

TWO LOCI AFFECTING B CELL RESPONSES TO B CELL MATURATION FACTORS

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Cytokines are molecules affecting cell growth and differentiation and are considered to be important intercellular regulatory signals (1). Within the immune system, lymphokines are leukocyte-derived cytokines with effects on B and T cell differentiation and proliferation (2, 3). Among these are a family of molecules termed B cell maturation factors (BMFs)¹ (4–8). Three distinct BMF molecules have been identified: (a) IFN- γ , produced by T lymphocytes, (b) a non-IFN- γ BMF, also produced by T lymphocytes, and (c) a second non-IFN- γ BMF, derived from the B cells of an autoimmune mutant mouse called viable motheaten (*me^v/me^v*) (9). These molecules share the property of causing the maturation of B lymphocytes and certain comparable B cell tumor lines from the resting state to active Ig secretion. They are, however, distinguishable from each other and from other recognized lymphokines by molecular, antigenic, and functional criteria.

As in many other areas of biology, the availability of genetic mutants would be of great value in deciphering the mechanism of action and biological relevance of BMFs. In this paper, we show that the inbred DBA/2Ha mouse strain has a B cell-specific defect manifested by an inability to generate actively Ig-secreting cells (PFC) in response to BMFs *in vitro*. This defect depends on the combined action of two genetic loci, one of which may relate to sex steroid hormone levels. The DBA/2Ha mouse thus demonstrates genetic and hormonal regulation of B cell responsiveness to a family of lymphokines, and should prove valuable as a tool for examining the mechanisms and *in vivo* relevance of this lymphokine-driven pathway of B cell triggering.

Materials and Methods

Mice. Our inbred DBA/2HaSmn colony (referred to here as DBA/2Ha) (F69+7) was established from mice obtained from the Roswell Park Memorial Institute, Buffalo, NY. Other mice used were obtained from the research and production colonies at The Jackson Laboratory.

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¹ *Abbreviations used in this paper:* BMF, B cell maturation factor; PEC, peritoneal exudate cell; TRF, T cell replacing factor.

Lymphocyte Cultures. Resting spleen cells (cells secreting minimal amounts of Ig after culture without added lymphokine) were obtained by discontinuous Percoll density gradient fractionation, using cells banding at the 1.085–1.095 g/ml interface. Spleen cells were cultured at 200–1,000 per 0.2 ml in flat-bottom 96-tray wells, in Iscove's Modified Dulbecco's Medium supplemented with albumin, transferrin, and soybean lipids (prepared according to Schreier and Tees [10]). Culture media were further supplemented with supernatant of S26.5 helper T cells stimulated with appropriate antigen and accessory cells (4), with supernatant from cultured spleen cells from mice homozygous for the mutation viable motheaten (me^v/me^v) (8), or with supernatant derived from Chinese hamster ovary (CHO) cells transfected with the cloned IFN- γ gene (7), as indicated. Cultures were assayed, after 3 d of incubation, by the polyclonal anti-IgM-protein A reverse-plaque assay (11). For the repeated testing of individual backcross animals, 100 μ l of peripheral blood was collected into 0.9 ml of 0.9% NaCl, 10 mM EDTA, and diluted to a final concentration of 1:10,000 with culture medium. Of this, 200 μ l, containing $\sim 10^3$ PBL, were cultured per well. Responder mice usually showed a fivefold increase in PFC levels in cultures of PBL with vs. without BMF. A >2:1 ratio of stimulated/unstimulated PFC from PBL cultures was required for typing a mouse as a BMF responder.

Macrophage Cultures. Peritoneal exudate cells (PEC) were collected 3 d after the injection of 1.5 ml of FBS into the peritoneum. Macrophages were obtained by incubating 2×10^5 PEC for 2 h at 37°C on a 15-mm glass coverslip in 1 ml of RPMI-1640 medium supplemented with 10% FBS, 10 mM Hepes, 2 mM L-glutamine, and 100 U/ml penicillin/streptomycin, after which the nonadherent cells were washed away, leaving the macrophages bound to the cover slip. After 3 d of culture with or without natural or cloned IFN- γ , 0 or 2×10^5 heat-killed *Listeria monocytogenes* bacteria were added as a phagocytic challenge. After a fourth day of culture, macrophage morphology was visually inspected, and the cells were fixed and stained with anti-Ia^d mAb MK-D6 followed by 100 μ g/ml of fluoresceinated F(ab')₂ rabbit anti-mouse Ig (12).

