the antigens H-Y (12), beef insulin (13), and the random polypeptides poly(Glu,Ala) and poly(Glu,Tyr) (14). However, there are other *I-A*^{b}-restricted immune responses [e.g., multichain poly(amino acid) poly(Tyr,Glu)-poly(Ala)--poly(Lys)—called (T,G)-AL—and collagen] in which B6 and bm12 mice respond comparably (12). Therefore, studies using bm12 mice provided direct genetic evidence that Ia molecules are themselves the antigen-specific mediators of the immune response, the so-called *Ir* gene products, and that each Ia molecule has multiple functional domains (12, 15), only one of which was altered in the bm12 mutant. More recent studies with bm12 mice and cells have defined the mutation site as a discrete functional domain seen by allogeneic (8) and antigen-specific (16, 17) T lymphocytes. In studies by Hochman and Huber (16) and Mengle-Gaw *et al.* (8), this functional domain was found to be shared between A^{bm12}_β and E^b_β molecules, thus correlating with the putative gene conversion event. These authors suggested that the conversion event resulted in the transfer of a "minigene" that maintains its functional integrity and that other hypervariable regions of Ia may also function as independent domains. Such an interpretation would have significant implications on genetic evolution and medicine. For example, once the precise function of a so-called "minigene" was determined, this knowledge could be used to genetically diagnose or therapeutically manipulate the immune system in an exquisitely specific manner.

To address these questions regarding T-cell recognition of Ia, we analyzed the B6 anti-bm12 proliferative response using mixed lymphocyte reaction assays and numerous T-cell clones. In contrast to the aforementioned studies, our data suggest that the *bm12* mutation is seen as a unique conformational determinant not shared with the E^b_β molecule or Ia molecules of other standard haplotypes. Therefore, our results suggest that the putative *bm12* gene conversion event resulted in the creation of a unique functional domain on Ia,

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Abbreviations: B6, C57BL/6; bm12, the spontaneous *I-A* mutant mouse strain, B6.C-*H-2*^{bm12}; TCGF, T-cell growth factor.

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thus providing genetic evidence that alloreactive T cells see predominantly conformational determinants.

MATERIALS AND METHODS

Animals. C57BL/6, B10.D2, B10.BR, B10.G, and B10.RIII were purchased from The Jackson Laboratories. bm12 mice originally provided by R. Melvold (Northwestern University) were bred in our own facilities. B10.S mice were kindly provided by C. David (Mayo Clinic). All mice were used between ages 2 and 6 months. The mouse strains used and their salient properties are summarized in Table 1.

Generation of Alloreactive T-Lymphocyte Colonies. The methods of Sredni *et al.* (20) were used with slight modification. Briefly, T lymphocytes were enriched by passage of normal spleen cells over nylon–wool columns. Responder T lymphocytes (3×10^6) were cultured with equal numbers of irradiated stimulator spleen cells in a 1-ml vol of RPMI 1640 medium (GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (Reheis Chemical, Phoenix, AZ; 50 units of penicillin, 50 μg of streptomycin, and 100 μg of gentamycin per ml (all from GIBCO); 2 mM L-glutamine (GIBCO); and 50 μM 2-mercaptoethanol in 24-well culture plates (Limbro, Flow Laboratories). Cultured cells were recovered 5 days later, washed, and seeded in soft agar containing irradiated stimulator spleen cells. Colonies were picked between days 3 and 5 and were expanded *in vitro* with 1×10^5 stimulator cells in complete RPMI 1640 medium supplemented with 10% T-cell growth factor (TCGF or interleukin 2). TCGF activity was provided by the culture supernatants of EL-4 cells (provided by John Farrar, Department of Immunopharmacology, Hoffmann–La Roche) stimulated with 4β-phorbol 12-myristate 13-acetate (Sigma) at a final concentration of 15 ng/ml. Cultures were fed with fresh medium and stimulator cells every week.

