B6.C-*H-2*^{bm12}

A New *H-2* Mutation in the *I* Region in the Mouse*

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Mutations in the *H-2* complex in the mouse have proven to be of considerable interest and have led to a clearer conceptual definition of the murine major histocompatibility complex (MHC)[1] (1, 2). Thus far there have been 24 spontaneous *H-2* mutations described, and one which may have been chemically induced (1, 2). These mutations have been detected by the rejection of parental skin grafts and thus may be recorded as a gain, loss or both a gain and loss mutation (1, 2). All of the *H-2* mutants described thus far have been found by the gene-complementation and other, less direct methods, to involve the *H-2K* or *H-2D* loci of the *b*, *d*, *f*, and *k* haplotypes, although *H-2K*^{b} is the most common site of mutation, as 14 *H-2K*^{b} mutants have already been described (1, 2). Another mutant, BALB/c-*H-2*^{dm2}, is of interest in that it is a loss mutation involving the *H-2L*^{d} locus (3). Studies of these *H-2* mutants have revealed: (*a*) extensive T-cell-mediated effects can be produced by alteration of H-2D or H-2K molecules without accompanying serologically detected changes (1–5); (*b*) the T-cell effects are a result of the presence of multiple specificities, possibly in the order of 19 or more (5, 6); and (*c*) the presence of the *H-2L* locus—already described by extensive serological testing (7). The mutants have also been of value in defining the relationship of the T-cell specificities detected in cell-mediated lympholysis (CML) assays involving H-2K, D, and L target cells bearing these antigens in normal, trinitrophenyl-modified and in virus-infected cells (8–10). The *H-2* mutants have clearly given a new dimension in studies of the murine MHC, particularly with regard to phenomenon associated with *H-2K*, *H-2D*, and *H-2L*. We now describe another spontaneous mutation in the *H-2* complex of C57BL/6. The mutation is of the gain and loss type as defined by skin graft-rejection patterns. However, in contrast to the *H-2K*^{b} mutants of C57BL/6, this new mutation appears to have occurred in the *IA* subregion.[2]

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[1] *Abbreviations used in this paper*: CML, cell-mediated lympholysis; *Lad*, lymphocyte-activating determinants; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; RC, rabbit complement; RFC, rosette-forming cell.

[2] The bm^{12} mutant was shown in early tests to have an apparent alteration of the H-2.33 specificity detected by the serum (B10.A × A)F_1 anti-B10.A(5R). It was assumed therefore to be a new mutation in the *K* region and was verbally reported as such by I. McKenzie in meetings at Wood's Hole, Mass., 1978 and London, 1979. This assumption was incorrect as shown by the data presented herein.

Materials and Methods

Mice. The mice used are listed in the Tables.[3] The inbred strains were maintained by strict brother × sister breeding in Melbourne, Australia. The mutant, produced as C57BL/6-*H-2*^{bm12} (henceforth referred to as bm[12]) was freighted to Melbourne from the Harvard Medical School and Warren Radiation Center, New England Deaconess Hospital, Boston, Mass. The bm[12] mutant arose in a (C57BL/6Kh × BALB/cKh)F_1 and the mutation was shown to be in the *H-2*^{b} haplotype of C57BL/6. The original F_1 hybrid mouse bearing the mutation was backcrossed and made congenic with C57BL/6Kh (12).

Serological Studies. Antisera were produced as previously described (3). Some H-2 and Ia antisera were obtained from Dr. J. Ray of the Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md. and are described in the Institute's Catalog of Mouse Alloantisera. Cytotoxicity, and absorption testing using cytotoxicity, were performed as previously described (5). For these tests, selected rabbit sera were used as a source of complement. Rosetting assays for direct testing and absorption were also performed, using sheep anti-mouse IgG coupled to sheep erythrocytes with chromic chloride (11). The spleen cells had their surface Ig removed by capping before use (11). All sera used and their cytotoxicity and rosetting titers are shown in the Tables. Erythrocytes were also tested by the hemagglutination method (3, 5).

Skin Grafting and Complementation Studies. Skin grafts were performed as described elsewhere (3, 11) using either tail skin to dorsum (Melbourne) or tail skin to tail (Boston). To genetically map the site of the mutation, the standard gene-complementation test was performed (2). This involved grafting C57BL/6Kh skin to F_1 recipients formed by mating a C57BL/10 recombinant strain (differing from the *H-2*^{b} haplotype at known loci) with the bm[12] mutant. In these circumstances, graft rejection indicates a failure of the recombinant strain to complement the bm[12] mutant for a C57BL/6 graft, and so the recombinant and mutant strain both lack the necessary *H-2*^{b} gene required for complementation. Graft acceptance indicates that the recombinant strain carries the appropriate *H-2*^{b} gene so that (recombinant × bm[12])F_1 is identical to the parental C57BL/6 strain.

Mixed Lymphocyte Reaction (MLR)

CELL PREPARATION. Spleen and lymph node cells were teased in RPMI-1640 medium (Commonwealth Serum Laboratories, Melbourne, Australia) supplemented with 2.5% human serum, 20 mM Hepes buffer, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM/L-glutamine and 5×10^{-5} M 2-mercaptoethanol. Cell suspensions were centrifuged at 1,000 rpm for 5 min and washed twice.

CULTURE CONDITIONS. Responder lymph node cells were adjusted to 1×10^6 viable cells/ml. Spleen cells at 5×10^6/ml were treated with mitomycin C (Sigma Chemical Co., St. Louis, Mo.) at 25 μg/ml for 25 min at 37°C, washed three times and finally adjusted to 2×10^6/ml and used as stimulating cells in the MLR. Responder cells (0.1 ml) were cultured with 0.1 ml of stimulator cells in U-bottom Cooke microtiter plates (Cooke Engineering Co., Alexandria, Va.). Cultures were set up in quadruplicate incubated at 37°C in a humidified atmosphere of 10% CO_2/90% air for 4 d, labeled with [^{3}H]thymidine (2 μCi/well; 5 Ci/mmol sp act) and incubated for a further 20–24 h. Cells were harvested, using a Skatron multiple cell harvester (Flow Laboratories, Sydney, Australia) on to glass fiber filters (Titertek filter mats, catalogue No. 77-300-06, Flow Laboratories) which were then dried and placed into vials containing 10 ml of scintillation fluid [1.1 g POPOP, (Kochlight Laboratories, Buckinghamshire, England) 5 g PPO (Packard, Melbourne, Australia) per l of toluene].