Surgical Procedures. Gonadectomy or adrenalectomy of brown (*b/b*) backcross mice younger than 1 mo of age, which had been previously typed as BMF nonresponsive (*Bmfr-1^{nr}/Bmfr-1^{nr}*), was performed under aseptic conditions. Mice were anesthetized with Avertin (13) and ovariectomized or adrenalectomized via bilateral flank incision. Castration was accomplished via midline abdominal incision. Muscle layers were closed with 4-0 chromic gut, and skin incisions were closed with stainless steel wound clips. Mice were allowed to recover from anesthesia under extra heat, and were then returned to the animal rooms. Adrenalectomized mice were maintained after surgery on water containing 0.9% NaCl. Mice were retested for BMF responsiveness from 2–4 mo after surgery.

Mup-1 Electrophoresis. Methods described previously (14) were used, with minor modifications. Urine samples were collected from mice at 6–10 wk of age. Samples from males were diluted 1:5 urine/distilled water, while samples from females were diluted 1:1 urine/water. The incubation of samples in Amberlite MB-1 (14) was omitted, since this step was found not to affect the results. Samples were applied five times to the cathodal end of Sepapore X cellulose acetate plates. The electrophoresis buffer consisted of 21.8 g Tris base, 6.18 g boric acid, and 0.58 g EDTA (free base) per liter. Electrophoresis was conducted for 25 min at 200 V. Gels were stained with 0.5% Ponceau S in 5% TCA and destained in 5% acetic acid.

Statistics. χ^2 goodness-of-fit and contingency tests were used, as indicated, to assess the significance of various sets of data.

Results

To assess B cell responses, resting spleen cells (Percoll-separated dense cells) of several strains of mice were cultured in either unsupplemented serum-free medium, or in medium supplemented with one of three BMFs or the B cell mitogen LPS. After 3 d the cultures were assayed using the polyclonal anti-IgM-protein A reverse-plaque assay (11), which measures the number of actively

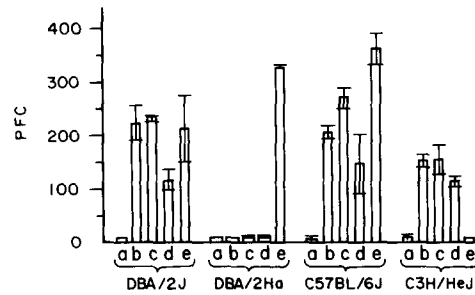


FIGURE 1. PFC responses per culture from resting spleen cells of several mouse strains cultured in medium with (a) no supplement, (b) 10% S26.5 helper T cell supernatant, (c) 10% viable motheaten spleen cell supernatant, (d) 10 U IFN- γ , and (e) 50 μ g/ml LPS.

TABLE I
Macrophage Responses

Lymphokine	LM*	DBA/2Ha		DBA/2J	
		Percent Ia ⁺⁺	Spreading [§]	Percent Ia ⁺	Spreading
—	—	<1	+	<1	+
—	+	4	+	5	+
IFN- γ [¶]	—	11	+	39	+
IFN- γ	+	43	+++	46	+++
T-CM [¶]	—	22	+	48	+
T-CM	+	70	+++	71	+++

* Heat-killed *L. monocytogenes* added on day 3 as a phagocytic challenge.

† Determined by immunofluorescent staining followed by visual counting.

§ Determined by visual inspection.

¶ 10 U/culture recombinant IFN- γ .

¶ 10% T cell-conditioned medium containing 5 ng/ml IFN- γ .

