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Immune Response Deficiency of BSVS Mice

I. Identification of Ir Gene Differences Between A/J and BSVS Mice in the Antistreptococcal Group A Carbohydrate Response

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Abstract. The genetic control of the low response of BSVS mice to streptococcal Group A carbohydrate (GAC) was studied in crosses with responder A/J mice. F_1 mice were responders. In the backcross (BSVS × A/J)F_1 × BSVS mice, there were equal numbers of anti-GAC responder and nonresponder mice, indicating genetic control by a small number of major loci. The anti-GAC responses of the backcross mice showed no obligate linkage between responder status and A/J *H-2* or IgC_H alleles. However, it was observed that the average anti-GAC titers were higher in backcross mice heterozygous at these loci. The above data, a lack of low-responder F_2 animals, and the segregation of a non-*H*-2-, non-IgC_H-linked locus in the first and second backcross mice, indicate that the defect in the BSVS anti-GAC responsiveness involves three loci: one linked to *H-2*, another linked to IgC_H, and a third locus—tentatively named *Ir-GAC*—not linked to *H-2*, IgC_H, or *Hbb*.

Introduction

Humoral antibody levels in the mouse have been shown to involve genes at several loci, including the following: *Ir-1*, within the *H-2* histocompatibility gene complex, chromosome 17 (Shreffler and David 1975); *Ir-5*, located about 19 map units from *H-2* (Zaleski and Klein 1974); *Ir-2* of chromosome 2 (Gasser 1969); genes linked to the immunoglobulin heavy-chain allotype locus (IgC_H) (Blomberg *et al.* 1972); and genes of unknown linkage, including *Ir-3* (Mozes *et al.* 1969), *Ir-4* (Lilly *et al.* 1971), and others (Cramer and Braun 1975, Gasser and Silvers 1974). While it seems likely that genes at many or all of these loci may be involved in most antibody responses, their involvement may be apparent only when the proper allelic combinations are available for genetic studies with the antigens being examined. In the present report, using BSVS and A/J mice, we present evidence for the involvement of three loci in the antibody response to streptococcal Group A carbohydrate (GAC), a low molecular weight cell wall component (Krause 1970). Two of these loci, linked to *H-2* and IgC_H,

respectively, exert only moderate effects on the anti-GAC immune response, while the third locus, which we will refer to as *Ir-GAC*, is not linked to either *H-2* or IgC_H and exerts a major effect on the anti-GAC response.

BSVS mice were chosen as a low-responder strain for this study since they had a markedly reduced capacity to make IgM and IgG anti-GAC antibody in response to intravenous injection of streptococcal Group A vaccine (Briles and Davie 1975). The relative immune deficiency of BSVS mice may be related to the fact that they were bred for susceptibility to certain bacterial and viral infections (Webster 1937). A/J mice were selected as high responders since they consistently produce higher levels of anti-GAC antibody than most other strains when injected intravenously with Group A vaccine (Eichmann 1972, Briles and Davie 1975)

Materials and Methods

Mice. A/J, BALB/cJ, C3H/HeJ, C57BL/6J, SJL/J, and SWR/J were obtained from Jackson Laboratory, Bar Harbor, Maine. BSVS and BSVR mice were bred in our own colony from stock obtained from the National Cancer Institute, Bethesda, Maryland. BRVR mice were purchased from the Animal Facilities of the State University of New York at Buffalo. This last source can also supply BSVS mice, which are no longer available from the NCI. All F_1 mice were produced by mating A/J females with BSVS males. Mating F_1 females with BSVS males produced, 25 female and 24 male backcross mice and an additional 4 female and 4 male backcross mice were produced by mating BSVS females with F_1 males. No major differences were observed between the anti-GAC titers of backcross animals made by these 2 procedures. F_2 mice were produced by mating F_1 males and females.