Generation of Long-Term Alloreactive T-Lymphocyte Lines. Long-term alloreactive T-lymphocyte lines were generated by the methods of Kimoto and Fathman (21). Briefly, primary mixed lymphocyte cultures were set up in 24-well plates with 3×10^6 responders and 3×10^6 irradiated stimulators in a 1-ml vol. On day 7, cultures were replenished with fresh medium and allowed to rest for another week. After that, cultures were split and restimulated with 1×10^6 irradiated stimulator spleen cells for 5 days. Cultures were then allowed to rest for 10 days, and the cycle was repeated. Subsequently, viable cells were isolated by Ficoll-Paque purification and cultured at 4×10^5 cells per well. From then on, cells proliferated rapidly when stimulated by the appropriate stimulators.

Assay for Alloreactivity. Cloned T lymphocytes (5×10^3) were cultured in a 0.2–3 ml vol of complete RPMI 1640 medium in 96-well flat-bottomed plates together with 2×10^5 irradiated normal spleen cells from various mouse strains as stimulator cells. Cultures were maintained in 95% air/5% CO_2 at 37°C for 4 days. Ten to 20 hr before harvesting, 1 μCi of tritiated thymidine (New England Nuclear) was added to each culture. Incorporation of thymidine was determined in a Beckman liquid scintillation counter (model L5900). All cultures were established in triplicates, and the results were expressed as the mean number of cpm.

Table 1. Mouse strains used, their *I* region genotypes, and their Ia antigens expressed on the cell surface

Strain	*I*-region genotypes*				Ia antigens expressed on cell surface†
	A_β	A_α	E_β	E_α	
B6	*b*	*b*	*b*	*b*	$A^b_\alpha \cdot A^b_\beta$
bm12‡	*bm12*	*b*	*b*	*b*	$A^b_\alpha \cdot A^{bm12}_\beta$
B10.BR	*k*	*k*	*k*	*k*	$A^k_\alpha \cdot A^k_\beta$, $E^k_\alpha \cdot E^k_\beta$
B10.D2	*d*	*d*	*d*	*d*	$A^d_\alpha \cdot A^d_\beta$, $E^d_\alpha \cdot E^d_\beta$
B10.S	*s*	*s*	*s*	*s*	$A^s_\alpha \cdot A^s_\beta$
B10.G	*q*	*q*	*q*	*q*	$A^q_\alpha \cdot A^q_\beta$
B10.RIII	*r*	*r*	*r*	*r*	$A^r_\alpha \cdot A^r_\beta$, $E^r_\alpha \cdot E^r_\beta$
B10.A(5R)§	*b*	*b*	*b/k*	*k*	$A^b_\alpha \cdot A^b_\beta$, $E^k_\alpha \cdot E^{b/k}_\beta$

*The haplotype origin of each gene is designated by a lowercase letter in the table.

†The *b*, *s*, and *q* haplotypes fail to express a second Ia molecule due to a genetically aberrant E_α or E_β gene (19).

‡The bm12 strain has a mutation in its A_β gene and thus is designated A^{bm12}_β.

§B10.A(5R) is an intra-*I*-region recombinant with the left half of its E_β gene derived from the *b* haplotype, whereas the right half was derived from the *k* haplotype (18). The exon in B10.A(5R) encoding the E_β outer domain (that region involved in the putative gene conversion event) was derived from the *b* haplotype.

RESULTS

Analysis of B6 Anti-bm12 Alloreactive Clones. Since the three amino acid differences in the A_β polypeptide chain of bm12 Ia antigen created a determinant that is recognized as foreign by B6 T lymphocytes, we investigated the uniqueness of this determinant by raising B6 anti-bm12 alloreactive T-lymphocyte clones and testing for crossreaction with cells from standard haplotypes. T-lymphocyte clones were obtained by the soft-agar methodology (20). Seventeen bm12-specific alloclones were raised and tested in mixed lymphocyte cultures with spleen cell stimulators bearing the Ia alloantigens of the *d*, *k*, *r*, *q*, or *s* haplotypes. Table 2 summarizes the reactivities of these clones 2 months after their clonal expansion in liquid culture. Crossreactivity in our system was defined as proliferation that was minimally 10% that of the response to bm12 Ia antigen. This definition was based on the fact that the standard errors of the means of control culture responses were usually <10%. Based on this criteria, the majority of the B6 anti-bm12 clones were specific for the bm12 determinant and exhibited no extensive crossreaction with other haplotypes. Four of the 17, however, did show low levels of crossreactivity. These 4 clones were subjected to further analysis.