Ig-secreting cells. As shown in Fig. 1, B cells from DBA/2J and C57BL/6J mice responded vigorously to all three BMFs and LPS, whereas the B cells from C3H/HeJ mice, which are genetically unresponsive to LPS (15), responded normally to the three BMFs but not to LPS. In contrast, the B cells from DBA/2Ha mice responded well to LPS but not to any of the three BMFs. DBA/2Ha B cells therefore are capable of differentiating into actively Ig-secreting plasma cells, but show a specific defect in response to BMFs.

Unlike the non-IFN- γ BMF from T cells (4) and the BMF from motheaten B cells (8), the BMF IFN- γ affects many other cell types besides B cells. These effects include induction of a virus-resistant state (16), increased expression of MHC antigens (17), and increased activation of macrophages (18). To test whether IFN- γ is active on other cell types of the DBA/2Ha mouse, peritoneal macrophages from DBA/2Ha and DBA/2J mice were cultured with or without IFN- γ and an additional phagocytic challenge (heat-killed *Listeria monocytogenes*), and then assayed for Ia expression and the enhanced spreading and membrane ruffling characteristic of the activated state (12). Table I shows that macrophages from both strains responded to IFN- γ . The defect in the response of DBA/2Ha

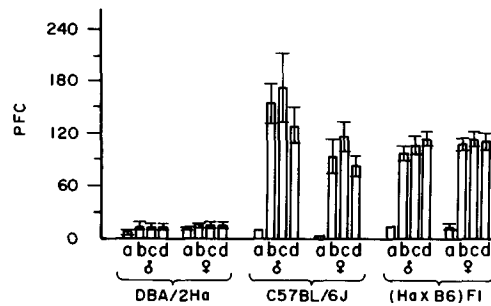


FIGURE 2. PFC responses per culture for resting spleen cells from parental and (responder \times nonresponder) F_1 mice. (a)–(d) as in Fig. 1.

B cells to IFN- γ is therefore not present, or is much weaker, in DBA/2Ha macrophages.

To characterize the genetic basis for the unresponsiveness of DBA/2Ha B cells to BMF, F_1 progeny were produced by crossing nonresponsive DBA/2Ha and responsive C57BL/6J parents (Fig. 2). When challenged with any of the three BMFs, B cells from both sexes of F_1 offspring responded as well as those from their C57BL/6J parents. Reciprocal F_1 hybrids were tested, with equivalent results. Similar results regarding BMF responses were obtained with (DBA/2Ha \times BALB/cByJ) F_1 , (DBA/2Ha \times DBA/2J) F_1 , (DBA/2Ha \times C3H/HeJ) F_1 , and (DBA/2Ha \times CBA/N) F_1 mice (female parent designated first, according to convention; data not shown). These results indicate both that the lack of responsiveness of DBA/2Ha B cells is not simply determined by a single sex-linked locus and that responsiveness is dominant to nonresponsiveness.

To determine the inheritance and linkage of the gene(s) responsible for the DBA/2Ha defect in BMF responsiveness, responsive F_1 hybrids were backcrossed to nonresponsive DBA/2Ha mice. In this and all further backcross experiments, four types of backcross mice were tested: (C57BL/6J \times DBA/2Ha) F_1 \times DBA/2Ha; DBA/2Ha \times (C57BL/6J \times DBA/2Ha) F_1 ; (DBA/2Ha \times C57BL/6J) F_1 \times DBA/2Ha; and DBA/2Ha \times (DBA/2Ha \times C57BL/6J) F_1 . Since both segregation and linkage results from the four groups of backcross animals were similar, data from all four groups of backcross mice were pooled. Among 91 randomly selected backcross animals tested for BMF responsiveness at or before 1 mo of age, 49 were nonresponsive and 42 were responsive. This near 1:1 ratio indicates Mendelian inheritance of a single locus, and confirms that responsiveness to BMFs is dominant over nonresponsiveness in heterozygotes. We have termed this locus BMF responsiveness-1 (*Bmfr-1*) with nonresponsive and responsive alleles *nr* and *r*, respectively. Furthermore, as shown in Fig. 3, BMF responsiveness in these young backcross mice was tightly linked to the *brown* (*b*) coat color locus. This indicates that *Bmfr-1* is located near the *b* locus on chromosome 4.