Immunization. Mice were injected intravenously (i.v.) with Group A streptococcal vaccine on days 0, 7, 14, and 49 with 0.1 ml vaccine diluted to contain 3, 10, 30, and 30 μg, respectively, of the cell wall component rhamnose per injection. They were bled on day 59 as described previously (Briles and Davie 1975). In addition, the immunization protocol of Cramer and Braun (1975) was used; thus, mice were immunized intraperitoneally (i.p.) with 0.1 ml vaccine, diluted to contain 30 μg of cell wall rhamnose, on days, 1, 2, 3, 8, 9, 10, 15, 16, 17, 22, 23, and 24 and bled on day 29.

Assays. Anti-GAC antibodies were detected by a modified Farr assay including ^{22}Na as a volume marker (Gotschlich 1971). Antisera were diluted in a PBS-protein diluent [0.056 M Na_2HPO_4, 0.016 M KH_2PO_4, 0.077 M NaCl, pH 7.2; 1% bovine serum albumin (BSA), 0.01% bovine gama globulin] to make serial threefold dilutions. Twenty μl of each dilution were mixed with 20 μl of the PBS-protein diluent containing 2×10^4 cpm of 4 μCi/μg ^{125}I-GAC (Briles and Krause 1974) and 1×10^4 cpm of $^{22}NaCl$ (Amersham/Searle, Arlington Heights, Illinois). After at least 10 minutes at room temperature, 50 μl of 85% saturated (25° C) $(NH_4)_2SO_4$ solution were added with mixing After 1 hour at room temperature, the tubes were centrifuged at $2000 \times g$ for 10 minutes; most of the supernatant fluid in each tube was aspirated. The tubes were then counted in a 2-channel gamma counter, and the data were calculated according to the method of Gotschlich (1971). Antibody titers were coverted to mg antibody/ml serum by comparison to a standard pooled mouse anti-GAC serum having 8 mg anti-GAC antibody/ml serum, as determined by quantitative precipitin assay (McCarty and Lancefield 1955).

Mouse IgC_H haplotypes a^4 and a^3 (Potter and Lieberman 1967) were detected with SWR/J anti-A/J and A/J anti-SWR/J antisera, respectively, produced by immunization with washed pertussis antipertussis agglutinates (Potter and Lieberman 1967) made with pertussis vaccine (Eli Lilly and Co., Indianapolis, Indiana). The allotypic assays were based on the radioimmune assay described by Bosma and coworkers (1975) and used ^{125}I-labeled normal mouse immunoglobulin from A/J and SWR/J mice.

H-2 haplotypes were detected by direct hemagglutination assay. Blood was taken orbitally in heparinized capillary tubes (Curtin Matheson Scientific, Inc., Houston, Texas), and the erythrocytes were washed once with citrate-saline (1% sodium citrate, 0.85% sodium chloride) and twice with saline and were suspended to 0.8% in PBS. One tenth ml of the erythrocyte suspension was mixed with 0.1 ml of anti-H-2 serum, diluted in PBS, which contained 2% polyvinylpyrrolidine and 0.1% BSA (Stimpfling 1966). After 3 to 4 hours at room temperature, the hemagglutination patterns were read, after gentle agitation, under 7× magnification with dark field illumination. The *a* and *t5 H-2* haplotypes were detected, respectively, with BSVS anti-A/J and A/J anti-BSVS antisera, prepared by immunizing mice with tissue suspensions (Möller 1961). The results obtained with these sera were confirmed with anti-19 and anti-11 sera obtained from Dr. George D. Snell, The Jackson Laboratory, Bar Harbor, Maine.