One possible circumstance that can give rise to a false positives is the clonality of the cell population. Since the soft-agar colonies were not recloned, it was possible that some of these colonies contained, in addition to bm12-specific cells, other alloreactive cells that were also propagated due to nonspecific recruitment (22). But since the majority of cells were specific, these nonspecific contaminants would eventually be diluted out. Thus, 51E5, 52E8, 53G6, and 54B8 were stimulated and passed for 2 additional months. Table 3 shows the reactivity of these four clones 4 months after establishment. In this experiment, titrating numbers of T lymphocytes were used. It can be seen that three of the four originally crossreactive clones now no longer exhibited any crossreactivity, indicating that the original 2-month cultures actually contained nonspecific contaminating cells rather than a crossreactive clone. One clone, 51E5, continued to show crossreactivity to B10.D2. Table 4 shows the results of an experiment in which the four clones were tested again 6 months after establishment. It confirms the observations of Table 3 and further shows that no new specific responses arose in the 4 months of culture.

Taken together, we concluded that the "gain" antigenic determinant on bm12 Ia represents a private specificity. The minor crossreactivity with Ia^d was only 1 of 17, suggesting that 51E5 was a rare clone.

Conformational Recognition of the bm12 Ia Determinant. Recent studies have suggested that the *bm12* mutation represents a gene conversion whereby at least 14 nucleotides from an E^b_β gene replaced homologous information in an A^b_β

Table 2. Alloantigen specificity of B6 anti-bm12 T-cell colonies

T-cell colonies	Proliferative response in cultures with stimulators, cpm							
	Medium	B6	bm12	B10.BR	B10.D2	B10.S	B10.G	B10.RIII
63F5	134	177	*9992*	337	358	261	292	367
50B5	98	228	*2362*	343	208	223	149	199
50E10	162	324	*6031*	624	443	863	691	539
51B2	209	160	*3816*	276	217	289	216	260
51B3	75	133	*2855*	206	202	292	139	210
51B5	171	384	*2043*	444	337	235	255	448
51B8	40	193	*4228*	328	221	329	593	508
51B9	1152	1304	*11554*	1341	1633	1858	1882	1889
51E4	43	291	*5604*	442	326	644	515	515
52B5	150	221	*7309*	235	216	703	239	317
52B10	108	241	*3466*	317	414	297	337	389
53G11	76	249	*11542*	302	316	358	222	195
54E10	121	130	*10815*	383	270	275	203	204
51E5	53	106	*2787*	*434* (12.2%)	*648* (20.2%)	235	175	213
52E8	52	102	*2209*	291	172	276	188	*536* (20.6%)
53G6	86	143	*4387*	*1052* (21.4%)	269	332	258	251
54B8	153	455	*5863*	*1003* (10.1%)	436	530	614	607

Two months after picking the colonies and after expansion in liquid cultures, 5×10^3 T cells were cultured with 2×10^5 irradiated normal spleen cells from various mouse strains as the source of stimulator cells in complete culture medium supplemented with 10% TCGF. Proliferate responses were measured on day 4. Crossreactivities above 10% were indicated in parentheses. Crossreactivity was calculated by the following formula in which stimulation is measured as cpm incorporation:

$$\frac{\text{stimulation by crossreactive strain} - \text{stimulation by syngeneic B6}}{\text{stimulation by bm12} - \text{stimulation by syngeneic B6}} \times 100\%.$$

gene, thus creating the A_β^{bm12} gene (7–9). This putative conversion resulted in three amino acid differences between A_β^b and A_β^{bm12} polypeptides in a hypervariable region of the outer molecular domain (β1). Since B6 and bm12 mice fail to express E_β^b chains (19), the *bm12* gene conversion resulted in the expression of a new Ia sequence expressed by bm12 and not by B6 cells. To determine whether T cells see the site of the *bm12* mutation as a separate functional domain, we used the intra-*I* region recombinant mouse strain B10.A(5R). As listed in Table 1, B10.A(5R) cells express both cell surface Ia dimers $E_\alpha^k \cdot E_\beta^{b/k}$ and $A_\alpha^b \cdot A_\beta^b$. Since the *β1* exon of the E_β gene of B10.A(5R) was derived from the *b* haplotype (18), bm12 and B10.A(5R) both express the same Ia sequence involved in the conversion event, albeit bm12 in the context of A_β and B10.A(5R) in the context of E_β.