To determine the gene order and recombination frequencies between *Bmfr-1*, *b*, and other loci on chromosome 4, backcross progeny were typed for *Bmfr-1*, major urinary protein (*Mup-1*), and *b*. As this study progressed, however, it became apparent that BMF responsiveness was not always associated with the *b* allele of the *brown* locus. Unlike the data shown above from backcross mice <1

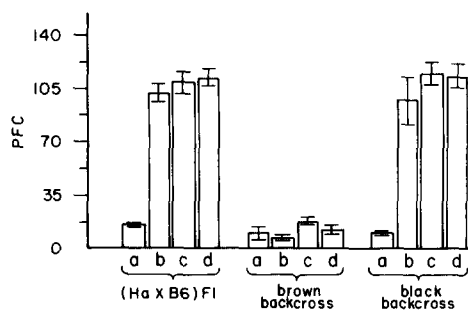


FIGURE 3. PFC responses per culture for resting spleen cells from parental and (responder \times nonresponder F₁) backcross mice. (a)–(d) as in Fig. 1.

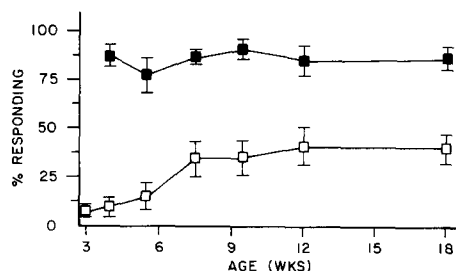


FIGURE 4. Percentage of BMF-responsive black (■) and brown (□) (responder \times nonresponder F₁) backcross mice as a function of age.

mo old, the segregation ratio of BMF responsiveness in mice tested from 2–6 mo of age was not consistent with the Mendelian inheritance of a single autosomal locus (76:35 responders vs. nonresponders, which is significantly different [$p < 0.001$ in χ^2 goodness-of-fit test] from the 1:1 ratio expected according to simple Mendelian inheritance). In comparison, the segregation ratios of *b* (52:59 for the *b*:*B* alleles) and *Mup-1* (54:57 for the *a*:*b* alleles) were normal, i.e. 1:1, in the same mice. These results suggested that BMF responsiveness in young mice was controlled by a single autosomal locus (*Bmfr-1*) linked to *brown* on Chromosome 4, and that another, unlinked locus modified this response in older mice.

When BMF responsiveness was analyzed according to the age of the backcross mice tested, the pattern shown in Fig. 4 emerged. At all ages tested, 80–90% of black (*B/b*) backcross mice were BMF responsive. However, while only ~5% of brown (*b/b*) backcross mice were BMF responsive at <1 mo of age (see Fig. 3), ~40% of this population was BMF responsive when assayed at ≥ 2 mo of age. These results suggested that some BMF nonresponsive mice convert to responsiveness. To test this hypothesis, individual mice were successively tested at several different ages by examining the BMF responses of their PBL. With increasing age, many brown and a few black backcross mice converted from BMF nonresponsiveness to responsiveness (data not shown). This conversion usually occurred between one and two months of age, and was stable to ≥ 6 mo of age.

With the phenomenon of conversion in mind, only backcross mice that were BMF-typed before 1 mo of age (whether BMF responsive or nonresponsive), and mice that were found not to respond to BMF after 1 mo of age (these were

TABLE II
Mapping Summary for Chromosome 4

Chromosome type	Loci typed*			Numbers of mice [‡]		Total
	<i>Mup-1</i>	<i>b</i>	<i>Bmfr-1</i>	<4 wk	>4 wk	
Three-point cross						
Parental	<i>a</i>	<i>b</i>	<i>nr</i>	12	43	78
	<i>b</i>	<i>B</i>	<i>r</i>	23	[58] [§]	
<i>Mup-1</i> recombinant	(<i>b</i>)	<i>b</i>	<i>nr</i>	2	6	9
	(<i>a</i>)	<i>B</i>	<i>r</i>	1	[9]	
<i>b</i> recombinant	<i>a</i>	(<i>B</i>)	<i>nr</i>	0	3	3
	<i>b</i>	(<i>b</i>)	<i>r</i>	0	[3]	
<i>Bmfr-1</i> recombinant	<i>a</i>	<i>b</i>	(<i>r</i>)	0	[28]	14
	<i>b</i>	<i>B</i>	(<i>nr</i>)	3	11	
					Total:	104

