GAIN/LOSS OF POLY(GLU^{50}TYR^{50})/ POLY(GLU^{60}ALA^{30}TYR^{10}) RESPONSIVENESS IN THE *bm12* MUTANT STRAIN*

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Specific immune responses to thymus-dependent antigens involve multiple stages of cell interaction. Initiation of an immune response requires the presentation of antigen by macrophages, as well as the successful interaction of multiple subsets of T cells. In the mouse, both antigen recognition and the production of antigen-specific T cell factors by helper (Th)[1] or suppressor (Ts) T cells appear to be controlled by *I*-region genes of the major histocompatibility complex (*H-2*) (1, 2). Immune response-associated (Ia) molecules found on most B cells, and to varying degrees on macrophages and T cells, are encoded by genes in the *I-A* and *I-E* subregions and may well be products of immune response (*Ir*) and/or immune suppressor (*Is*) genes (3–5). Successful T-B cell collaboration requires identity of genes, or their products, in the I-A subregion (3).

The development of inbred strains of mutant mice has proven useful in ascribing specific gene fractions to particular genetic loci within the regions and subregions of the H-2 complex (6). The B6.C-*H-2*^{bm12} (*bm12*) strain is of particular interest in that it bears a presumptive single gene mutation altering the A^b_β chain, encoded in the I-A subregion, and is characterized by changes in serologically defined Ia determinants, by strong graft rejection, and by mixed lymphocyte response between parent and mutant (7–14). Mice of the *bm12* strain have impaired immune responses, relative to the parental strain C57Bl/6Kh (B6), to beef insulin (15), to H-Y antigen (11), and to the alpha chain of human hemoglobin (16). In contrast, *bm12* immune responses to other antigens, such as poly(L-Tyr,L-Glu)-poly(DL-Ala)--poly(L-Lys) [(T,G)-A--L], poly(L-Phe,L-Glu)-poly(DL-Ala)--poly(L-Lys) [(Phe,G)-A--L], and poly(L-His,L-Glu)-poly(DL-Ala)--poly(L-Lys) [(H,G)-A--L], do not differ markedly from parental B6 mice (11, 15, 17). Although the immune responsiveness to beef insulin, H-Y antigen, human

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[1] *Abbreviations used in this paper:* ANOVA, analysis of variance; B6, C57BL/6 (H-2^b) mice; *bm12*, B6.C-*H-2*^{bm12}; (C × B6)F_1, (BALB/c × B6)F_1; DTH, delayed-type hypersensitivity; FCS, fetal calf serum; GA, poly(Glu^{60}Ala^{40}); GAT, poly(Glu^{60}Ala^{30}Tyr^{10}); GT, poly(Glu^{50}Tyr^{50}); HBSS, Hanks' balanced salt solution; (H,G)-A--L, poly(L-His,L-Glu)-poly(DL-Ala)--poly(L-Lys); *Ir*, immune response gene(s); *Is*, immune suppressor gene(s); MBSA, methylated bovine serum albumin; PFC, plaque-forming cell; (Phe,G)-A--L, poly(L-Phe,L-Glu)-poly(DL-Ala)--poly(L-Lys); SRBC, sheep erythrocytes; T_{prolif}, proliferating T cell; Th, helper T cell; Th_{eff}, Th_{aff} efferent and afferent Th, respectively; Ts, suppressor T cell; TsF, T cell-derived suppressor factor; (T,G)-A--L, poly(L-Tyr,L-Glu)-poly(DL-Ala)--poly(L-Lys).

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alpha-hemoglobulin, (T,G)-A--L, (Phe,G)-A--L, and (H,G)-A--L map to the I-A subregion, alteration of the A^b_β molecule (12) does not affect all of these responses.

Primary immune responses to the thymus-dependent synthetic antigens poly-($Glu^{50}Tyr^{50}$) (GT) and poly($Gln^{60}Ala^{30}Tyr^{10}$) (GAT) are controlled by *I*-region genes (18–21). GAT stimulates the development of Th in responder strains (H-$2^{a,b,d}$ haplotypes) and Ts in nonresponder strains (H-$2^{p,q,s}$ haplotypes) (21). On the other hand, >40 inbred strains of mice have been shown to be nonresponders to GT (18, 19, 22, 23). Nonresponsiveness to GAT and GT is not a result of a general B cell defect because all strains make a good antibody response to these antigens when coupled to the immunogenic carrier molecule, methylated bovine serum albumin (MBSA) (18, 20). GT-nonresponder strains of mice can be further classified as either GT-suppressor (H-$2^{d,k,s}$ haplotypes), in which administration of GT renders the animals incapable of responding to subsequent immunizations with GT-MBSA, or as GT nonsuppressors (H-$2^{a,b,q}$ haplotypes) (18, 19, 24–26). Mice of the H-$2^{b,d,k}$ haplotypes, when injected with GT, produce a first-order T cell-derived suppressor factor (GT-TsF_1) that induces a second, distinct subset of Ts (Ts_2) in H-$2^{a,d,k}$ haplotype mice (24, 26).