B6 and bm12 cells mount a strong primary mixed lymphocyte response not only to each other but to third party cell types as well (Table 5). Cells from this experiment also were used to make a B6 anti-bm12 alloreactive line by the method of Kimoto and Fathman (21). This line was repeatedly stimulated with bm12 cells for 3 months and then was tested on a panel of cell types. The B6 anti-bm12 line showed a strong response to bm12 stimulators but a negligible response to Ia^k (B10.BR), Ia^d (B10.D2), and Ia^q (B10.G) antigens (Table 6). It is noteworthy that only background levels of response were observed when this line was stimulated by B10.A(5R) cells, suggesting that the *bm12* mutation is seen by T cells as a conformational determinant.

Table 3. Alloantigen specificity of B6 anti-bm12 T-cell colonies 4 months after establishment

T-cell colonies	T cells per well, no.	Proliferative response in cultures with stimulators, cpm				
		B6	bm12	B10.BR	B10.D2	B10.RIII
51E5	5.0×10^3	268	5441	161	1430 (22.9%)	
	7.5×10^3	505	8502	343	2131 (20.3%)	
	10.0×10^3	341	11547	761	2821 (22.1%)	
	15.0×10^3	457	14309	1117	3002 (18.4%)	
52E8	1.25×10^3	187	2601			266
	2.50×10^3	177	7750			95
	5.00×10^3	253	13457			144
	7.50×10^3	210	16971			103
53G6	5.0×10^3	185	9051	956		
	7.5×10^3	283	15376	1131		
	10.0×10^3	300	17261	1485		
	15.0×10^3	517	22839	1684		
54B8	2.5×10^3	180	2948	199		
	5.0×10^3	207	8171	349		
	7.5×10^3	184	11039	424		
	10.0×10^3	248	14713	498		
	15.0×10^3	266	18878	511		

Four months after picking the colonies and following *in vitro* expansion, various numbers of T lymphocytes from each cell line were cultured with 2×10^5 irradiated normal spleen cell from the indicated strains in complete culture medium supplemented with 10% TCGF. Cells were harvested on day 4, and [^{3}H]thymidine was added 16 to ≈18 hr before harvesting. Crossreactivities above 10% were indicated in parentheses. Crossreactivity was calculated in the same manner as in Table 2.

Table 4. Alloantigen specificity of B6 anti-bm12 T-cell colonies 6 months after establishment

T-cell colonies	Proliferative response in cultures with stimulators, cpm						
	B6	bm12	B10.BR	B10.D2	B10.S	B10.G	B10.RIII
51E5	651	*32171*	683	*6079* (17.2%)	145	213	228
52E8	124	*8241*	95	65	245	101	49
53G6	62	*14139*	141	29	81	99	95
54B8	103	*12330*	196	110	99	180	36

Six months after picking colonies and following *in vitro* expansion, 5×10^3 T lymphocytes from each cell line were cultured with 2×10^5 irradiated stimulator cells from various mouse strains in complete culture medium supplemented with 10% TCGF. Cells were harvested on day 4 and [^{3}H]thymidine was added 16 to ≈18 hr before harvesting. Crossreactivity of >10% was indicated in parentheses and crossreactivity was calculated from the same formula as in Table 2.

Table 5. Primary mixed lymphocyte reactions *in vitro*

Responder	Proliferative response in cultures with stimulators, cpm					
	B6	bm12	B10.A(5R)	B10.BR	B10.D2	B10.G
B6	1265	12523	14323	22656	35496	25239
bm12	12603	2061	15079	18917	17793	15369
B10.A(5R)	3372	26312	2593	32550	26509	13284

Normal responder spleen cells (3×10^5) were cultured in a 0.2-ml vol of complete RPMI 1640 medium with 3×10^5 irradiated spleen stimulator cell populations from various strains. Stimulator cells were irradiated with 2000 R. [^{3}H]Thymidine incorporation was assayed on day 4.