Recombination frequency:

$$Mup-1 - b = (9 + 3)/104 = 12 \pm 3\%$$

$$Mup-1 - Bmfr-1 = (9 + 14)/104 = 22 \pm 4\%$$

$$b - Bmfr-1 = (3 + 14)/104 = 16 \pm 4\%$$

Two-point cross

Parental	<i>b</i>	<i>nr</i>	133	161
	<i>B</i>	<i>r</i>	28	
Recombinant	<i>b</i>	<i>r</i>	7	25
	<i>B</i>	<i>nr</i>	18	
			Total:	186

Recombination frequency:

$$b - Bmfr-1 = 25/186 = 13 \pm 2\%$$

* Alleles in parentheses showed apparent recombination relative to the parental alleles of the other two loci typed in the three-point cross.

[‡] These totals are valid for estimating map distances but not for segregation analysis, since some of these mice were preselected by coat color.

[§] Samples in brackets were not included in the calculations of recombination frequencies, since they were old enough to have converted from BMF nonresponsiveness to responsiveness.

assigned as BMF nonresponsive) were included in the mapping analysis. In this way, mice that may have converted from BMF nonresponsiveness to responsiveness were not incorrectly typed as BMF responsive. Those mice that were not used in the calculations of recombination frequencies are indicated by the square brackets in Table II. The results (Table II) from typing three markers in single animals (three-point cross) indicate that the gene order and recombination frequencies are: *Mup-1*-12 ± 3%-*b*-16 ± 4%-*Bmfr-1*. If mice typed for only *b* and *Bmfr-1* are included with those typed for all three markers (two-point cross), the sample size nearly doubles (*n* increases from 104 to 186), and the best estimate of the *b* to *Bmfr-1* distance is 13 ± 2%, a result not significantly different from that obtained above.

The DBA/2Ha strain was recognized and established following a spontaneous reversion of the *dilute* locus (*d*) on chromosome 9 from *d* to *d*^{+Ha}. To examine

TABLE III
Intercrosses with DBA/2Ha

F ₁ parents used for intercross	Color (genotype)	Frequency of BMF responders	Significance*
(DBA/2Ha × C57Bl/6)F ₁ (<i>b/B</i>)	Brown (<i>b/b</i>)	2/7 = 29%	<i>p</i> < 0.01
	Black (<i>B/-</i>)	23/28 = 82%	
	Total:	25/35 = 71%	
(DBA/2Ha × BXD29)F ₁ (<i>b/B, d^{Ha}/d</i>)	Brown (<i>b/b, d^{Ha}/-</i>)	5/21 = 24%	<i>p</i> < 0.001
	Tan (<i>b/b, d/d</i>)	12/14 = 86%	
	Black (<i>B/-, d^{Ha}/-</i>)	8/9 = 89%	NS
	Gray (<i>B/-, d/d</i>)	11/11 = 100%	
	Total:	36/55 = 65%	

* X² contingency test between indicated groups in cross. NS, not significant.