Results

Description of bm^{12} *Mutant.* The bm[12] mutant was first detected as a gain and loss mutation in grafting between (C57BL/6 × BALB/c)F_1 mice. The mutation was

[3] The most recent listing of all available mutants, their haplotypes, properties and origin will be found in (*a*) Kohn, H. I., J. Klein, R. W. Melvold, S. G. Nathenson, D. Pious, and D. C. Shreffler. 1978. The first H-2 mutant workshop. *Immunogenetics.* **8**:279 and (*b*) Melvold, R. W. 1979. Mutant H-2 haplotypes of the H-2 gene complex. *In* FASEB Handbook of Inbred and Genetically Defined Strains of Laboratory Animals. 129.

TABLE I
*Graft Rejection Studies in the bm^{12} H-2 Mutant Strain**

Donor	Recipient	Median survival time	Conclusion
		d ± *SD*	
C57BL/6Kh	bm^{12}	16.2 ± 2.8	Loss mutation in bm^{12}
C57BL/6	(bm^{12} × LP.RIII)F$_1$	12.2 ± 1.2	Loss mutation in bm^{12}
C57BL/6	bm^{12} (immune)‡	7.2 ± 1.4	Loss mutation in bm^{12}
bm^{12}	C57BL/6	14.8 ± 2.1	Gain mutation in bm^{12}

* Groups of 6–10 female mice grafted with tail skin from donors of the same sex.
‡ Mice immunized by a preceding skin graft.

shown to be in the *H-2^b* haplotype of C57BL/6 (see below) and the strain was then made homozygous on a C57BL/6Kh background. Both C57BL/6 and bm^{12} reject each other's skin grafts in 14–16 d (Table I) indicating that the mutation is of both the gain and loss type, and further, the mutation has induced histogenic changes of similar strength to the strongest responses found with the *H-2K^b* mutants (4).

Mapping the bm^{12} Mutation to the IA subregion of C57BL/6. The results of the original studies (12) and of the complementation studies indicate several points of importance (Table II). Firstly, the finding that the (B6.C-*H-2^d* × bm^{12})F$_1$ hybrid rejects C57BL/6 grafts indicates that the mutation is not in the *H-2^d* haplotype of BALB/c but has occurred in the *H-2^b* haplotype, particularly as the original F$_1$ mutant rejected C57BL/6 and accepted BALB/c grafts. Secondly, crosses with other *H-2^b* mutants indicate that the bm^{12} mutant occurred at a third mutational site within the *H-2^b* haplotype. This was shown by the cross with bm^{13} which we have mapped to *H-2D^b* (G. M. Morgan, H. Dellos, I. F. C. McKenzie, D. W. Bailey, and R. W. Melvold. Studies of the bm^{13} and bm^{14} mutants of C57BL/6. Manuscript in preparation.). The ability of bm^{13} to complement the bm^{12} mutant indicates that the bm^{12} mutation occurred at a site other than *H-2D^b* locus. Crosses with four different *H-2K^b* mutants (bm^1, bm^5, bm^6, and bm^8), which are all mutant alleles at the one (*H-2K^b*) mutational locus, also complemented the mutant bm^{12} strain for a C57BL/6 graft. These results demonstrate that this second mutational site, probably *H-2K^b* itself, is also not the site of the bm^{12} mutation, which must therefore have occurred at a third *H-2^b* mutational site, distinct from both *H-2K^b* and *H-2D^b*. Complementation occurred in the cross with B10.A(5R) which localized the mutation either to *K^b*, *IA^b* or, *IB^b* subregion. Finally, complementation studies in two other crosses with B10.A(4R) or D2.GD excluded the *IB^b* subregion. The bm^{12} mutation therefore occurred in either the *IA^b* subregion or in the *H-2K^b* region, but at a different site to the *H-2K^b* locus. (This was our first, but incorrect conclusion.[2]) However, from the serological evidence it is extremely likely that the mutation occurred in the *IA* subregion.

Serological Studies Comparing C57BL/6 and bm^{12} with H-2 Alloantisera. The sera used and the results obtained are shown in Table III. When tested directly using cytotoxicity with rabbit serum as a source of complement, all sera, with the exception of an H-2.33 antiserum gave identical or similar results with the C57BL/6Kh parental strain and the bm^{12} mutant, i.e., there was no difference in the reaction of the two strains with sera detecting the H-2.2, 5, 27, 28, 29, 35, and 36 specificities (Table III). With the H-2.33 antisera, several different patterns of reaction were observed with the two different antisera (Table III and Fig. 1). The D33 and AS924 sera both gave

TABLE II

Complementation Studies to Map the Site of the bm[12] Mutation

Strains crossed with bm[12] to make F_1 hybrid recipients for C57BL/6 skin grafts*	H-2 haplotype									Rejection of parental C57BL/6 graft	Complements (C) or not (NC)	Conclusion
	K	*IA*	*IB*	*IJ*	*IE*	*IC*	*S*	*G*	*D*			
B6.C-H-2[d]	d	d	d	d	d	d	d	d	d	yes	NC	Mutation not in *H-2[d]*
C57BL/6	b	b	b	b	b	b	b	b	b	no	—	Control
bm[13]	b	b	b	b	b	b	b	b	b‡	no	C	Mutation not in *H-2D[b]*
bm[1], bm[8], bm[5], bm[6]	b‡	b	b	b	b	b	b	b	b	no	C	Mutation not in same locus as *H-2K[b]* mutants, therefore probably not in *H-2K[b]*
B10.A(5R)	b	b	b	k	k	d	d	d	d	no	C	Mutation in *K[b]*, *IA[b]*, *IB[b]*
B10.A(4R)	k	k	b	b	b	b	b	b	b	yes	NC	Mutation in *K[b]* or *IA[b]*
D2.GD	d	d	b	b	b	b	b	b	b	yes	NC	Mutation in *K[b]* or *IA[b]*

* The strains listed in the column were crossed with bm[12] and the F_1 hybrid grafted with C57BL/6Kh skin from a donor of the same sex. Groups of 6–10 mice were used. Grafts either did not reject in periods of observation >60 d or were rejected in 12–18 d.