The determination of d and s hemoglobin of the *Hbb* locus (Popp and Amand 1960) were performed using the lysate from 75 µl whole blood. Erythrocytes were washed as described for the *H-2* assay, lysed with 0.1 ml of water, and diluted to 0.25 ml total volume with 0.043 M sodium barbital, pH 8.6. Each sample (0.25 µl) was subjected to electrophoresis at 300 volts for 30 minutes in a model R101 Microzone Cell on cellulose acetate membranes (Beckman Instruments, Fullerton, California) in the barbital buffer. Three *Hbb* phenotypes—*ss*, *sd*, and *dd*—could be detected on the unstained membranes. A/J and SWR mice, known to be *Hbb d* and *s* (Green 1968), respectively, were used as standards. BSVS mice were found to be Hbb^s.

Results

The antistreptococcal Group A carbohydrate (GAC) antibody response of BSVS mice (Briles and Davie 1975) was compared to those of mice from several other strains (Table 1). After either i.v. or i.p. immunization, strains A/J and C57Bl/6J were high or moderate responders and BSVS and C3H/HeJ were low responders. However, the amount of antibody produced by SJL/J and BALB/cJ was dependent on the route of immunization used. SJL/J mice responded better to i.v. than to i.p. injection; conversely, BALB/cJ mice responded much better when injected i.p. Of all strains tested—including BRVR and BSVR mice, which were derived from the same outbred population as

Table 1. Anti-GAC Response of Female Mice to Streptococcal Group A Vaccine

Strain	IgC_H	*H-2*	*Ir-1*			Anti-GAC (mg/ml)			
			A	*B*	*C*	n	After Four i.v. Injections	n	After Ten i.p. Injections
A/J	a^4	*a*	*k*	*k*	*d*	9	6.18 (1.4)[a]	8	5.47 (1.5)
C57BL/6J	a^2	*b*	*b*	*b*	*b*	6	5.25 (1.4)	10	5.45 (5.5)
SJL/J	a^2	*s*	*s*	*s*	*s*	5	4.67 (1.8)	6	1.69 (1.1)
SWR/J	a^3	*q*	*q*	*q*	*q*	7	3.09 (1.3)		N.D.[b]
BALB/cJ	a^1	*d*	*d*	*d*	*d*	6	1.29 (1.6)	6	8.47 (1.4)
C3H/HeJ	a^1	*k*	*k*	*k*	*k*	6	0.98 (1.6)	5	1.07 (1.3)
BRVR	?	*k*	*k*	*k*	*k*	6	0.60 (1.2)		N.D.
BSVR	?	?	?	?	?	3	0.73 (1.2)		N.D.
BSVS	a^3	*t5*	*s*	*s*	*s*	5	0.26 (1.4)	6	0.75 (1.2)

[a] Mean and, in parentheses, standard error factor of the anti-GAC response of n mice.
[b] N.D. = not done.

BSVS (Webster 1937)—BSVS mice produced the lowest levels of antibody with either injection route.

A comparison of the antibody responses of the nine inbred strains tested and their *Ir-1* and IgC_H haplotypes (Table 1) shows that the low anti-GAC response of BSVS mice is probably not solely the result of *Ir-1* or IgC_H genes. Thus, BSVS mice have the same IgC_H haplotype ($^{21,25}G^{3,8}H^{9,11}F^{8,19}A^{-}$) as that of SWR/J mice (Rose Lieberman, personal communication), but produce only 0.1 as much antibody with i.v. immunization. The *H-2* type of the BSVS mouse is thought to be a natural recombinant of $H\text{-}2^s$ and $H\text{-}2^a$, with *Ir-1* regions derived from the $H\text{-}2^s$ haplotype (Shreffler and David 1975). Since SJL/J mice ($H\text{-}2^s$) and A/J mice ($H\text{-}2^a$) are both responders and make over 10 times as much antibody to i.v. injection as do BSVS mice, it is unlikely that the low antibody response of BSVS is solely the result of *Ir* genes with in the *H-2* complex.