the effect of loci in the DBA/2Ha-derived chromosome 9 segment marked by *d*, we tested the BMF responses of animals segregating normal and DBA/2Ha-derived chromosome 9, i.e., in F₂ animals derived by intercrossing (DBA/2Ha × BXD29)F₁ mice. The BXD29 recombinant inbred strain carries the *B* allele of its C57BL/6J progenitor at the *b* locus and the *d* allele of its DBA/2J progenitor at the *d* locus (B. A. Taylor, unpublished observations), both of which are distinguishable from the *b* locus allele *b* and *d* locus allele *d^{Ha}* of DBA/2Ha. The population of intercross (DBA/2Ha × BXD29)F₂ mice contained four color phenotypes (brown, [*b/b, d^{Ha}/-*]; black, [*B/-, d^{Ha}/-*]; tan, [*b/b, d/d*]; and gray, [*B/-, d/d*]) from the interaction of the two coat-color genes *brown* and *dilute*. As shown in Table III, significantly fewer brown F₂ mice of this population were BMF responsive, when tested at 1–4 mo of age, than were F₂ mice of the other three coat-color groups. Comparable data from F₂ intercross mice derived from (DBA/2Ha × C57BL/6J)F₁ parents are also shown for comparison in Table III. The difference in BMF responsiveness between the brown (*b/b, d^{Ha}/-*) and tan (*b/b, d/d*) groups of mice indicates that a second genetic locus, termed BMF responsiveness-2 (*Bmfr-2*), with responder and nonresponder alleles *r* and *nr*, respectively, is located near *d* on chromosome 9. Experiments to determine the gene order and recombination frequencies between *Bmfr-2* and other loci on chromosome 9 are under way.

Since the conversion of BMF nonresponsive backcross mice to BMF responsiveness usually occurred about the time of sexual maturity (1–2 mo of age), manipulation of steroid hormone levels was explored to try to modulate this conversion. Groups of typed BMF-nonresponsive brown backcross mice <1 mo of age were subjected to various surgical procedures and were then retested several months later for BMF responsiveness. As shown in Table IV, ovariectomy significantly reduced the frequency of female mice converting to BMF responsiveness. In contrast, adrenalectomy (of mice of either sex) and castration of

TABLE IV
Effect of Surgical Treatments on BMF Responsiveness of Brown (DBA/2Ha × C57BL/6)F₁ × DBA/2Ha Backcross Mice

Surgical treatment	Nonresponders	Responders	Frequency of responders	Significance*
None	34	23	40%	—
Adrenalectomy	13	9	41%	NS
Castration	11	9	45%	NS
Ovariectomy	15	3	17%	$p < 0.05$

* χ^2 contingency test relative to control group (no surgery). NS, not significant.

males did not significantly affect the percentage of brown backcross mice becoming BMF responsive.

Discussion

The results reported here demonstrate that the action of lymphokines in the immune system is subject to both genetic and hormonal regulation. Two loci that determine B cell responsiveness to the BMF family of lymphokines were identified in studies using the DBA/2Ha mouse strain. The DBA/2Ha alleles of these loci lead to a specific defect in B cell responses to BMFs. However, B cells from this strain respond normally to the B cell mitogen LPS, and macrophages of this strain respond to one of the three BMFs (IFN- γ) that is inactive on B cells from the same mice. One gene responsible for the B cell defect in DBA/2Ha mice was named BMF responsiveness-1 (*Bmfr-1*), assigned to chromosome 4, and mapped ~13 cM telomeric to the *brown* locus. Mice homozygous for the nonresponder allele of *Bmfr-1* (*Bmfr-1^{nr}/Bmfr-1^{nr}*) are BMF-nonresponsive at birth, while mice with one or two responder alleles (*Bmfr-1^{nr}/Bmfr-1^r* or *Bmfr-1^r/Bmfr-1^r*) are BMF responsive throughout life. A second gene, BMF responsiveness-2 (*Bmfr-2*), was found to be closely linked to the *dilute* locus on chromosome 9, and affects conversion of initially BMF-nonresponsive mice (*Bmfr-1^{nr}/Bmfr-1^{nr}*) to BMF responsiveness at about the time of puberty. Mice with genotype *Bmfr-1^{nr}/Bmfr-1^{nr}*, *Bmfr-2^{nr}/Bmfr-2^{nr}* convert to BMF responsiveness significantly less frequently than mice with genotypes *Bmfr-1^{nr}/Bmfr-1^{nr}*, *Bmfr-2^{nr}/Bmfr-2^r* or *Bmfr-1^{nr}/Bmfr-1^r*, *Bmfr-2^r/Bmfr-2^r*. Preliminary results indicate that occasional offspring (<10%) derived by crossing two backcross mice of genotype *Bmfr-1^{nr}/Bmfr-1^{nr}*, *Bmfr-2^{nr}/Bmfr-2^{nr}* will convert to BMF responsiveness, a result which may be due to additional, as yet unidentified, genes, incomplete penetrance of *Bmfr-2*, etc. Table V summarizes the expected BMF response phenotypes of mice with various *Bmfr-1* and *Bmfr-2* genotypes.