‡ Mutational site.

similar direct reaction patterns and titers. These two sera, made as (B10.D2 × A)F_1 anti-B10.A(5R) could potentially contain anti-Ia.9 and 20 antibodies. The titer of bm[12] with these sera was lower than found in C57BL/6 although the shape of the titration curve did not differ in the two strains. Furthermore, AS924 absorbed with A.TH, to remove the Ia.9 antibody, gave similar titers on both C57BL/6 and bm[12]. Clearly the H-2.33 specificity is represented equally on both strains. However two other sera, AS1117 and a similar serum (kindly provided by Dr. C. S. David, Mayo Clinic, Rochester, Minn.) and made as (B10.A × A)F_1 anti-B10.A(5R) gave a different reaction (Fig. 1). These sera could and do contain anti-Ia antibodies. On testing the parental C57BL/6 strain, there were two plateaus of reaction: (*a*) >90% lysis indicative of lysis resulting from H-2.38 antibodies, and (*b*) ~60% lysis, presumably a result of the Ia antibodies. When these sera were tested on the bm[12] mutant, only one plateau with >90% lysis was observed and the second Ia plateau was absent (Fig. 1). This finding suggested that the bm[12] mutant was not reacting with the anti-Ia component in these sera and therefore might have altered Ia specificities, the alteration being produced by the mutation. Further studies with the H-2 antisera involved testing by absorption using the same sera. Except for the H-2.33 sera, identical absorption patterns were obtained with all the H-2 antisera. Two of these are shown in Fig. 2 a, b; H-2.5, a public *H-2[b]* specificity, and the serum D28 contains antibodies to the H-2.28 family of specificities. There is obviously no difference in the absorption capacity of either strain for serum D28 (Fig. 2 a) or for D5 (Fig. 2 b) and identical absorption of C57BL/6 and bm[12] were apparent for the other H-2 specificities (data not shown). By contrast, the two different anti-H-2.33 sera gave different absorption patterns (Fig. 3 a, b). One serum, AS924, demonstrated that bm[12] had slightly less absorptive capacity when compared with C57BL/6 (Fig. 3 a). The other serum (from C. S. David) gave very different results in that C57BL/6 could absorb

TABLE III

Comparison of C57BL/Kh and bm^{12} Using H-2 Alloantisera

Specificity	H-2K/D	Antiserum No.	Other specificities potentially reactive with *H-2^b*	Donor	Recipient	Cytotoxic titer on spleen cells	
						C57BL/6*	bm^{12}
Public							
H-2.5	K	D5	—	B10.PL(73NS)	(B10.M × DBA/2)F$_1$	1/32	1/64
		AS235	—	B10.PL(73NS)	(B10.M × DBA/2)F$_1$	1/32	1/64
H-2.27, 28, 29	K/D	D28	Ia.9, H-2.36	A.SW	(A.CA × B10.BR)F$_1$	1/128	1/128
		AS274	Ia.9, H-2.36	A.SW	(A.CA × B10.BR)F$_1$	1/128	1/128
		AS834	H-2.2, 56	B10.A(2R)	(B10.BR × LP.RIII)F$_1$	1/256	1/256
		D28b	H-2.2, 56	B10.A(2R)	(B10.BR × LP.RIII)F$_1$	1/64	1/64
		D29	H-2.56	AKR.M	(SJL × AKR)F$_1$	1/32	1/32
		AS276	H-2.56	AKR.M	(SJL × AKR)F$_1$	1/128	1/128
H-2.35	K	D35	—	B10.Y	(B10.A(2R) × A.CA)F$_1$	1/128	1/128
		AS190	—	B10.Y	(B10.A(2R) × A.CA)F$_1$	1/64	1/64
H-2.36	K	D36	Ia.9	A.SW	(B10.A(2R) × A.CA)F$_1$	1/8–1/16	1/8–1/16
Private							
H-2.2	D	AS946	H-2.56	B10.A(2R)	(B10.D2 × A)F$_1$	1/128	1/128
H-2.33	K	D33	H-2.39, 53, 54, 62, Ia.9, 20	B10.A(5R)	(B10.D2 × A)F$_1$	1/800	1/400
		AS924	H-2.39, 53, 54, 62, Ia.9, 20	B10.A(5R)	(B10.D2 × A)F$_1$	1/512	1/512
		AS924 abs. A.TH	Ia.20	B10.A(5R)	(B10.D2 × A)F$_1$	1/512	1/512
		C. S. David	Ia.8, 9, 20	B10.A(5R)	(B10.A × A)F$_1$	1/128	1/128
		AS1117	Ia.8, 9, 20	B10.A(5R)	(B10.A × A)F$_1$	1/128	1/64

* C57BL/6 has these specificities: H-2: 2, 5, 27, 28, 29, 33, 25, 26; Ia: 3, 8, 9, 15, 20.

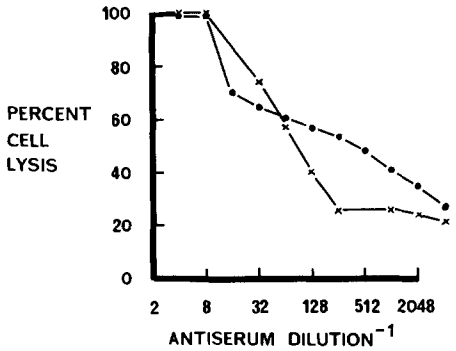

FIG. 1. Direct cytotoxicity testing on C57BL/6 (●) and the bm^{12} mutant (×). The percentage of lysis is shown and dilution ($\log_2$). C57BL/6 gives two plateaus of reaction: one with 90% lysis, one with ~60% lysis. The mutant bm^{12} has only the one plateau at 90% lysis. The rabbit complement (RC) control (not shown) was <20% lysis.

this serum, but the bm^{12} mutant had little absorptive capacity (Fig. 3b). (These results caused us initially to consider bm^{12} to be a new *H-2K^b* mutant. See footnote 2.) The results with the H-2 sera suggest that all the H-2 specificities (except H-2.33) were identical in the C57BL/6 and bm^{12} mutant and that a specificity in the H-2.33 serum, probably not H-2.33 itself, was lacking in the bm^{12} mutant. Testing by hemagglutination revealed no differences between C57BL/6Kh and bm^{12}.

Serological Studies with Anti-Ia Sera. When the parental C57BL/6Kh and bm^{12} mutant were examined with sera of the known Ia specificities it was apparent that the

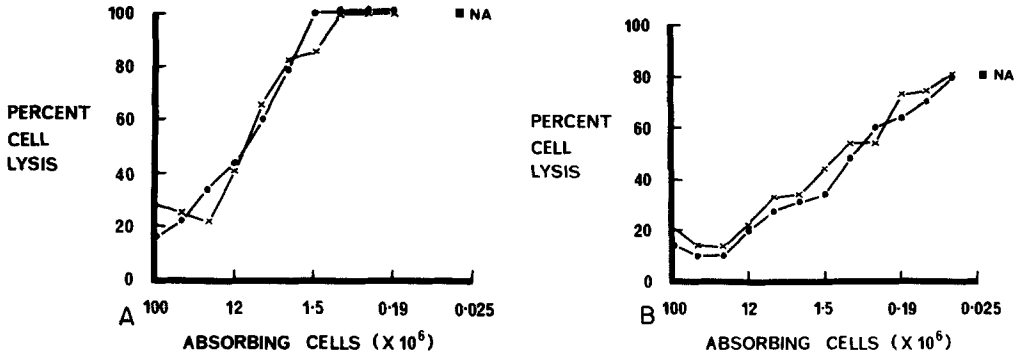

FIG. 2. Absorption of two H-2 antisera with C57BL/6 (●) and bm[12] (×), and testing, by cytotoxicity on C57BL/6 spleen cells showing the percentage of lysis and the number of absorbing cells used. (■) nonabsorbed control (NA). The RC control was <20%. (a) absorption of serum D28; (b) absorption of serum D5. Both strains absorb equally.