In this study we have investigated GAT and GT responsiveness of mutant *bm12* mice. We find that, in contrast to the parental B6, *bm12* mice are responders to GT and low or nonresponders to GAT and are unable to produce GT-TsF_1.

Materials and Methods

Animals. BALB/cCum (H-2^d) mice were purchased from Cumberland View Farms, Clinton, TN. B10.A(5R) (5R; H-2^{i5}), B6 (H-2^b), BALB/cKh (H-2^d), and (*bm12*) mice as well as F_1 and F_2 hybrid mice derived from these strains, were bred in the authors' colonies at Northwestern University, Chicago, IL. Mice used in these studies were 2–4 mo old and were maintained on standard laboratory chow and water *ad lib.*

Antigens. GT, lot 9, originally purchased from Miles Laboratories, Inc., Elkhart, IN was the generous gift of Dr. Baruj Benacerraf, Harvard Medical School, Boston, MA. Poly($Glu^{60}Ala^{40}$) (GA), with average 45,000 mol wt, was purchased from Miles Laboratories, Inc. GAT, lot 1, and MBSA were purchased from Vega Biochemicals, Tucson, AZ. In some experiments, GAT, lot 31F5040, from Sigma Chemical Corp., St. Louis, MO was used with identical results. GAT, GT, GA, and their MBSA complexes were prepared as previously described (26).

Immunizations. Mice were immunized with either 100 μg GT or 10 μg GT as GT-MBSA in Maalox (aluminum-magnesium hydroxide gel; Wm. Roher, Ft. Washington, PA) and *Bordetella pertussis*, intraperitoneally, as adjuvant. 7 d later, the number of GT-specific plaque-forming cells (PFC) per spleen was determined. For delayed-type hypersensitivity (DTH) and in vitro proliferation studies, mice were immunized subcutaneously at the base of the tail with 20 μg of GAT contained in 50 μl of complete Freund's adjuvant (containing H37Ra) as previously described (27).

GT-TsF Preparation. Lymphoid cell extracts were prepared from GT-primed mice as previously described (24, 25). Briefly, B6 or *bm12* mice were intraperitoneally primed with 100 μg GT in Maalox 3 d before they were killed. Single cell suspensions of their spleens were prepared, adjusted to 6×10^8 cells/ml in Hanks' balanced salt solution (HBSS), and sonicated at 50 W for 5 min by a Sonifier Cell Disruptor (Ultrasonic Industries, Westbury, NY) equipped with a microtip. Sonicated material was centrifuged at 40,000 *g* for 45 min at 4°C and the resultant supernates stored at −85°C until use.

Spleen Cell Cultures. Single cell suspensions of unimmunized mice were prepared aseptically and washed once with HBSS. Cells were resuspended to a final concentration of 16×10^6 cells/ml in Mishell-Dutton medium (28) containing 5×10^{-5} M 2-mercaptoethanol and 10% fetal calf serum (FCS; lot 07; Bioserum, Inc., Great Neck, NY) (25). GT (2.5–10 μg), GA (10 μg), GAT (2.5–10 μg), or GT (2.5 μg) as GT-MBSA antigens were added to 0.5 ml of cell suspension in individual wells of a multiwell culture plate (76-033-05; Linbro Chemical Co., Hamden,

CT) as indicated in the Table legends. Extracts from GT-primed B6 or *bm12* mice were added at final concentrations as indicated in Table legends. Cultures were incubated for 5 d in a humidified atmosphere of 83% N_2, 10% CO_2, and 7% O_2 at 37°C and fed daily with a mixture of 50% nutritional cocktail and 50% FCS (28).

Hemolytic Plaque Assay. 7 d after in vivo injection of antigen, or 5 d after culture initiation, spleen cells were washed three times in HBSS, and PFC responses were assayed using sheep erythrocytes (SRBC) coupled with the cross-reacting copolymer, GAT (29). GA-, GAT-, and GT-specific PFC were determined by subtracting the number of PFC detected in the presence of suitable dilution of GAT from the number of PFC detected on GAT-SRBC in the absence of the specific inhibitor. All assays were performed in duplicate and the number of PFC per culture are reported.

Induction and Elicitation of Delayed-Type Hypersensitivity (DTH) Responses. 6–7 d after immunization, mice were challenged by intracutaneous injection of the dorsal surface of the ear with 10 μg of GAT (contained in 10 μl of saline) using a 100-μl Hamilton syringe fitted with a 30-gauge needle. 24 h after antigen challenge, increases in ear thickness were determined using an engineer's micrometer, and the results were expressed in units of 10^{-4} in. (27).