To further explore the role of Ia conformation, we also analyzed the B10.A(5R) anti-bm12 proliferative response. B10.A(5R) cells mounted a strong primary mixed lymphocyte response to bm12 cells and, as expected, no response to B6 cells (Table 5). Cells from these same cultures were propagated by the above described procedures to establish an alloreactive cell line. This B10.A(5R) anti-bm12 line was found to be highly specific for Ia^{bm12} antigens (Table 6). Since B10.A(5R) cells are incapable of recognizing the primary sequence of the *bm12* mutation, these data underscore the importance of conformation in T-cell recognition of Ia.

In summary, three lines of evidence are presented that suggest that T cells see predominantly the secondary structure of Ia: (*i*) none of the B6 anti-bm12 clones reacted with cells expressing E^k_β polypeptides that share the same amino acid sequence with A^{bm12}_β and E^b_β in the region involved in the mutation; (*ii*) a B6 anti-bm12 line failed to react with E^b_β- or E^k_β-bearing cells; and (*iii*) B10.A(5R) cells mounted a strong anti-bm12 response even though they should be tolerant to all linear determinants.

DISCUSSION

We studied the T-cell recognition of mutant Ia^{bm12} antigens by parental B6 cells. This assay system has several unique features that make it of interest in studies of immune recognition. First, it permits the study of a select portion of the Ia molecule that has been structurally defined; second, recognition of the *bm12* mutation is known to have functional importance in both allogeneic and antigen-specific T-cell responses; and third, given the structure of the *bm12* mutation, the B6 anti-bm12 response is tantamount to studying recognition of a functional domain of an E_β molecule transferred to the homologous site on an A_β molecule. Our studies of the B6 anti-bm12 response support several conclusions.

Table 6. Mixed lymphocyte reactions of B6 anti-bm12 and B10.A(5R) anti-bm12 cell lines

Responder cell line	Cells $\times 10^{-3}$ per well	Proliferative response in cultures with stimulators, cpm					
		B6	bm12	B10.A(5R)	B10.BR	B10.D2	B10.G
B6	5	148	*41654*	247	463	155	423
anti-bm12	10	663	*51031*	538	2160	722	705
B10.A(5R)	5	652	*161930*	456	3111	988	957
anti-bm12	10	412	*240113*	827	3246	2215	1070

Three months after repeated stimulation and resting periods *in vitro* and 10 days after the last stimulation with bm12 stimulator cells, various numbers of cells were cultured with 2×10^5 irradiated spleen stimulator cells from the strains indicated. [^{3}H]Thymidine incorporation was assayed on day 4.

The Gain-Specificity of the *bm12* Mutation Is Unique. Analyses of B6 anti-bm12 clones and lines indicate that the overwhelming majority of this response is directed against a unique Ia determinant. Sixteen of 17 B6 anti-bm12 clones showed no reproducible crossreactions with the other haplotypes tested (*k,d,s,q,r*), whereas only 1 showed a minor crossreaction with Ia^d antigens. Similarly, B6 anti-bm12 and B10.A(5R) anti-bm12 bulk culture (or line) showed principal reactivity with bm12. Concordantly, the humoral response against Ia^{bm12} was found previously to define a unique serological determinant not shared with other haplotypes (23). In contrast to these findings, the reciprocal response (bm12 anti-B6) was found to recognize both private and public Ia^b determinants in proliferative (24) and humoral responses (23).