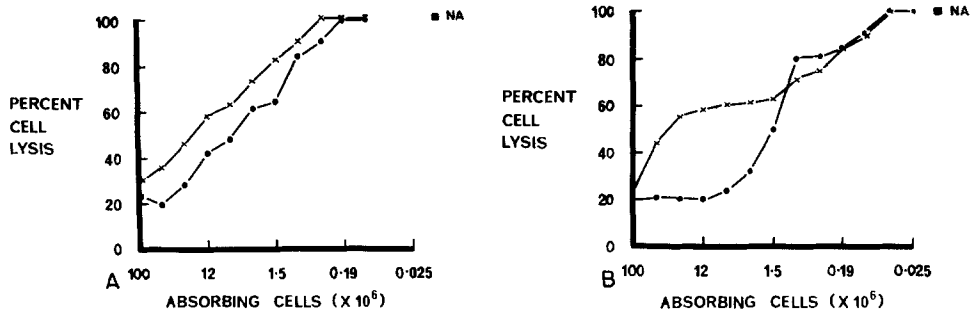

FIG. 3. Absorption of two different H-2.33 antisera with C57BL/6 (●) and the bm[12] mutant (×) and testing back on C57BL/6 by cytotoxicity. The nonabsorbed sera (■, NA) gave >90% lysis. (a) absorption of AS924: (B10.D2 × A)F_1 anti-B10.A(5R); (b) absorption of (B10.A × A)F_1 anti-B10.A(5R).

mutant strain had a marked decrease or absence of the Ia^b specificities (Table IV, Figs. 4–8).

Ia.3, 15. The serum A.TH anti-A.TL (AS1070), containing Ia.3, 15 antibodies reactive with C57BL/6 had a reduced cytotoxic reaction with bm[12] (titer 1/32) compared with C57BL/6 (titer 1/256) (Table IV, Fig. 4a), but this was not so apparent with the more sensitive rosetting procedure (Table IV, Fig. 4b, i.e., titers of 1/1,500 vs. 1/3,000). A similar result was obtained using AS760 (B10.HTT × A.SW)F_1 anti-A.TL. The known specificities in these sera were then examined individually.

Ia.3. The Ia.3 specificity was tested by absorption using the A.TH anti-A.TL serum with B10.T(6R) as the target cell ($Ia.3^+15^-$). The results are shown in Fig. 5 where it is clear that the mutant strain has a decreased amount of this specificity although it still appears to be present. By comparing the number of cells required to absorb an equal amount of antibody, bm[12] carries ¼–⅛ of the Ia.3 antigen that C57BL/6Kh carries.

Ia.15. The Ia.15 specificity was tested directly with AS767 where the bm[12] mutant was nonreactive by cytotoxicity and only weakly reactive by rosetting (Table IV, Fig. 6a). These reactions were also examined by absorption (Fig. 6b) and this demon-

TABLE IV

*Reactions of C56BL/6 and bm^{12} Mutant with Anti-Ia Sera**

Specificity	Other Ia specificities present in sera‡	Antiserum No.	Donor	Recipient	Titers			
					C57BL/6		bm^{12}	
					Cytotoxic	RFC	Cytotoxic	RFC
Sera reacting with I^b (Ia.3, 8, 19, 15, 20)								
Ia.3, 15	1, 2, 7, 22	AS1070	A.TL	A.TH	1/256	1/3000	1/32	1/1500
Ia.8	16	AS742	B10.D2	(B10.A × A)F$_1$	1/128	1/640	0–1/4	1/32
Ia.9	4, 5, 12	AS935	A.TH	(A.TL × AKR)F$_1$	1/32	1/64	0	0–1/16
Ia.20(?)	8, 9	AS1117 absorbed with E14	B10.A(5R)	(B10.A × A)F$_1$	1/128	1/320	1/16	1/20
Ia.15	2, 19, 22	AS767	A.TL	(A.TH × B10.RIII[71NS])F$_1$	1/16	1/64	0	1/2
?	—	AS1070 absorbed with bm^{12}	A.TL	A.TH	1/64	1/640	0–1/4	1/160
Ia.3, 15	1, 2, 19	AS760	A.TL	(B10.HTT × A.SW)F$_1$	1/32	—	1/8	—
Ia.8	Monoclonal antibodies from G. Hämmerling				1/16	—	0	—
Ia.9	Monoclonal antibodies from G. Hämmerling				1/16	—	0	—
Sera reacting with specificities not usually found in I^b								
Ia.1, 2	—	NIH	A.TL	(A.BY × B10.HTT)F$_1$	0	0	0	0
Ia.4	5, 12	NIH	A.TH	(A.TL × B10.A(5R))F$_1$	0	0	0	0
Ia.6	—	AS915 absorbed with CBA§	B10.(2R)	(B10.A(4R) × 129)F$_1$	0	0	0	0
Ia.7	22	NIH	B10.A(5R)	(C57BL/10 × HTT)F$_1$	0	0	0	0
Ia.10	5, 10, 13, 16	AS133	B10.T(6R)	(C57L × AQR)F$_1$	0	0	0	0
Ia.11	16, 23	AS307	B10.D2	(C57BL/6 × A)F$_1$	0	0	0	0
Ia.13	5, 21	AS681	BDP	(C57BL/6 × A)F$_1$	0	0	0	0
Ia.17	1, 2, 6, 7, 15, 18, 19, 22	AS538	AQR	B10.T(6R)	0	0	0	0
Ia.22	6, 7	AS851	B10.A(2R)	(B10.A[4R] × 129)F$_1$	0	0	0	0
Ia.7	6, 22	—	B10.A(2R)	B10.A(4R)	0	0	0	0

* Assignment of specificities and combinations used are according to published charts (21).

‡ All of the sera listed react with the donor; the specificities in this column could potentially be present; not all are present; e.g., AS538 contains no Ia.15 reactivity as C57BL/6 is negative.

§ The Ia.6 specificity is difficult to produce and the sera usually contain only Ia.7, 22. This serum does contain an Ia.6 antibody for, after absorption with CBA, it is B10.D2(+).

strated that bm^{12} carries 1/32–1/64 the amount of Ia.15 carried by C57BL/6. This specificity was also examined by using the A.TH anti-A.TL serum with D2.GD target cells (Ia.3$^-$15$^+$) so that absorption resulting from the Ia.15 specificity only is examined (Fig. 6c). It was found that bm^{12} has a very low absorptive capacity (1/64th) compared with C57BL/6Kh. The Ia.15 specificity is therefore present in very small amounts, and may indeed be absent.