To investigate further the genetic differences in the ability of A/J and BSVS mice to produce anti-GAC antibody following i.v. immunization with Group A streptococcal vaccine, (A/J × BSVS)F_1, (A/J × BSVS)F_2, and backcross (A/J × BSVS)F_1 × BSVS mice were immunized (Fig. 1). Among mice of each of these genetic combinations, as well as the BSVS, the average responses of females were higher than those of males, although considerable overlap was observed. For the BSVS, F_1, F_2, and F_1 × BSVS backcross mice, the differences in antibody titers by gender were significant by the Student's t-test at less than 0.025. For

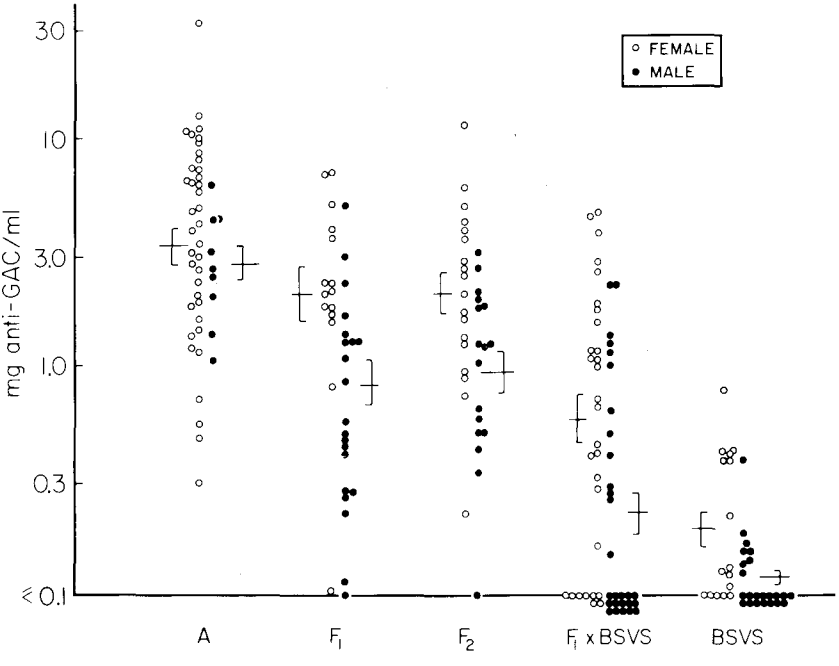

Fig. 1. Anti-GAC response of A/J, BSVS, (A/J × BSVS)F_1, (A/J × BSVS) × (A/J × BSVS)F_2, and backcross F_1 × BSVS mice injected with four i.v. injections of streptococcal Group A vaccine. Mice were injected at two months of age

A/J mice the difference in the anti-GAC titer by gender was not statistically significant. Therefore, the data for each of the genetic crosses have been analyzed separately for females and males.

The F_1 mice produced about half as much antibody on the average as did A/J mice of the same sex, but more than 10 times as much antibody as did BSVS mice. This incomplete dominance of the A/J response suggests that, at one or more loci, A/J genes should be homozygous in order to achieve maximum responsiveness. However, further study of the loci responsible for the differences in anti-GAC titers of F_1 and A/J mice was not made, since the extensive overlap between F_1 and A/J mice would make difficult the unequivocal assignment of the F_1 or A/J phenotypes to $F_1 \times$ A/J backcross progeny.

On the other hand, the slight overlap in the anti-GAC responses of BSVS and F_1 mice allowed us to study further the genetic control of the differences in anti-GAC responsiveness of BSVS and F_1 mice. Mating F_1 with BSVS mice produced approximately equal numbers of progeny with F_1 and BSVS phenotypes, which suggests that a single locus might regulate the expression of the F_1 phenotype on a BSVS background. However, a strict one-locus interpretation of the A/J and BSVS anti-GAC responses appears to be an oversimplification, since the anti-GAC responses of F_2 mice are virtually identical to those of F_1 mice. Not seen are the 25 percent of low responders that would be expected in a strict one-locus system. This finding indicates that A/J genes at more than one locus, either singly or in combination, can result in anti-GAC titers characteristic of F_1 mice. The difference in the findings with F_1 and F_2 mice may be the result of the more extensive A/J gene contribution in F_2 mice, which might in turn give increased importance to minor loci.