In Vitro T Cell Proliferation. After determination of in vivo ear swelling, draining lymph nodes (inguinal and periaortic) were used as a source of cells for determining the level of antigen-induced T cell proliferation. 4×10^5 cells were cultured in flat-bottomed microwell plates in a vol of 0.2 ml of modified Click's medium (30) supplemented with fresh 0.5% syngeneic mouse serum. Cultures were stimulated with the indicated doses of GAT or GA. The plates were incubated in a humidified atmosphere of 5% CO_2, 95% air for 120 h, and were pulsed with 1 μCi of [^{3}H]thymidine (6 Ci/mM; Research Products International Corp., Mt. Prospect, IL) for the final 24 h. Cultures were harvested with a semiautomatic sample harvester, and measurements of trichloroacetic acid-insoluble radioactive material were determined in a Beckman liquid scintillation counter (Beckman Instruments, Inc., Mt. View, CA). Cultures were performed in triplicate and results expressed as Δ cpm [(mean cpm of antigen-containing cultures) − (mean cpm of antigen-free-"nil"-cultures)], as previously described (27).

Results

Elicitation of Primary GT Responses in bm12 Mice. In >40 inbred strains of mice, representing 23 different H-2 haplotypes and 15 different non-H-2 backgrounds tested by primary or secondary injection of GT, none responded to GT (18, 19, 22, 23). Merryman and Maurer (22) and Debré et al. (18) demonstrated that some outbred Swiss mice responded to GT, although it was not possible to attribute GT responsiveness to a particular haplotype or locus. Although it is possible to selectively inbreed GT responder Swiss mice to ultimately identify the locus or loci involved in GT responsiveness, such procedures, though straightforward, are both time-consuming and costly. Rather than trying to produce a GT-responder phenotype by backcrossing and selection, we chose to look at inbred strains that differ from parental strain mice only by mutation at one or a few loci. Preliminary in vitro screening experiments showed that *bm12* mice appeared to make a primary PFC response to GT. As shown in Table I, this was confirmed in vivo (group a) when the injection of *bm12* mice with 100 μg GT resulted in a primary antigen-specific PFC response. In contrast, injection of parental B6 mice (group B) with GT does not elicit a primary PFC response. B6 mice injected with 10 μg GT as GT-MBSA (group C) show a response of similar magnitude to the *bm12* GT response. In fact, the *bm12* anti-GT response is significant ($P = 0.001$) when compared with nonresponder-nonsuppressor B6 (group B) mice and suppressor BALB/c (group D), 5R (group E), and (BALB/c × B6)F_1, (C × B6)F_1, (group G) mice. One could always make the argument that as the *bm12* mutation was first detected in a (B6 × C)F_1 mouse, BALB/c passenger genes may account for GT responsiveness. In fact, this cannot be the case since (C × B6)F_1 mice are nonresponders

TABLE I
GT Responsiveness In Vivo

Group	Strain	Antigen*	Number of mice per group	Antigen-specific PFC per spleen‡
				arithmetic mean ± SE
A	*bm12*	GT	9	20,750 ± 5,520
B	B6	GT	13	2,090 ± 685
C	B6	GT-MBSA	8	22,120 ± 1,345
D	BALB/c	GT	5	<250
E	5R	GT	4	<250
F	(C × B6)F_1	GT-MBSA	5	9,020 ± 1,910
G	(C × B6)F_1	GT	5	1,030 ± 780
H	(*bm12* × BALB/c)F_1	GT	3	6,000 ± 1,995
I	(*bm12* × 5R)F_1	GT	5	22,400 ± 5,340

* Mice were intraperitoneally immunized with either 10 μg GT as GT-MBSA or 100 μg GT in Maalox and *B. pertussis* as adjuvant.

‡ 7 d after GT or GT-MBSA injection, the number of antigen-specific PFC per spleen was determined. *bm12* (group A) GT responsiveness differs significantly ($P < 0.005$, as determined by ANOVA) from groups B, D, E, and G.

to GT (group G). Furthermore, in (responder × suppressor)F_1 hybrid mice of both sexes, GT-responsiveness is dominant as seen in groups H and I of Table I.

Responsiveness of bm12 and B6 Spleen Cells to GT and GAT In Vitro. During the course of our preliminary screening experiments, we noticed that *bm12* mice were not responsive to GAT in vitro, as illustrated in Table II. Cultures of B6 spleen cells are responsive to GAT in a 2.5–10 μg dose range (in our laboratory 10 μg is optimum for all GAT responder strains), whereas they are nonresponsive to GT over a similar dose range, although they respond to 2.5 μg GT as GT-MBSA. *bm12* mice, on the other hand, respond to GT over a 2.5–10 μg dose range, while remaining totally unable to make an anti-GAT PFC response. These data suggest that the mutation allowing *bm12* mice to respond to GT also has an adverse effect on GAT PFC responses.