The conversion from BMF nonresponsiveness to responsiveness, which is regulated by *Bmfr-2*, may operate through or be affected by the levels of steroid sex hormones, since ovariectomy of brown female backcross mice (*Bmfr-1^{nr}/Bmfr-1^{nr}*, *Bmfr-2^{nr}/Bmfr-2^r*) significantly reduced the percentage of animals converting to BMF responsiveness. This conversion was not influenced by removal of the testes in males, however. Development of gonadal function in female rodents

TABLE V
Phenotypes Expected from Various Bmfr-1 and Bmfr-2 Genotypes

<i>Bmfr-1</i> genotype	<i>Bmfr-2</i> genotype		
	<i>r/r</i>	<i>nr/r</i>	<i>nr/nr</i>
<i>r/r</i>	r-r*	r-r	r-r
<i>nr/r</i>	r-r	r-r	r-r
<i>nr/nr</i>	nr-r	nr-r	nr-nr

* Expected BMF response phenotypes: <1 mo of age; >2 mo of age

(19) is characterized by maturation of hypothalamic gonadotropin releasing hormone and pituitary follicle stimulating hormone secretion capabilities at the end of the third week of life (20, 21). Although ovarian secretion of progesterin and androgen begins during that period, significant estrogen secretion appears only in the fourth, and later, weeks of life (22–25). Cyclic estrogen secretion, so essential for female reproduction, is not needed or present in male rodents. Instead, masculine requirements for estrogen are met by a fairly constant peripheral conversion of androgen precursors such as testosterone to estradiol and estrone (26). Gonadectomy at 4 wk of age deprives both male and female mice of their major sources of sex steroids, leaving only adrenal androgens, such as androstenedione and dehydroepiandrosterone, for peripheral conversion to estrogens in modest amounts. We hypothesize that the reduced levels of estrogen remaining in gonadectomized male but not in female *Bmfr-2^{nr}/Bmfr-2^r* mice are adequate to support the conversion from BMF nonresponsiveness to responsiveness.

The DBA/2Ha strain was tested for BMF responsiveness because of the work of Takatsu and Hamaoka (reviewed in 27), who reported that the B cells of DBA/2Ha mice are unresponsive to a family of lymphokines termed T cell replacing factors (TRF). However, these authors concluded that the TRF defect was caused by an X-linked recessive gene, because they found that B cells from male (DBA/2Ha × BALB/c)_{F1} mice were nonresponsive to TRF, while B cells from female siblings responded. Several possibilities might explain the discrepancy between their results and those reported here. First, since the biochemical relationship between the BMF and TRF families of lymphokines is not yet clear, and since BMF and TRF assays differ markedly in the B cell populations used, culture conditions, response kinetics, antigen requirements, etc., the DBA/2Ha strain in fact may have multiple independent abnormalities affecting B cell behavior. A second possibility is that the sexual dimorphism demonstrated here, which may be based on steroid sex hormone involvement in the operation of the *Bmfr-2* gene, led to an erroneous conclusion regarding the genetic basis for the DBA/2Ha defect. Phenotypic variation between males and females is not necessarily based on sex chromosome-linked genes. A third possibility is that separate sublines of DBA/2Ha mice may exist. It is interesting to note that the DBA/2Ha strain originated about the time when the DBA/1 and DBA/2 strains were established (28). Various alleles were still segregating in the foundation population, since DBA/2Ha mice resemble DBA/1J mice at the *Car-2* and *Gpd-1* loci