Additional insight into the number of genes affecting anti-GAC titers of backcross mice was gained by examining the effects of strain A/J *H-2* and IgC_H loci on the amount of antibody produced. As can be seen in Tables 2 and 3, an obligate relationship between antibody production and inheritance of the A/J allotype or *H-2* type was not observed in the backcross mice. However, it was observed that mice which inherited the A/J alleles at either locus exhibited higher anti-GAC responses than mice which did not. These effects were greater

Table 2. Anti-GAC Antibody Responses of Backcross (A/J × BSVS) × BSVS Mice by *H-2* Type

H-2[a]	*a/t5*		*t5/t5*	
	Anti-GAC mg/ml	Responder[b] / Nonresponder	Anti-GAC mg/ml	Responder / Nonresponder
Females	0.67[c]	12/6	0.40	7/4
Males	0.37	6/6	0.17	3/12

[a] A/J mice are *H-2*^{a}; BSVS mice are *H-2*^{t5}.
[b] Responders are mice producing more than 0.3 mg/ml.
[c] Geometric mean. See Table 4 for individual values.

Table 3. Anti-GAC Antibody Responses of Backcross (A/J × BSVS) × BSVS Mice by Allotype

IgC_H[a]	a^4/a^3		a^3/a^3	
	Anti-GAC mg/ml	Responder[b] / Nonresponder	Anti-GAC mg/ml	Responder / Nonresponder
Females	0.85[c]	7/1	0.42	12/9
Males	0.46	6/7	0.16	2/11

[a] A/J mice are IgC_H a^4; BSVS mice are a^3.
[b] Responders are mice producing more than 0.3 mg/ml.
[c] Geometric mean. See Table 4 for individual values.

Table 4. Anti-GAC Antibody Response of Backcross (A/J × BSVS)BSVS Mice by Sex, and *H-2* and IgC_H Type

Loci	*H-2* and IgC_H Haplotypes							
H-2	*a/t5*[a]		*t5/t5*		*a/t5*		*t5/t5*	
IgC_H	a^4/a^3		a^4/a^3		a^3/a^3		a^3/a^3	
Sex	Anti-GAC mg/ml	R/N[b]	Anti-GAC mg/ml	R/N	Anti-GAC mg/ml	R/N	Anti-GAC mg/ml	R/N
Females	1.09[c]	5/0	0.59	2/1	0.55	7/6	0.35	5/3
Males	0.82	3/1	0.22	3/6	0.22	2/5	0.12	0/6

[a] A/J mice are *H-2*^{a}, a^4; BSVS mice *H-2*^{t5}, a^3.
[b] $\text{R/N} = \frac{\text{mice producing more than 0.3 mg anti-GAC/ml}}{\text{mice producing less than 0.3 mg anti-GAC/ml}}$.
[c] Geometric mean. Anti-GAC values for individual mice are: females a/t^5, a^4/a^3, 0.42, 0.66, 1.1, 1.2, 4.7; females *t5/t5*, a^4/a^3, 0.16, 1.0, 1.1; females *a/t5*, a^3/a^3, <0.1, <0,1, <0.1, <0.1, <0.1, 0.29, 0.32, 1.8, 1.9, 2.5, 2.9, 3.9, 4.6; females *t5/t5*, a^3/a^3, <0.1, <0.1, <0.1, 0.40, 0.45, 0.71, 1.2, 1.6; males *a/t5*, a^4/a^3, 0.27, 0.63, 1.2, 2.2; males t^5/t^5, a^4/a^3, <0.1, <0.1, <0.1, <0.1, <0.1, <0.1, 0.40, 1.3, 2.2; males *a/t5*, a^3/a^3, <0.1, <0.1, <0.1, 0.15, 0.29, 0.50, 1.1, males *t5/t5*, a^3/a^3, <0.1, <0.1, <0.1, <0.1, <0.1, 0.26. Not included in Table 4 is a male mouse, allotype a^3/a^3, which made less than 0.1 mg anti-GAC per ml serum, and which was not *H-2* typed.