The *bm12* Conversion Resulted in the Creation of a New Functional Domain, Not the Transfer of a Preexisting One. Recent studies by Hochman and Huber (16) and Mengle-Gaw *et al.* (8) defined Ia determinants that are shared between A^{bm12}_β and E^b_β polypeptides and are recognized by either antigen-specific or allogeneic T cells, respectively. Because this crossreaction correlates with the putative gene conversion, both groups proposed that the *bm12* mutation defines a functional unit for T-cell recognition of Ia. In the study by Hochman and Huber (16), immune responsiveness of *H-2*^{k} mice to sheep insulin was shown to be mediated by the E^k_α·E^k_β molecule, whereas the A^b_α·A^b_β molecule of *H-2*^{b} mice conferred nonresponsiveness to sheep insulin. Since bm12 mice were found to be responders to sheep insulin and E^k_β and E^b_β polypeptides are identical in the region involved in the putative conversion, they postulated that this mutation defines an immune response epitope on Ia for sheep insulin. In the studies by Fathman and colleagues, a single A anti-(B6 × A)F_1 alloreactive T-cell clone defined a determinant shared by A^{bm12}_β and E^b_β polypeptides (8). Based on this correlation with the putative *bm12* conversion event, they also proposed that this clone defines a T-cell recognition unit on Ia, or a so-called minigene product.

If the bm12 determinant represents the transfer of a functional unit from E^b_β to A^{bm12}_β, one would predict that B6 anti-bm12 alloreactive T cells should also be stimulated by E^k_β- or E^b_β-bearing cells. However, this is clearly not the case. None of the B6 anti-bm12 clones could be stimulated by E^k_β-positive cells, nor did the B6 anti-bm12 culture show B10.A(5R) (E^b_β-positive) reactivity. Therefore, an A^{bm12}_β-E^b_β crossreactive determinant was not detectable in these responses, suggesting that the *bm12* mutation site is seen as a conformational determinant. In other words, the *bm12* mutation is recognized not as a separate structural domain but rather as a conformational determinant seen in the context of other parts of the A_β or E_β polypeptide or as a combinatorial determinant seen in the context of the A_α or E_α polypeptide. Therefore, we suggest that the published correlations of T-cell function with the putative *bm12* conversion represent fortuitous findings and that Ia^{bm12} is no more likely to share determinants with E^k_β or E^b_β polypeptides than with any other Ia alloantigen.

T Cells Recognize Predominantly Conformational Determinants on Ia. The T-cell receptors that recognize allogeneic Ia are apparently a subset of those that recognize nominal antigen in the context of self-Ia (6). Therefore, the results reported here raise important questions regarding functional recognition of Ia in the immune response. For example, is the mutated region of Ia^{bm12} a predominant site for T-cell recognition? A series of previous observations suggested this was true. First, several lines of evidence indicated that Ia molecules have multiple functional sites (e.g., refs. 13, 15, and 17); second, a high proportion of relevant T-cell clones and hybridomas can discriminate between Ia^b and Ia^{bm12} molecules in either allogeneic or antigen-specific immune respons-

es (17). However, the results reported here suggest that this discrimination between Ia^b and Ia^{bm12} is probably due to recognition of secondary structural differences between these molecules. Therefore, the high frequency of T cells that can distinguish mutant from wild-type Ia may be a reflection of the extent of the conformational change ensuing from the *bm12* mutation rather than the importance of the primary structure in this region of the molecule.

Another question raised by the studies reported here is whether Ia-restricted, antigen-specific T cells also recognize conformational determinants. Several recent findings could be interpreted as evidence that immune T cells see the primary structure of Ia or nominal antigen. The aforementioned studies of Hochman and Huber (16) suggest that immune response to sheep insulin is dependent upon recognition of the five amino acid sequence involved in the putative *bm12* gene conversion. However, we were unable to reproduce these results (unpublished observation). Studies from several laboratories have reported that a small peptide can replace native antigen for the stimulation of certain T cells (e.g., ref. 25). However, clones reactive with peptide might represent rare clones, and it is unclear what size fragment would be necessary to attain a recognizable conformation. It also seems possible that a peptide (or antigen-processed fragment of native antigen) interacts with Ia in a manner necessary to attain a conformation required for T-cell recognition. There is also some evidence that T cells can see secondary structure of nominal antigen. Studies using T cells immune to cytochrome *c* (26), myoglobin (27), or insulin (28) suggested that Ia-restricted T cells see conformational determinants on nominal antigen. Taken together with the data presented here, we feel the evidence supports the conclusion that T cells recognize *predominantly* the conformation of Ia in both allogeneic and syngeneic responses. This conclusion is also consistent with previous studies using K^b mutants that demonstrated that cytotoxic T cells see conformational determinant on class I major histocompatibility complex molecules (29).