Ia.8. Testing for the Ia.8 specificity with AS742 demonstrated an extremely weak reaction with bm^{12} by cytotoxicity (0–¼ on different occasions) (Table IV, Fig. 7a). In addition direct testing using a monoclonal Ia.8 antibody (Table IV), C57BL/6Kh was found to be reactive (titer 1/16), whereas bm^{12} gave a barely detectable response (0—½ titer). (This antibody was provided by Dr. G. Hämmerling, Institute for Genetics, University of Cologne, Cologne, Federal Republic of Germany; full details of these tests will be provided elsewhere.) Direct testing by rosetting indicated that bm^{12} gave a greater reaction than by cytotoxicity (Fig. 7b). However, when testing the AS742 serum on C57BL/6 after performing quantitative absorptions with bm^{12}

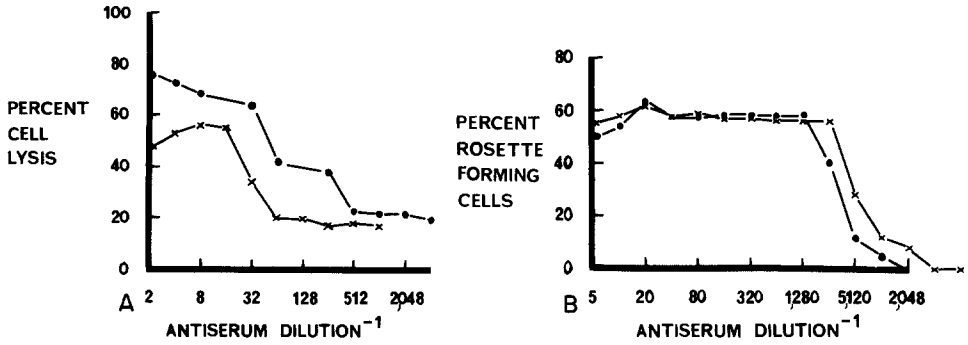

FIG. 4. Direct testing of A.TH anti-A.TL serum on C57BL/6Kh (●) and the bm^{12} mutant (×). (a) testing by cytotoxicity, RC control <20% (b) rosetting testing.

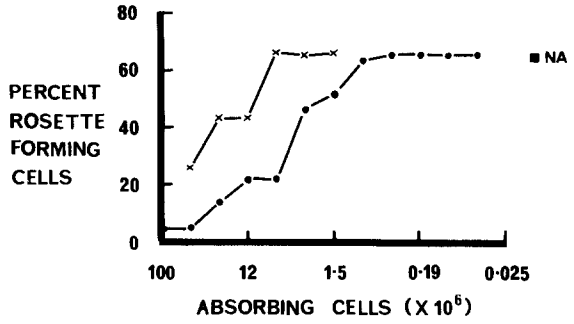

FIG. 5. Absorption testing for Ia.3 using A.TH anti-A.TL antiserum on B10.T(6R) target cells, and testing by inhibition of RFC. C57BL/6 (●) bm^{12} (×). (■) not absorbed (NA).

and C57BL/6 (Fig. 7c), it was clear that bm^{12} carried no Ia.8 specificity, especially by rosetting (Fig. 7b). The weak reaction of the serum directly on bm^{12}, which is Ia.8$^-$ by absorption, indicates that there are other antibodies in this serum, reactive with the bm^{12} mutant. This is considered further below and studies are currently in progress to define these specificities.

Ia.9. The Ia.9 specificity could not be detected on the bm^{12} mutant by cytotoxicity using AS735 (Table IV) but gave a weak reaction by rosetting (Fig. 8a). Absorption testing indicated that the bm^{12} serum carried (1/16–1/32) the amount of Ia.9 as the parental C57BL/6 strain (Fig. 8b). A monocloncal anti-Ia.9 antibody (obtained from Dr. G. Hämmerling) was also nonreactive on the bm^{12} mutant (Table IV).

Ia.20. The Ia.20 specificity was tested for by using AS1117: (B10.A × A)F$_1$ anti-B10.A(5R) absorbed with EL4 to remove H-2 antibodies. The serum could putatively contain antibodies to Ia.8, 9, 20; it was nonreactive with bm^{12} but still reacted with C57BL/6 (titer 1/64) and we therefore presume that Ia.20 is also missing but we have no independent proof that the Ia.20 specificity is represented in this antisera.

The results presented in this section indicate that a dramatic alteration has occurred in the structure of the *IA*-coded Ia-bearing molecule in that the specificities, Ia.3, 8, 9, 15, and possibly 20 are either totally absent, or present in very much reduced amounts in the bm^{12} mutant. The loss mutation detected by skin grafting therefore has a serological counterpart in that there are losses of the Ia^b specificities. Is there any evidence of other losses or of new or gained specificities in the bm^{12} mutant? We

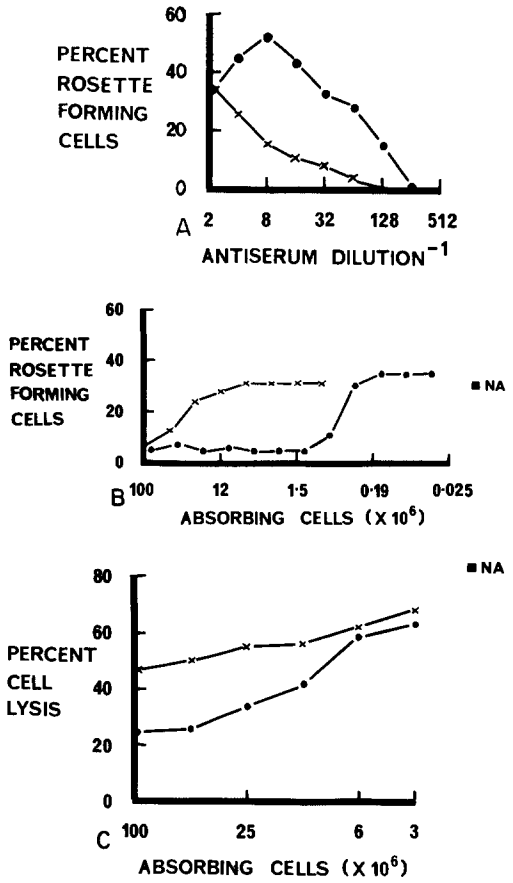

FIG. 6. Testing for the Ia.15 specificity with C57BL/6 (●) and the bm^{12} mutant strain (×) using AS767 (Ia.15) (a, b) with C57BL/6 target cells, and AS1070 with D2.GD target cells (c). In (a), direct testing by rosetting is shown and in (b), testing is shown by absorption using the same assay system. In (c), absorption using cytotoxicity is recorded. (■) nonabsorbed (NA) serum reaction.