Table 5. Results of Least-Squares Analysis of Variance

	Sex	P-Values	
		IgC_H	*H-2*
All mice	<0.025	<0.025	<0.025
Males		<0.05	<0.05
Females		>0.1	>0.1

in males than in females and were even more striking when examined together (Table 4). Mice inheriting both the A/J *H-2* type and allotype showed the highest average responses, and mice homozygous for BSVS alleles at these loci showed the lowest responses. Using a least squares analysis of variance, the effects of *H-2* types, allotype, and sex were all significant at <0.025 (Table 5). There was no statistically significant association between a third marker, *Hbb*, in mouse linkage group 1 (Popp and Amand 1960), and anti-GAC responsiveness.

A third genetic factor, unlinked to *H-2* and IgC_H, was suspected, since in $F_1 \times$ BSVS mice, especially the females, the segregation of strain A/J IgC_H- and *H-2*-linked alleles affects the amount of anti-GAC antibody produced, but does not account completely for the anti-GAC responder and nonresponder status of individual mice (Tables 2, 3, and 4). In fact, among eight backcross females lacking both the A/J *H-2* type and allotype, five produced more than 0.4 mg antibody per ml serum (average 0.75 mg/ml), and two of these produced more than 1 mg antibody per ml serum (Table 4). Further evidence for a third locus comes from female second-backcross mice obtained by crossing first-backcross responder mice to BSVS mice. Out of 20 second-backcross females, ten were responders producing more than 0.3 mg antibody per ml serum (range 0.31–2.9 mg/ml) and ten were classified as nonresponders, producing less than 0.3 mg antibody per ml serum (range 0.04–0.25 mg/ml). Five of the responders (four were expected, based on parental *H-2* and IgC_H genotypes) had not inherited A/J alleles at either the *H-2* or IgC_H loci and had antibody concentrations of 0.31, 1.0, 1.1, 1.4, and 2.7 mg per ml serum. Six female second-backcross progeny of nonresponder females all produced less than 0.2 mg antibody per ml serum. Male second-backcross mice were generally unresponsive, following the pattern observed in first-backcross males homozygous for BSVS alleles at both the *H-2* and IgC_H loci.

Since 30 of the 32 F_2 mice were responders (>0.3 mg/ml), an examination of their *H-2* and IgC_H types was relatively uninformative; however, of the two F_2 mice that produced less than 0.3 mg antibody per ml, one was a male with $H\text{-}2^a/H\text{-}2^{t5}$, a^3/a^3 genotype and the other, a female, was homozygous at both the *H-2* and IgC_H loci for BSVS alleles.

Discussion

In this report we have presented evidence that BSVS and A/J mice differ by at least three genetic loci which affect anti-GAC antibody production. Two of these loci, linked to *H-2* and IgC_H, respectively, exert moderate effects on the antibody response, while the third locus, not linked to *H-2* or IgC_H, exerts a major effect on the anti-GAC response and appears to segregate as a single gene in first- and second-backcross mice. For purposes of discussion, this locus will be referred to as *Ir-GAC*. A fourth factor affecting antibody production in these mice was their sex, which had an effect equivalent to that of the genes associated with the *H-2* or IgC_H loci. It is possible that additional genetic differences exist between A/J and BSVS mice, with effects as large as those associated with the *H-2* and IgC_H loci, which could, in part, account

for the lack of low responders in the F_2 mice. In fact, the small differences in anti-GAC responsiveness produced by genes linked to the IgC_H and *H-2* loci could not have been associated with individual loci had it not been possible to compare the average immune response of backcross mice of known genotypes at the *H-2* and IgC_H loci.