Failure of bm12 Mice to Make GT-TsF_1. The GT-MBSA PFC responses of B6 (H-2^b) mice cannot be suppressed by preimmunization with GT. B6 mice do, however, produce GT-TsF_1 as a consequence of GT priming, although they cannot be suppressed by this or any other GT-TsF_1, because they are deficient in the ability to produce functional Ts_2 (24–26). Although *bm12* mice are able to respond to GT challenge, it is possible that they, likewise, make a GT-TsF_1. The data in Table III show that B6 mice make a GT-TsF_1 that suppresses >80% of the GT-MBSA PFC response of BALB/c mice that normally generate Ts_2 as a consequence of exposure to B6 GT-TsF_1 (24). Lymphoid cell extracts from GT-primed *bm12* mice show no such suppressive activity. In fact, experiment 2 of Table III shows that *bm12* GT-extract markedly enhances the BALB/c GT-MBSA PFC response.

PFC Responses of (BALB/c × bm12)F_1 and F_2 Mice to GT, GA, and GAT In Vitro. The data in Table I show that *bm12*-related GT responsiveness is inherited as a dominant trait. The data in Table II indicate that *bm12* is a GAT-nonresponder strain. Previous reports (20, 21) have shown that responsiveness to GAT is inherited as a dominant trait: a (suppressor × responder)F_1 hybrid animal responds to GAT. *bm12* mice appear to be GT responders and GAT nonresponders, whereas BALB/c mice are the exact

TABLE II

Responses of bm12 and B6 Spleen Cells to GT and GAT In Vitro

Experiment	Antigen	Antigen-specific PFC per culture*	
		C57BL/6	*bm12*
	μg		
1	10.0 GAT	1,010	<20
	5.0 GAT	920	<20
	2.5 GAT	270	<20
	10.0 GT	<20	2,540
	5.0 GT	<20	2,760
	2.5 GT	<20	840
	2.5 GT-MBSA	1,270	1,170
2	20.0 GAT	ND‡	50
	10.0 GAT	ND	40
	2.5 GAT-MBSA	ND	1,410
	20.0 GT	ND	1,410
	10.0 GT	ND	1,200
	2.5 GT-MBSA	ND	690

* GAT- or GT-specific PFC responses to GAT, GT, and GT as GT-MBSA or GAT as GAT-MBSA per culture of B6 or bm12 spleen cells on day 5 of culture.

‡ Not determined.

TABLE III

Effect of Extracts from GT-primed bm12 Mice on BALB/c GT-MBSA Responses In Vitro

Extract*	GT-specific PFC per culture‡	
	Experiment 1	Experiment 2
Control GT-MBSA response	1,170	2,685
B6 GT-TsF$_1$		
1:400	<25	440
1:800	<25	480
bm12 GT-extract		
1:400	975	3,740
1:800	1,250	5,620

* B6 or *bm12* mice were injected with 100 μg GT in Maalox; 3 d later, extracts were prepared from their spleens and thymuses, filter-sterilized, and the extract equivalent to 1.5×10^6 cells (1:400) or 7.5×10^5 cells (1:800) was added at culture initiation.

‡ GT-specific PFC responses of BALB/c spleen cells to 2.5 μg GT as GT-MBSA on day 5 of culture.

opposite (18–21). We asked whether (BALB/c × *bm12*)F_1 hybrid mice are responders to both GT and GAT. The data in Table IV show that (BALB/c × *bm12*)F_1 mice are able to respond equally well to in vitro immunization of GT or GAT and to the closely related polymer GA. One would predict that if a single or multiple, but closely linked, mutation(s) affects PFC responsiveness to GT and GAT, then the intercross of (BALB/c × *bm12*)F_1 should result in [GT nonresponder-GAT responder]:[GT/GAT responder]:[GAT nonresponder-GT responder] F_2 hybrid mice in the ratio of 1:2:1. (BALB/c × *bm12*)F_2 mice were immunized in vitro with GT, GA, or GAT, (Table IV). Of the 21 mice so far tested, 5 were GAT responders, but nonresponders to GT (as compared with GT-MBSA controls), 12 were responsive to both GAT and GT, whereas 4 were nonresponders to GAT, although remaining responsive to GT. Mice that were nonresponsive to GAT were also nonresponders to GA. Our observed 5:12:4

TABLE IV

PFC Responsiveness of (BALB/c × bm12)F_2 Mice to GT, GA, and GAT In Vitro

Animal number	Sex	Antigen-specific PFC per culture*				H-2 genotype
		GT	GA	GAT	GT-MBSA	
(BALB/c × *bm12*)F_1						Known
1	M	3,900	3,340	3,140	810	d/bm12
2	F	420	420	400	400	d/bm12
3	F	520	460	440	440	d/bm12
(BALB/c × *bm12*)F_2						Presumptive§
1	M	845	1,950	1,625	400	d/bm12
2	M	565	940	1,090	975	d/bm12
3	M	**200**‖	1,005	875	1,400	d/d
4	M	1,120	1,010	1,200	870	d/bm12
5	M	1,145	905	1,065	830	d/bm12
6	M	940	1,060	825	730	d/bm12
7	M	800	835	765	1,160	d/bm12
8	M	375	**<20**	**<20**	560	bm12/bm12
9	M	345	**<20**	**<20**	340	bm12/bm12
10	M	360	355	335	340	d/bm12
11	F	695	960	585	530	d/bm12
12	F	600	725	350	625	d/bm12
13	F	**90**	1,125	605	675	d/d
14	F	1,390	1,725	1,155	1,175	d/bm12
15	F	830	1,010	1,130	1,185	d/bm12
16	F	260	**25**	**25**	320	bm12/bm12
17	F	540	**70**	**<20**	260	bm12/bm12
18	F	**<15**	210	235	190	d/d
19	F	240	210	195	195	d/bm12
20	F	**<15**	270	255	210	d/d
21	F	**<15**	495	375	360	d/d