sought these answers by three different approaches. Firstly, C57BL/6 and bm^{12} were tested with a range of antisera detecting known Ia specificities of other haplotypes, e.g., for Ia.1, 2, 4, 6, 7, 10, 11, 13, 15, 16, 17, 18, 19, and 22. All were nonreactive on both C57BL/6 and the bm^{12} mutant, although not all the sera used can be guaranteed to contain all the specificities listed. However the mutation did not appear to give rise to a new specificity which had previously been described. The second approach involved absorbing sera with the bm^{12} mutant and testing on C57BL/6. Although these studies are still in progress, it is clear that serum 1070 (A.TH anti-A.TL) does contain extra specificities, which can especially be detected by the rosetting procedure (Table IV; Figs. 4a, b). This was confirmed when this serum, absorbed with bm^{12}, still gave a substantial reaction on C57BL/6 (Table IV). The third approach involved immunization. In several different combinations extensive immunizations were performed but no anti-Ia antibody resulted (Table V). This result is not too surprising as it has proven difficult to produce antisera by immunizing between mutant and parental strains (5).

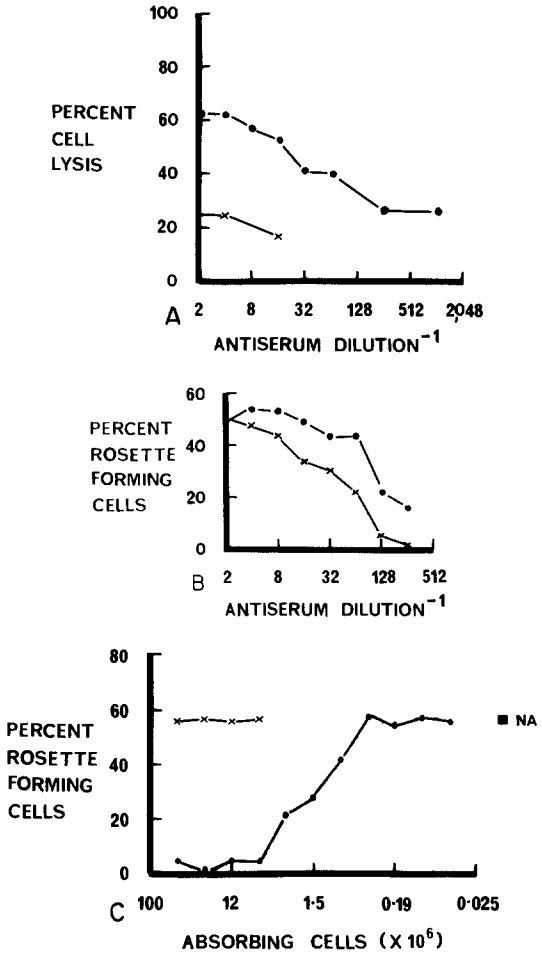

FIG. 7. Testing for the Ia.8 specificity with AS742 on C57BL/6 (●) and bm[12] (×). (a) direct testing by cytotoxicity, RC control <20%; (b) direct testing by rosetting; (c) absorption testing on C57BL/6 target cells, by rosetting. (■) number of RFC with nonabsorbed sera (NA).

The bm[12] Mutant has Normal B-Cell Numbers. As Ia specificities were detected only with difficulty in the bm[12] mutant, it could be considered that this mouse (which survives as a healthy strain but which breeds poorly) had low numbers of B cells—the cell bearing Ia specificities in mouse spleen. Several spleen cell suspensions were examined for the proportion of Ig$^+$ rosette-forming cells (RFC) using sheep anti-mouse Ig coupled to sheep erythrocytes. The results (percentage of Ig$^+$ cells) were: C57BL/6 (52–58%); bm[12] (51–56%); i.e., the bm[12] mutant has a normal content of B cells in the spleen.

MLR Studies with C57BL/6 and bm[12]. The MLR data is shown in Table VI. The mutation in bm[12] has not only led to histocompatibility and serological changes in the *IA* subregion, but also to an alteration that leads to a reaction in the mixed lymphocyte culture. Firstly, bm[12] spleen cells are able to stimulate C57BL/6 lymph node cells (experiment 1, Table VI), the degree of stimulation being similar to those obtained when either C57BL/6 (experiment 7) or bm[12] (experiment 8) stimulate

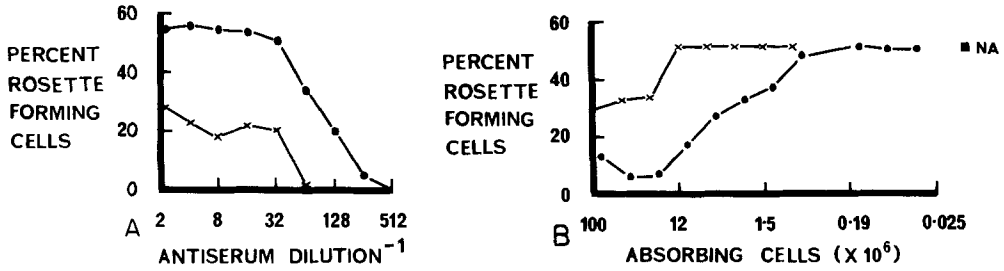

FIG. 8. Testing for the Ia.9 specificity with AS935 by rosetting on C57BL/6 (●) and bm^{12} (×). (a) direct testing; (b) absorption testing on C57BL/6. (■) nonabsorbed (NA) serum reaction.

B10.A(4R) or when B10.A(4R) stimulates C57BL/6 (experiment 5). In the experiments involving B10.A(4R), there is a strong degree of stimulation as a result of lymphocyte-activating determinants (*Lad*) loci present in both the *K* and *IA* subregion so that the stimulation of C57BL/6 produced by bm^{12} (experiment 1) is as great, or greater than that produced by a K + IA region difference. In experiment 1, C57BL/6 is presumably responding to the gained specificities on bm^{12}, as bm^{12} is both a gain and loss mutation (Table I). In the reciprocal experiment (experiment 3, Table VI), bm^{12} is able to respond to C57BL/6, although in this study, not as strongly as does C57BL/6 to bm^{12}. The presumption in this experiment is that bm^{12} has lost specificities, which are present in the parent C57BL/6 and which lead to stimulation. It should be noted that B10.A(4R) both stimulates, and responds to C57BL/6 and bm^{12} in a similar manner (experiments 5–8). It is clear that the one mutational event has simultaneously effected the MLR (*Lad* loci) as much as the other parameters discussed above.