Previous studies, using strains other than BSVS, revealed no obvious correlation among *H-2* type, allotype, and the amount of anti-GAC antibody produced (Eichmann 1972, Cramer and Braun 1974). Our results, which include data from the exceptionally low responding BSVS mouse, confirm this general finding, both in the comparison of inbred strains and in the backcross (A/J × BSVS)F_1 × BSVS mice, in the sense that no obligatory relationships were observed among allotype, *H-2* type, and responder status. However, the observation that backcross (A/J × BSVS)F_1 × BSVS mice heterozygous at the *H-2* or IgC_H loci generally produce more antibody than mice homozygous for BSVS alleles at these loci indicates that genes linked to these loci do have a modifying effect on anti-GAC antibody production.

It is anticipated that the *H-2*-linked effect on the anti-GAC response is the result of *Ir-1* genes located within the *H-2* gene complex. The fact that these genes play only a minor role in the anti-GAC response, as compared to the major role of *Ir-1* genes in many other systems (Shreffler and David 1975), might be explained as being either because only minor differences exist between A/J and BSVS anti-GAC *Ir-1* genes, or because the intensive anti-GAC immunization protocols which have been designed to maximize serum antibody responses minimize any *Ir-1* differences, as has been seen previously with mouse anti-BGG (Vaz and Levine 1970) and guinea pig anti-BSA (Green *et al.* 1970) responses.

It is now well-established that genes controlling the expression of antibody idiotypes are linked to the IgC_H locus (Capra and Kehoe 1975). Among the idiotypes of anti-GAC antibodies, in particular, the A5A (Eichmann and Berek 1973) and L-12 (Briles and Krause 1974) determinants have been shown to be linked to the a^4 *and* a^3 haplotypes, respectively. The inheritance of idiotypes appears to reflect the inheritance of structural genes for antibody heavy-chain variable regions (Capra and Kehoe 1975). However, in most cases, the genes regulating idiotypes and allotypes have not been shown to affect the total amount of antibody produced. The most notable exception to this observation is the murine response to α-(1→3) dextran, where both the immune response to dextran and the J558 idiotype of the antidextran antibody are linked to the IgC_H locus. It has been suggested that this IgC_H-linked effect on total anti-α-(1→3) antibody production is mediated by the inheritance of the J558 idiotype, which is more successfully used in anti-α-(1→3) antibody production than are other anti-α-(1→3) antibodies (Blomberg *et al.* 1972). It is possible that the IgC_H-linked anti-GAC immune response genes described here may act by a similar mechanism. Genes of the IgC_H locus have also been shown to have a major effect on total antibody levels to other antigens, such as ρ-aminobenzoic acid and sulphanilic acid coupled to BGG (Říhová-Škárová and Říha 1974), and the in vitro response to sheep erythrocytes (SRBC, McCarthy and Dutton 1975). About 10 percent of the difference in anti-SRBC responsiveness of Biozzi high

and low mice can be accounted for by genes at the IgC_H loci (Lieberman *et al.* 1972, Biozzi *et al.* 1975). It is of particular interest here that a small but significant effect of *H-2*-linked genes on the responses was also observed in Biozzi mice (Stiffel *et al.* 1974). Since breeding studies indicated that between seven and 13 loci control the ability to respond to SRBC, it was assumed that two different genes closely linked to IgC_H and *H-2*, respectively, were among the multiple regulatory genes known to exist (Feingold *et al.* 1976). To us, this explanation is the simplest, and we favor it as an interpretation of the correlations of anti-GAC response with inheritance of *H-2* and IgC_H haplotypes seen in our own data. For our data, a three-locus system—*Ir-1*, IgC_H and *Ir-GAC*—is also consistent with our F_2 results which indicate involvement of multiple loci.