* Spleen cells from normal (BALB/c × *bm12*)F_1 or F_2 mice were cultured in the presence of 10 μg GT, GA, GAT or 2.5 μg GT as GT-MBSA; antigen-specific PFC per culture were determined on day 5.

‡ (BALB/c × *bm12*)F_1 were the parents of the (BALB/c × *bm12*)F_2 mice, below.

§ H-2 genotype as predicted from a [GT nonresponder]:[GAT-GT responder]:[GAT nonresponder] ratio of 1:2:1. Actual F_2 data show a 5:12:4 ratio which yields a χ^2 value of 0.5238 ($P = 0.773$).

‖ Boldface PFC values indicate low or nonresponder as compared with control (GT-MBSA) PFC responses.

ratio does not differ significantly ($\chi^2 = 0.524$, $P = 0.773$) from the predicted 1:2:1 ratio. Yet, because of the small sample size, our data do not exclude a 9:3:3:1 ratio that one would predict if the gene(s) responsible for GT responsiveness and GAT/GA unresponsiveness segregated as two independent loci.

Cell-mediated Immune Responses to GA and GAT in bm12 Mice. The data shown in Tables II and IV above indicate that *bm12* mice respond to GT and not to GA or GAT in the PFC assay. We asked whether this responder/nonresponder PFC status of *bm12* is generalizable to other immunological phenomena. Accordingly, GAT PFC responder [B6, BALB/c, (C × B6)F_1] and GAT PFC nonresponder (*bm12*) mice were immunized with 20 μg GAT emulsified in Freund's complete adjuvant at the base of the tail. 6 d after immunization, the mice were challenged with 10 μg GAT (in 10 μl phosphate-buffered saline) subcutaneously in the dorsal surface of the ear. DTH reactions were assayed by ear swelling 24 h after challenge. The draining inguinal and periaortic lymph nodes were cultured in the presence of 5 or 0.5 μg GAT for 96 h, whereupon T proliferative responses were measured by tritiated thymidine incorporation. In addition, GAT-specific splenic PFC of these same mice were enumerated on the day of DTH measurement. Data from these experiments show GAT PFC responder mice [B6, BALB/c, and (C × B6)F_1] show very good DTH and T proliferative responses as well as anti-GAT PFC responses (Table V). *bm12* mice, on the other hand, show significantly diminished DTH responses and background levels of GAT-specific PFC, but no decrease in the T proliferative response to either 5 or 0.5 μg of GAT in vitro. These results indicate that *bm12* mice are GAT nonresponders for PFC and DTH responses, while showing T proliferative responses equal to that of B6 mice. There are several important points that have to be dealt with in regard to these findings. First, the ear swelling assay is not a measurement of anti-GAT DTH responses, but rather is a result of an Arthus-type reaction, and lack of ear swelling would be a result of the lack of antibody that would mediate the immune complex reaction. Experiments performed in GAT-responder mice (not shown) indicate that ear swelling is not antibody-mediated; no ear swelling is seen at 4 h postchallenge, the

TABLE V

DTH, PFC and T_{prolif} Responses to GAT in bm12

Strain	Antigen*	Number of mice	Ear swelling‡	*P* value	GAT-specific PFC per spleen§	*P* value	T cell proliferation			
							5.0 μg GAT		0.5 μg GAT	
							cpm‖	*P* value	cpm‖	*P* value
			× 10^{-4} *in*							
B6	"nil"	3	3.8 ± 1.0	—	1,130 ± 80	—	—	—	—	—
B6	GAT	4	65.9 ± 5.1	<0.001	22,000 ± 2,470	<0.001	34,520 ± 13,710	—	22,870 ± 10,570	—
bm12	GAT	6	15.4 ± 2.0	0.001¶	1,560 ± 640	0.717**	35,960 ± 11,450	0.962	32,640 ± 13,690	0.580
BALB/c	GAT	4	40.7 ± 4.9	<0.001	28,540 ± 12,130	<0.001	ND‡‡		ND	
(C × B6)F_1	GAT	3	38.2 ± 3.2	<0.001	27,000 ± 2,410	<0.001	ND		ND	

* Mice were injected with 20 μg GAT emulsified in complete Freund's adjuvant containing H37Ra subcutaneously at the base of the tail.