on chromosomes 3 and 4, respectively, but are like DBA/2J at the *H-2* and *Ce-2* loci on chromosome 17. (*Car-2*, *Gpd-1*, and *Ce-2* were generously typed by R. Fox, and *H-2* by G. Carlson, The Jackson Laboratory, data not shown.) The DBA/2Ha strain thus appears to be equally related to the DBA/1J and DBA/2J strains. To eliminate the potential effects of other DBA/2Ha genes, the *Bmfr-1* and *Bmfr-2* alleles of DBA/2Ha are now being transferred to other standard laboratory backgrounds. Since an extensive typing of other strains for *Bmfr-1* and *Bmfr-2* has not yet been done, and since the two nonresponder alleles are most easily recognized in combination with each other, it is possible that other strains have either the nonresponder allele of *Bmfr-1* or of *Bmfr-2*. Mice homozygous only for the nonresponder allele of *Bmfr-1* (*Bmfr-1^{nr/nr}*) can be recognized by their nonresponsiveness to BMFs at <1 mo of age. In contrast, strains bearing only the nonresponder allele of *Bmfr-2* (*Bmfr-2^{nr/nr}*) must be typed by testing for permanently BMF-nonresponsive progeny among the F₂ descendants derived from crossing and intercrossing a test strain with mice of genotype *Bmfr-1^{nr/nr}*, *Bmfr-2^{nr/nr}*.

An interesting aspect of these results is that the linkages that we have identified may provide insight into the genetic and evolutionary relationship between *Bmfr-1* and *Bmfr-2*. Specifically, we hypothesize that *Bmfr-1* and *Bmfr-2* arose as gene duplicates through genome tetraploidization, and that their functions have subsequently diverged. Several authors have argued that the twofold increment in DNA content between a variety of species (29, 30) and the conserved linkage of loci within duplicated chromosome segments in different species (31, 32) are evidence for a series of genome duplications during the early evolution of vertebrates but before the appearance of mammals. The functions and regulation of duplicated loci may have subsequently diverged, with some becoming paralogous loci and others eventually becoming pseudogenes (33). The linkages found in our study may reflect this phenomenon: *Bmfr-1* is linked to phosphoglucosyltransferase-2 (*Pgm-2*) on chromosome 4, while *Bmfr-2* is linked to *Pgm-3* on chromosome 9. Thus, *Bmfr-1* and *Bmfr-2* may be genetically as well as functionally related.

In preliminary experiments, striking differences were not seen in the serum immunoglobulin levels or the overall health of DBA/2Ha vs. other strains of mice. However, mice homozygous for several other previously characterized immunodeficiency mutations (*xid* [34], *nude* [35], *Lps* [15], *bg* [36], and *scid* [37]) have been surprisingly fit in conventional laboratory colonies. The fitness of mice with immunological defects probably results from the known multiplicity of B, T, and accessory cell subsets and triggering pathways that provide alternative routes to immunological protection. Further comprehensive studies are required to evaluate the effect of BMF unresponsiveness on the overall immune status of DBA/2Ha mice.

In conclusion, these studies add two new members to the growing collection of single gene mutations affecting the immune system. Such mutations have already proven to be of great value in work from many laboratories. In the future, the growing number of available mutants should be ever more valuable

in probing the mechanisms, regulation, and significance of individual components of the immune system.

Summary

B lymphocytes from DBA/2Ha mice have a genetic defect characterized by a failure to differentiate into antibody-secreting cells in response to a family of lymphokines termed B cell maturation factors (BMFs). By contrast, B cells from DBA/2Ha mice respond normally in PFC assays to the B cell mitogen LPS, and macrophages from these mice are activated by one of the three BMFs. Two loci are responsible for the B cell defect in DBA/2Ha mice. One locus (*Bmfr-1*) is constitutively expressed throughout life, and maps ~13 cM distal to the *brown* locus on chromosome 4. A second locus (*Bmfr-2*) becomes active only after sexual maturity and is closely linked to the *dilute* locus on chromosome 9. At both loci, alleles determining responsiveness to BMFs are dominant over nonresponder alleles. The effect of *Bmfr-2* on B cell responsiveness may be related to levels of the steroid sex hormones. DBA/2Ha mice offer a tool for studying the genetic and hormonal regulation of the immune system.

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