There have been several other reports of major immune response loci for which linkage to other known genetic markers has not yet been demonstrated. These include the *Ir-3* locus (Mozes *et al.* 1969), the *Ir-4* locus (Lilly *et al.* 1971), and the *Ir-A-CHO* locus, described by Cramer and Braun (1975). The *Ir-A-CHO* locus is particularly relevant to this discussion since it controls the ability of BALB/c mice to respond to i.p. injection of streptococcal Group A vaccine with higher antibody levels than other strains. At present, it is not known whether any of these loci or the *Ir-GAC* locus are related. Even though both the *Ir-GAC* and *Ir-A-CHO* loci regulate the anti-GAC response to streptococcal vaccine, it seems unlikely that they are identical. In particular, the two strains used most extensively by Cramer and Braun (1975) to study the *Ir-A-CHO* locus (high-responder BALB/c and low-responder C57Bl/6) reverse their responder status by the i.v. immunization used here (Table 1). Furthermore, the high-responder strain in this study, A/J, is classified as a low responder by Cramer and Braun (1974, 1975). Thus, it appears that different genes and probably different loci are involved in the high anti-GAC responsiveness induced by the i.p. and i.v. immunization. Since BSVS mice are the lowest responders observed with either i.v. or i.p. immunization, one might postulate that the *Ir-GAC* locus regulates responsiveness to Group A vaccine, while the *Ir-A-CHO* gene regulates the magnitude of the anti-GAC response in Ir-GAC-positive animals given i.p. immunization. Additional experiments underway in this laboratory will explore the genetic relationships between the *Ir-GAC* locus described here and the *Ir-A-CHO* and other non-*Ir-1*, non-IgC_H loci.

The higher anti-GAC titer of female versus male F_1 and backcross mice cannot be explained by assuming that the A/J *Ir-GAC* is located on the X chromosome, since all the F_1 males and females and half of the backcross progeny resulting from mating F_1 females with BSVS males would have inherited one such gene from their mothers. Furthermore, BSVS mice show the same sex-related anti-GAC response difference. The influence of gender on the immune response is not unique to this antigen or to these mouse strains, since numerous observations have been made of elevated antibody responses and accelerated graft rejections in females, as compared to males of several species, including the mouse (Goble and Konopka 1973, Graff *et al.* 1969). Investigations from other laboratories indicate that both androgens and estrogens can suppress antibody production (Batchelor 1968) and graft rejection (Graff *et al.* 1969),

and may account for the general immunological superiority of females (Eidinger and Garrett 1972). The failure of Braun and coworkers (1972) to observe higher anti-GAC responses in females after i.p. immunization may reflect differences in the mouse strains examined or in the route of immunization.

The anti-GAC immunodeficiency described here could be interrelated closely with the origin of the BSVS mouse, which was bred selectively by Webster (1937) for susceptibility to challenge with *Salmonella enteritidis* and St. Louis encephalitis virus. BSVS mice have been shown to be particularly susceptible to BSA anaphylaxis (Olitsky and Lee 1955) and to several induced autoimmune diseases, including encephalomyelitis and thyroiditis (Olitsky and Lee 1953, Boehme 1966, Rose *et al.* 1973). However, the extreme susceptibility of this strain to Salmonella and St. Louis encephalitis was not thought to be the result of a generalized deficit in humoral antibody production, since the anti-Salmonella agglutinin and anti-BSA responses of BSVS mice were indistinguishable from those of BRVR mice (Olitsky and Lee 1955), a closely related strain resistant to the above pathogens (Webster 1937). This conclusion of Olitsky and Lee may warrant a reappraisal, in view of the present and other data indicating a partial immunodeficiency of BSVS mice to T-dependent antigens (Briles and Davie, manuscript in preparation). These findings of immunodeficiency with the BSVS mouse stand in contrast to the results of the Biozzi mice, in which the high-responder line is markedly more susceptible to bacterial infection than are the low responders (Biozzi 1972). Thus, it appears that different mechanisms may be responsible for the immunodeficiency of the BSVS and Biozzi mice.

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