‡ Mice were challenged with 10 μg soluble GAT in 10 μl NaCl intracutaneously in the dorsal surface of the ear, 6 d after immunization, and responses measured by ear swelling 24 h later.

§ PFC responses were determined 7 d after immunization.

‖ See Materials and Methods for immunization, culture, and methods for determining net cpm.

¶ Comparison of B6 and *bm12* DTH responses to GAT are significantly different ($P < 0.001$) as determined by ANOVA.

** Comparison of B6 and *bm12* PFC responses to GAT are significantly different ($P < 0.001$) as determined by ANOVA.

‡‡ Not determined.

usual time period for appearance of Arthus. We feel that the lack of 4-h ear swelling and the ability to transfer the reaction by cells (data not shown) rules out an Arthus-type reaction. Further experiments are in progress to clarify this matter. Second, *bm12* mice show T proliferation to GAT, although they are unable to make an anti-GAT PFC or DTH response. This would indicate that the proliferating T (T_{prolif}) cell population is distinct from the DTH cell, raising the possibility that the genetic lesion in *bm12* mice is not a result of the lack of the T_{prolif} cell is generally held to be a Th cell. The lack of both GAT PFC and DTH responses in *bm12* may not be a result of a lack of T_{prolif}, but of the lack of a suitable target for the Th. The data in Table II show that *bm12* mice can make very good anti-GAT-MBSA PFC responses, indicating that they possess GAT-specific B cells. One would then have to hypothesize that there may be an intermediate cell between the Th and B cell, such as a Th_2 cell. Our laboratories are currently investigating this possibility.

Discussion

Mutant or abnormal genetic variants have been extremely useful in the genetic characterization of many biological systems. The *bm12* variant arose as a gain/loss type spontaneous mutation like other mutants of the *bm* series. However, *bm12* is unique among *bm* mutants in that it is the only strain so far described with a mutation localized to the *I-A*-subregion of the H-2 complex. The *bm12* mutant exhibits a number of functional differences relative to the parental B6 strain with regard to graft rejection (7), Ia antigen expression (8, 12, 13, 31, 32), mixed lymphocyte reaction (8), and loss of responsiveness to various antigens under *Ir* gene control (11, 14–17, 33). This study is the first to report the establishment of responder status as a consequence of mutation (to GT), while causing loss of responsiveness to a closely related antigen (GAT). Our data show that, because of a mutation affecting the A^b_β chain of the I-A molecule, *bm12* mice have the ability to respond to GT and lost the ability to make a PFC or DTH response to the closely related copolymer GAT. This is in sharp contrast to the parental B6 strain, which is a GT nonresponder and a GAT responder. Furthermore, this is the first report of an inbred GT-responder strain.

Previous reports (18, 22) have shown that some outbred Swiss mice were responsive to GT immunization, however, this trait could not be directly attributable to any particular H-2 or non-H-2 genes. In the present study, *bm12* mice have been shown to be PFC responders to GT in contrast to parental B6 mice. The *bm12* and B6 strains differ presumably only by the A_β chain of the I-A molecule. Therefore, we are able to ascribe GT responsiveness to the I-A subregion and, presumably, to the I-A molecule of H-2. At the same time, we have observed a greatly diminished ability to make a GAT-specific DTH responses or GA/GAT-specific PFC responses by *bm12*. GA/GAT responsiveness must also result from the same gene encoding the A_β molecule. Our observation confirms and extends the recent report of Baxevanis et al. (34) by mapping GA responsiveness to the A_β molecule encoded by the I-A subregion. GT responsiveness is transmitted as an autosomal dominant trait (Tables I and IV), much the same as has been reported for GAT responsiveness (19). GT responsiveness does not preclude GAT responsiveness in (C × B6)F_1 mice, Table IV, suggesting that the responses associated with the A_β molecules of both parental haplotypes are seen in the F_1 hybrid mice. This observation is in accord with classical observations that Ia antigens are co-dominantly expressed in F_1 animals (4). The segregation of GT and GA/GAT PFC

responses in (BALB/c × *bm12*)F_2 mice is exactly what one would predict with single or two closely linked genes (Table IV). In fact, the prediction of a [GT nonresponder-GAT responder]:[GAT-GT responder]:[GAT nonresponder-GT responder] ratio of 1:2:1 is not significantly different (χ^2 square = 0.524, $P = 0.773$) from the observed 5:12:4 ratio if only one gene or closely linked genes were involved. However, because of the limited sample size, we cannot exclude the possibility of two unlinked genes yielding a 9:3:3:1 [GAT-GT responder]/[GAT nonresponder-GT responder]/[GT nonresponder-GAT responder]/[GAT-GT nonresponder] ratio. Since evidence exists for I-A control of GA/GAT responsiveness (2, 34) and GT responsiveness (this paper), it is most likely that GT-GA/GAT responsiveness is controlled by a single locus or two closely-linked loci. We are currently making the appropriate backcrosses to resolve this question.