Discussion

The experiments reported here described the bm^{12} mutant—a spontaneous mutant occurring within the *H-2^b* complex of strain C57BL/6Kh. The mutation is of the gain and loss type—defined by skin grafting in that bm^{12} rejects parental C57BL/6 grafts (loss mutation) and C57BL/6 rejects bm^{12} skin grafts (gain mutation). The mutation presumably a single gene mutation,[4] has been mapped by the standard complementation method to the *K^b* or *IA^b* regions of the *H-2* complex (Table I). Further complementation studies with a series of *H-2K^b* mutants (Table I) have demonstrated that the mutation is not in the *H-2K^b* locus itself, but does not exclude the mutational site being adjacent in the *K* region. Only studies with newer recombinant strains will solve this question. However, the mapping by complementation also includes the *IA* subregion and in this subregion is another histocompatibility locus, *H-2IA*, and it is entirely possible that the bm^{12} mutation has effected this gene, particularly as there is also clear evidence for an alteration in the Ia specificities which are coded for by genes in the *IA* subregion. Taken together, the complementation and serological

[4] Whether bm^{12} has a point mutation is not clear at present. Most of the *H-2K^b* mutants appear to have single amino acid substitutions in their sequence indicating they probably arose as a point mutation (2, 13). By contrast, another mutant—*H-2^{dm1}* appears to have a frame shift or other type of mutation as there is an alteration in the *D^d* and *L^d* molecules revealed both serologically and by amino acid sequence studies (14, 15). Whether bm^{12} is a point mutation or involves a more extensive gene alteration awaits further serological study and amino acid analysis.

TABLE V

Attempts to Produce Antisera by Immunizing Between Parental C57BL/6 and the bm[12] Mutant

Antiserum No.	Donor	Recipient	Titers*	
			Cytotoxic	Rosetting
—	bm[12]	C57BL/6	0	0
AS1150	bm[12]	(C57BL/6 × LP.RIII)F_1	0	0
AS1180	bm[12]	(C57BL/10 × 129)F_1	0	0
—	C57BL/6	bm[12]	0	0
AS1074	C57BL/6	(bm[12] × LP.RIII)F_1	0	0
AS1097	C57BL/6	(BALB/c × bm[12])F_1	0	0

* Target cells were that of the donor used for the immunization.

studies place the mutational site in the *IA* subregion. The serological studies, performed both directly and by absorption with H-2 and Ia sera, and using two different techniques (cytotoxicity and rosetting) demonstrate very clearly that the bm[12] mutant has an extensive alteration in the *IA* subregion Ia specificities, but not in H-2K[b] (or D[b]) specificities. The initial studies were misleading (Figs. 3 a, b) as they demonstrated an alteration in the H-2K.33 specificity. However, this antiserum also contains the Ia.9 specificity and subsequent tests revealed that H-2.K33 of bm[12] and C57BL/6 were identical, but that the bm[12] lacked the Ia.9 specificity. All other direct and absorption studies with H-2 antisera to private and public H-2[b] specificities also demonstrated an identity of C57BL/6 and bm[12]. Although these findings with H-2 antisera do not prove that the mutation is in a site other than the *H-2K[b]* locus (because serologically detected alterations in H-2.33 in the *H-2K[b]* mutants are difficult to detect [1, 2]), with the accompanying demonstrable alteration in the *IA* subregion specificities, there is compelling evidence to conclude that the mutation occurred in the *IA* subregion and furthermore, in the *Ia-1* gene which codes for the Ia specificities Ia.3, 8, 9, 15, 20. All of these specificities appeared to be altered in some way so that they are either absent, or have a reduced amount in bm[12] compared with C57BL/6. These specificities are very likely to be on the same molecule, although coprecipitation analysis with sera to all specificities has not been simultaneously performed to conclusively demonstrate this. For example, Ia.8 and 9 have been shown by coprecipitation, to be on the same molecule coded for by the *IA* subregion (16). The same was shown to be true for specificities Ia.8, 9, and 20 (17). Specificity Ia.3 was mapped to the *IA[b]* subregion by coprecipitation with Ia.8 and 9 (17); and Ia.15, originally believed to be coded for by the *IE* subregion, was mapped to the *IA[b]* subregion because it also coprecipitated with specificities Ia.8 and 9 (18). It is therefore reasonable to conclude that specificities Ia.3, 8, 9, 15, and 20 are on the same molecule coded for by the *IA[b]* subregion. In bm[12], the specificities Ia.8, 9, 15 appear to be totally absent and Ia.3 and possibly Ia.20 present in reduced amounts. At this time, it is not clear whether Ia.3, 20 are indeed reduced, or absent, as the sera to these specificities are complex. It may be that multiple specificities are present, some decreased or absent in bm[12], e.g., Ia.3, 20, and others not previously detected may be present in similar amounts in C57BL/6 and bm[12]. This may explain the high activity of the polyspecific A.TH anti-A.TL antiserum, especially by the rosetting technique,

TABLE VI

MLR Reactions of C57BL/6 and bm^{12}

Experiment No.	Source of responder lymph node cells (1 × 10^6/ml)	Source of stimulator spleen cells (2 × 10^6/ml)	H-2 difference	[^{3}H]Thymidine uptake	Stimulation index
				mean cpm ± SE	
1	C57BL/6	bm^{12}	IA (gain)*	44, 830 ± 1,230	25.6
2	C57BL/6	C57BL/6	—	1,753 ± 527	—
3	bm^{12}	C57BL/6	IA (loss)‡	17,227 ± 1,892	18
4	bm^{12}	bm^{12}	—	956 ± 214	—
5	C57BL/6	B10.A(4R)	K^k, IA^k	34,688 ± 2,834	19.8
6	bm^{12}	B10.A(4R)	K^k, IA^k	30,520 ± 2,105	31.9
7	B10.A(4R)	C57BL/6	K^b, IA^b	44,978 ± 1.788	36.1
8	B10.A(4R)	bm^{12}	K^b, IA^b*	49,991 ± 4,698	39.3
9	B10.A(4R)	B10.A(4R)	—	1,246 ± 236	—

* C57BL/6 is presumably responding to the gained specificities in bm^{12}, as is B10.A(4R).

‡ bm^{12}, being a loss (as well as a gain) mutation is able to respond to these lost specificities present in C57BL/6.

which could in fact contain the elusive specificities to the thus-far undescribed *IE^b* and *IC^b* subregions. (The Ia specificities described so far for *H-2^b* all map in the *IA^b* subregion.) Further studies, currently in progress in our laboratory should provide an answer to this problem. However, it suffices for this initial serological description of the bm^{12} mutant to categorically state that the mutation has caused a partial to complete loss of all of the *IA^b* specificities. Furthermore, we have been unable to precipitate an Ia bearing chain with anti-Ia.8 or 9 antisera from the ^{125}I-radiolabelled cell surface of the bm^{12} mutant, in comparison with C57BL/6 (McKenzie et al. Manuscript in preparation.).