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1. Kaufman, J. F., Auffray, C., Korman, A. J., Shackelford, D. A. & Strominger, J. (1984) *Cell* **36,** 1–13.
2. Shevach, E. M. & Rosenthal, A. S. (1973) *J. Exp. Med.* **138,** 1213–1229.
3. Sprent, J. (1978) *J. Exp. Med.* **147,** 1159–1174.
4. Tse, H. Y., Mond, J. J. & Paul, W. E. (1981) *J. Exp. Med.* **153,** 871–882.
5. Janeway, C. A., Wigzell, H. & Binz, H. (1976) *Scand. J. Immunol.* **5,** 993–1001.
6. Kaye, J. & Janeway, C. A. (1984) *J. Exp. Med.* **159,** 1397–1412.
7. McIntyre, K. & Seidman, J. (1984) *Nature (London)* **308,** 551–553.
8. Mengle-Gaw, L., Conner, S., McDevitt, H. O. & Fathman, C. G. (1984) *J. Exp. Med.* **160,** 1184–1194.
9. Widera, G. & Favell, R. A. (1984) *EMBO J.* **3,** 1221–1225.
10. McKenzie, I. F. C., Morgan, G. M., Sandrin, M. S., Michaelides, M. M., Melvold, R. W. & Kohn, H. I. (1979) *J. Exp. Med.* **150,** 1323–1338.
11. deWaal, L. P., Melief, C. H. M. & Melvold, R. W. (1981) *Eur. J. Immunol.* **11,** 258–265.
12. Michaelides, M., Sandrin, M., Morgan, G., McKenzie, I. F. C., Ashman, R. & Melvold, R. W. (1981) *J. Exp. Med.* **153,** 464–469.
13. Lin, C.-C., Rosenthal, A. S., Passmore, H. C. & Hansen, T. H. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6406–6410.
14. Lei, H. Y., Melvold, R. W., Miller, S. D. & Waltenbaugh, C. (1982) *J. Exp. Med.* **156,** 596–609.
15. Beck, B. N., Nelson, P. A. & Fathman, C. G. (1983) *J. Exp. Med.* **157,** 1396–1404.
16. Hochman, P. S. & Huber, B. T. (1984) *J. Exp. Med.* **160,** 1925–1930.
17. Kanamori, S., Walsh, W. D., Hansen, T. H. & Tse, H. Y. (1984) *J. Immunol.* **133,** 2811–2814.
18. Steinmetz, M., Minard, K., Horvath, S., McNicholas, J., Srelinger, J., Wake, C., Long, E., Mach, B. & Hood, L. (1982) *Nature (London)* **300,** 35–42.
19. Mathis, D. J., Benoist, C., Williams, V. E., Kanter, M. F. & McDevitt, H. O. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 273–277.
20. Sredni, B., Tse, H. Y., Chen, C. & Schwartz, R. H. (1981) *J. Immunol.* **126,** 341–347.
21. Kimoto, M. S. & Fathman, C. G. (1981) *J. Exp. Med.* **153,** 375–385.
22. Tse, H. Y., Schwartz, R. H. & Paul, W. E. (1980) *J. Immunol.* **125,** 491–500.
23. Morgan, G. M., McKenzie, I. F. C. & Melvold, R. W. (1980) *Immunogenetics* **11,** 1–6.
24. Skelly, R. R., Pappas, F., Koprak, S., Ahmed, A. & Hansen, T. H. (1982) *J. Immunol.* **129,** 2094–2097.
25. Yoshioka, M., Bixler, G. S. & Atassi, M. Z. (1983) *Mol. Immunol.* **20,** 1133–1137.
26. Buchmuller, Y. & Corradin, G. (1982) *Eur. J. Immunol.* **12,** 412–416.
27. Berkower, I., Buckenmeyer, G. K., Gard, R. N. & Berzofsky, J. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4723–4727.
28. Glimcher, L. H., Schroer, J. A., Chan, C. & Shevach, E. M. (1983) *J. Immunol.* **131,** 2868–2874.
29. Sherman, L. A. (1982) *Nature (London)* **297,** 511–513.