Previous studies have shown a heterogeneity in *Is* gene defects in GT-nonsuppressor strains of mice (24, 25). Briefly, GT-nonsuppressor mice can be divided into two categories depending upon which *Is* gene they lack. H-2^a haplotype mice lack the ability to produce a suppressor T cell factor (GT-TsF_1) after GT injection, but can be readily suppressed when injected with GT-TsF_1. In H-2^b haplotype mice, such as B6, GT injection results in GT-TsF_1 production, although these mice lack a functional target cell for the factor (i.e., Ts_2). In the present study, we asked whether *bm12*, a GT-responder strain, can produce a GT-TsF_1 like parental B6 mice. The data in Table III above show that *bm12* mice do not produce GT-TsF_1. Since B6 GT-TsF_1 bears antigenic determinants of the I-J^b subregion (24), our present data indicate that a gene in the I-A subregion influences production of I-J^b-bearing GT-TsF_1. If the *bm12* variant bears a single mutation affecting only that gene encoding the A_β chain of the I-A molecule, then this same gene or its product(s) must influence gene(s) or the cells expressing gene products of the I-J subregion.

Although *bm12* mice are low or nonresponders to GAT by either the PFC or DTH responses, they must also be classified as GAT responders in the T cell-proliferation assay, when compared with parental B6 mice (Table V). Since we see a close parallel between lack of DTH responses and PFC against GAT in *bm12* mice, it could be argued that the ear swelling reaction reflects an Arthus-type reaction and not DTH. We don't believe this to be the case as we see no significant ear swelling above background within 4 h postchallenge (data not shown). In either case, lack of PFC or DTH responses in *bm12* mice cannot be predicted by the T cell-proliferation assay. A precedent already exists for the disparity between humoral immunity and proliferative responses to antigens under *Ir* gene control. Merryman and Maurer (22) reported that H-2^{b,d,f,k,r,s} haplotype mice make antibody responses to GA, whereas H-2^{j,p,q} do not. Baxevanis et al. (34), using the very same antigen in a T cell proliferation assay, reported that H-2^{r,s} mice are nonresponders to GA. From our present data, we propose several possible mechanisms that may account for the discrepancy between T cell proliferative responses and PFC vs. DTH responses: (*a*) The T_{prolif} is not the efferent Th cell (Th_{eff}) for PFC response, suggesting that the T_{prolif} is an afferent Th (Th_{aff}) cell. This would necessitate a two-step mechanism for help much the same as has been demonstrated for Ts, where a Ts_1 cell induces or stimulates a Ts_2 via TsF_1 (26). In fact, several investigators have proposed models involving multiple Th cells in regulatory cellular circuits for humoral immunity (35, 36) and DTH responses (37). We propose that the Th_{aff} is the GAT T_{prolif} cell and that its ability to incorporate

tritiated thymidine is not under *Ir* gene control and is not affected by the *bm12* mutation. The Th_{eff}, on the other hand, would appear to be the site of the functional *Ir/Is* gene lesion(s); this cell would be defective in its ability to deliver an adequate help signal to the DTH effector cell or to the B cell. Lack of functional Th_{eff} activity could be a result of either clonal deletion similar to the model recently proposed by Schwartz (38) or of active suppression of Th_{eff} function. Clonal deletion would necessitate that pre-Th_{eff} clones bearing receptors for GT/GAT plus A_β in B6/*bm12* mice mimic anti-self and are selectively deleted during ontogeny. It must be restated that the *bm12* does not appear to have a defective GAT-specific B cell, as *bm12* mice make a very good GAT-specific PFC response to GAT-MBSA (Table II). (*b*) If the T_{prolif} is the same as the Th cell for a PFC response, then it must be unable to communicate an effective help signal to the DTH effector cell or the B cell—indicating that the A_β molecule is involved in direct T-T cell (in DTH) or T-B cell (in PFC) interactions, and may serve as a recognition unit for this collaboration. (*c*) GAT proliferative response in *bm12* mice may not reflect the proliferation of T cells directly involved in T cell help, rather they may be cells involved in Ts induction. One would predict that the Ts-inducer cell proliferation would yield a GAT-specific TsF in *bm12* mice. Experiments currently in progress are addressing the question whether *bm12* mice produce GAT-TsF_1. On the hand, *bm12* mice do not make GT-TsF as parental B6 mice do (Table III), indicating that the proposed GT-specific Ts-inducer cell population must express the A_β molecule.