What then of the gain mutation which leads to graft rejection of bm^{12} by C57BL/6? We have used several approaches to study this. Firstly, antisera to almost all known Ia specificities (Table IV) were tested on C57BL/6 and bm^{12}. All were nonreactive by both cytotoxicity and rosetting, so that alteration to another known specificity, or mutation of the *Ia-1^b* allele to another known allele has not occurred. Rather, the mutation had led to the appearance of a new (*Ia-1^{bm}*) allele at this locus. The transition from one known allele to another has not been discovered for the *H-2* mutants, but there is recent and conflicting evidence that such changes can occur in tumours (19) and tumour cell variants (19, 20). The second approach to detect the gained specificities was standard: immunizations were performed between bm^{12}, C57BL/6, or F_1 hybrids (Table V) to attempt to produce an anti-bm^{12} cytotoxic or rosetting antibody. All such attempts have failed thus far but this is the usual finding (1) and we are continuing different approaches to attempt to produce specific anti-bm^{12} Ia antibodies. The immunization in the reverse direction such as bm^{12} anti-C57BL/6 also failed to produce an Ia.8, 9, or any other cytotoxic antibody. Attempts to introduce a different genetic background into these immunization procedures was also without effect (Table V) and it should be noted that except for the BALB/c-*dm^1* and *dm^2* mutations—where there is an alteration or loss of D^d + L^d and L^d molecules respectively, almost all attempts at immunization in mutants have failed to produce such an antibody (3). The MLR data (Table V) indicate that in addition to inducing

histocompatibility, and serological alterations in *IA* subregion specificities, there is also the induction of surface molecules which stimulate the parental C57BL/6 strain. Furthermore, the degree of stimulation is similar to that achieved with differences arising of the whole *H-2* complex (21–23), or more particularly, with known differences in the *IA* or *K* + *IA* regions (Table VI). At this stage, it should be noted that the strongest MLR-stimulating determinants are coded for by the *Lad-1* locus in the *IA* subregion and other *Lad* loci are found in *K* and *D* regions, in *IJ*, *IC/E*, and the non-*H-2* linked *Mls* locus (23). The finding that bm[12] stimulates C57BL/6 to respond equally well or better than B10.A(4R), a difference of the *K* + *IA* regions, provides strong evidence that the mutation has directly effected the *Lad-1* locus itself to provide new *Lad-1*-stimulating determinants. At this time, the nature and number of these determinants is unknown as is their relationship to determinants involved in the CML phenomenon but this is currently under intensive study (K. Melief, personal communication). However, based on studies with the *H-2K*^{b} mutants, it is likely that multiple specificities are involved (6).

The bm[12] mutation is of great importance in immunology—far beyond the formal description of another mutant. The initial studies reported herein, have clearly answered several important questions. Firstly, are serologically detected Ia specificities coded for by the same gene as the histocompatibility *H-2IA* gene or a closely associated *H*-gene? This question has remained unanswered although a clear association between the two was apparent. Both were products of the same subregion (*IA*) and no graft rejection occurred when there were no Ia incompatibilities; furthermore, anti-Ia sera could produce skin graft enhancement, and finally, anti-Ia antibodies were produced after graft rejection—compelling evidence for the close association, but not necessarily identity, of these two different moieties. The finding that the one mutation effected both graft rejection and serologically defined specificities indicates that the two are most likely to be present on the same molecule, and coded for by the same gene; although this may be an oversimplification and implies that T cells recognize the same specificities as antibody.

The second finding of importance is that bm[12] stimulates C57BL/6 in a MLR. This finding strongly suggests that the Ia determinants defined serologically, and the MLR-stimulating determinants, coded for by the *Lad-1* locus are present on the same molecule, insofar that they are coded for by the same gene (*Ia-1*). The studies therefore point to the identity of the *Ia-1*, *H-2(IA)*, and *Lad-1* genes. We should also question the relevance of the mutation in bm[12] to the definition of other *I* region products, particularly those detected serologically. We have not yet examined *IJ*^{b} but plan to do so. What of *IE*^{b} and *IC*^{b} products? These have not been defined with alloantisera in the mouse but we are currently investigating these specificities using both alloantisera and xenoantisera absorbed with the bm[12] mutant. These studies are of particular relevance as it has been suggested that a gene in the *IA* subregion (possibly *Ia-1*) also codes for one chain in the dimeric IC/IE molecules (24, 25). With appropriate antisera and by using both serological and biochemical approaches, it may be possible to demonstrate whether the bm[12] mutation has also affected these subregions, either directly or indirectly. The bm[12] mutant is therefore clearly of importance and should be of further help in determining the relationship of *Ir* genes to Ia specificities, factors involved in T-B-cell collaboration, and to other phenomena whose effects map to the *IA* subregion.

Summary

The B6.C-*H-2*^{bm12} mutant is described and evidence is presented for the mutational site occurring in the *IA* subregion. The mutant is of the gain and loss type as bm^{12} ⇄ C57BL/6 grafts are rejected in 14–16 d. Mapping studies by the gene-complementation method using *H-2* recombinant strains place the mutation in the *K* or *IA* regions of the *H-2* complex and furthermore, the use of this test and the use of other *H-2* mutants indicate that *H-2K*^{b} is not the site of the mutation, making the *IA* region the most likely site. Serological analysis with a battery of H-2^b, Ia^b, and other Ia sera, both by cytotoxicity, rosetting, and also by absorption analysis, indicated no alteration in H-2 specificities, particularly in H-2.K33. By contrast, all of the Ia^b specificities coded for by the *IA* subregion (Ia.3, 8, 9, 15, and possibly 20) are extensively altered and are either absent or greatly reduced in amount indicating an extensive alteration in the Ia-bearing molecule. The bm^{12} mutant strongly stimulates the parental C57BL/6 strain in an mixed lymphocyte reaction (MLR), and the reciprocal also occurs, the degree of stimulation being similar to that obtained with K + IA differences originating in another *H-2* haplotype and points to the mutation effecting the *Lad-1* locus. The presence of an extensive histocompatibility change, a marked alteration in the serologically detected Ia specificities, and a strong MLR, all produced by the one mutation, provides strong evidence for the identity of the *Ia-1*, *Lad-1*, and *H-2(IA)* loci in the *IA* subregion. The bm^{12} mutant should be of value in determining the relationship of Ia specificities, *Ir* genes, and other phenomena effected by the *I* region.

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