Several ramifications of the *bm12* genetic variation on GAT and GT immune responses become apparent. The alteration of the A_β molecule in *bm12* causes loss of primary GAT PFC and DTH responsiveness, while, at the same time, resulting in GT PFC responsiveness. That a single mutation would have opposite effects on immune responses to closely related antigens suggests a bottleneck in the cellular immune mechanisms responsible for GT and GAT responses. We know that *bm12* mice can make a B cell response to GAT-MBSA, indicating that these mice do not possess defective B cells (Table II). Furthermore, Fathman et al. (14) have shown that (*bm12* × B6)F_1 spleen cells can effectively present GAT to (B6 × A)F_1 GAT- or (T,G)-A--L-reactive T cell clones. They concluded that alteration of the A^b_β chain does not modify the T cell restriction site for some of the GAT epitopes, indicating normal antigen presentation (i.e., macrophage) function for GAT. Similarly, Pierce and Kapp (39, 40) and Clark et al. (41) have demonstrated that macrophages from nonresponder mice can stimulate GAT-specific helper T cells in responder mice. Since neither macrophages nor B cells appear to be responsible for GAT/GT responsiveness, we must assume that the bottleneck lies in the T cell component of the immune response. The evidence presented in this study suggests that the bottleneck is not at the level of the T_{prolif} cell. Strassmann et al. (37) have proposed two distinct Th in (T,G)-A--L DTH responses, one cell acting at the education stage (hence Th_{aff}) and the other at the efferent phase (Th_{eff}) of DTH. With regard to GAT responsiveness, we propose that *bm12* mice are functionally deficient in Th_{eff}, although they possess normal Th_{aff}. This GAT-specific Th_{eff} would be the efferent Th cell (or cells) for both DTH and PFC reactions, and functionally distinct from the Th_{aff} (or T_{prolif}) cell. The GT-specific Th_{aff} and Th_{eff} appear to function normally in the *bm12* strain, whereas one or both of these cells may be deficient in the parental B6 strain. On the other hand, the B6 mouse, a GT-nonsuppressor strain, possesses very inefficient GT-specific Th cells.

Evidence for this comes from the observations that B6 mice treated with cyclophosphamide can make a PFC response upon secondary challenge with GT in vivo (C. Waltenbaugh, unpublished observation) and that in vitro treatment of B6 spleen cells with monoclonal anti-I-J^b antibody allows the development of GT-specific PFC responses (42). We propose the following model to account for GAT/GT responsiveness for both B6 and *bm12* strains: (*a*) Both B6 and *bm12* strains possess normal Th_{aff} (i.e., T_{prolif}) for GAT (*bm12* proliferative T cell responses are, as yet, undetermined for GT). (*b*) That the A_β molecule is necessary for T-T cell interaction/recognition between the T_{prolif} and its target cell(s), either Ts_1 or Th_{eff}, for both GAT and GT. (*c*) Both GAT- and GT-specific Ts and Th_{eff} recognize different regions of the same A_β chain of the I-A molecule with opposite effects. We propose that the GAT-specific Th_{eff} must recognize the same portion of the A_β molecule as GT-specific Ts_1. Therefore, the mutation that would impair the generation of GAT-specific Th_{eff} would also render the *bm12* animal incapable of generating GT-TsF_1. Conversely, a different region of the A_β chain (or the A_α chain) of the I-A molecule, unaffected by the *bm12* mutation, must be responsible for generation of both GT-specific Th_{eff} and GAT-specific Ts_1. If B6 GT-specific Th_{eff} cells are weak or inefficient, they may be easily suppressed by B6 GT-TsF_1 directly without requiring amplifying Ts_2 cells. Pretreatment of B6 mice with either anti-I-J^b or cyclophosphamide may remove this low-level suppression, thus allowing B6 mice to make an anti-GT PFC response. We are currently investigating the possibility that both the *bm12* and B6 strains are capable of making GAT-TsF_1, indicating that neither strain has a defect in its ability to make GAT-TsF_1, as the model would predict. In the (BALB/c × *bm12*)F_1 animal, we see the dominance of response to both GT and GAT, which is in accord with the observations that in (responder × suppressor)F_1 hybrids, response predominates (2, 4, 20). Thus, our model would predict that different antigens may be recognized by different T cell populations in the context of the same Ia gene product.

Summary

The development of inbred strains of mutant mice has proven useful in ascribing specific gene functions to particular genetic loci within the regions and subregions of the H-2 complex. The B6.C-*H-2*^{bm12} (*bm12*) strain is of particular interest in that, compared to parental C57Bl/6Kh (B6) mice, it bears a presumptive single gene mutation altering the A^b_β chain encoded by the I-A subregion. Our data show that *bm12* mice have gained the ability to respond to poly(Glu^{50}Tyr^{50})(GT) and have lost the ability to make plaque-forming cell or delayed-type hypersensitivity responses to the closely related copolymer, poly(Glu^{60}Ala^{30}Tyr^{10})(GAT), although retaining the ability to mount a GAT-specific T cell proliferative response. This is in sharp contrast to the parental B6 strain, which is a GT nonresponder and a GAT responder. Thus, this study is the first to report the establishment of responder status as a consequence of mutation. Possible mechanisms accounting for the gain/loss of GT/GAT responsiveness in the context of a two-step helper T cell model are discussed.

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