IMMUNE RESPONSE AND THE *B* BLOOD GROUP LOCUS IN CHICKENS¹

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ABSTRACT

Chickens of three blood group genotypes, B^1B^1 , B^1B^2 and B^1B^{19} , were compared in their ability to produce antibodies. The *B* locus is the major histocompatibility locus in this species. Homozygous B^1 pullets had significantly higher adult mortality than did the B^1 heterozygotes. In two separate experiments the B^1B^1 females produced significantly fewer antibodies to *Salmonella pullorum* than did the B^1 heterozygotes. Also the B^1B^1 pullets responded with lower antibody titers following immunization with ferritin, bovine serum albumin and parainfluenza-3 virus, although the differences were not significant. The results of this study suggest that an immune response gene is associated with the major histocompatibility locus in chickens, paralleling the *H-2* locus and *Ir* genes in mice.

THE *B* blood group is the major histocompatibility locus in the chicken (SCHIERMAN and NORDSKOG 1961, 1963, 1964). *B* genes may also influence fertility, hatchability, egg production and livability (GILMOUR 1960; BRILES 1960).

Resistance to Marek's disease of the avian leukosis complex may be associated with *B* blood group alleles. Using both natural and artificial exposure to Marek's, HANSEN, VAN ZANDT and LAW (1967) reported that hens carrying the B^{19} allele had higher mortality than those carrying B^{21} .

NORDSKOG, RISHELL and BRIGGS (1973) reported lower egg production and higher adult mortality of the B^1B^1 genotype. Over a five-year period, the B^1B^1 pullets averaged 54% mortality compared with 10.6% for the B^1 heterozygotes. The rate of mortality, however, decreased 4.4% per generation. They suggested that the *B* locus is neutral but closely linked to a fitness locus.

Linkage between immune response (*Ir*) genes and histocompatibility genes have been described in both mice and guinea pigs. According to new genetic nomenclature recently adopted for mice (KLEIN *et al.* 1974), the *H-2* histocompatibility complex is divided into four regions, *K*, *I*, *S* and *D*, with the loci *H-2K* and *H-2D* defining the bounds, and the genes *Ss* and *Slp* in the *S* region dividing the complex into two "ends". Immediately adjacent to the *K* region is the *I* region which contains at least three *Ir* loci, *Ir-1A* and *Ir-1B* and *Ir-1C*, the latter only

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TABLE 1
Immune response of B blood group genotypes (mean titers)

Antigen	Test year	Genotypes														
		<i>B₁B₁</i> ^a					<i>B₁B₂</i> ^a					<i>B₂B₂</i> ^a				
		Days post-inoculation					Days post-inoculation					Days post-inoculation				
		4	7	10	14	21	4	7	10	14	21	4	7	10	14	21
<i>S. pullorum</i>	1972	...	172	...	58	20	...	682	...	90	32	...	404	...	85	35
<i>S. pullorum</i>	1972	9	180	106	57		37	328	258	80						
BSA	1972		176	207	84			191		214	169		174		177	121
Ferritin	1973	1933	4662	3520	741		2667	6811	5531	1130						
PI-3*	1973		32	248	38	36		40	72	46	52					

* Measured in hemagglutination inhibition units.

recently discovered. In addition, there may be *Ir* loci outside the *I* region (KLEIN 1973).

Susceptibility to the Gross Leukemia virus in mice is controlled by two independent loci of which one may be within the *I* region (LILLY 1966; BATTISTO and LILLY 1972). In guinea pigs, MARTIN *et al.* (1970) reported that the immune response gene, *PLL*, was linked to the major histocompatibility complex.

The specific aim of this study was to determine whether the *B* locus is associated with differences in immune response to both soluble and cellular antigens.

MATERIALS AND METHODS

A Leghorn population, S1, segregating for four *B* alleles, *B*¹, *B*², *B*¹⁹ and *B*²¹, was started in 1963 from an F₁ inbred cross. For this study, only the genotypes *B*¹*B*¹, *B*¹*B*² and *B*¹*B*¹⁹ were used. In each year since 1963 from 12 to 24 males were mated to 144 females in a controlled mating scheme designed to avoid subline formation and to minimize inbreeding (NORDSKOG, RISHELL and BRIGGS, 1973). Matings were arranged strictly on blood types with no deliberate selection for viability or for other performance traits.

In 1972, twenty adult hens of each genotype *B*¹*B*¹, *B*¹*B*² and *B*¹*B*¹⁹ were inoculated subcutaneously three times, two days apart, with a mixture of *Salmonella pullorum* bacterin and bovine serum albumin (BSA). *S. pullorum* bacterin, prepared according to standard procedures (DAVIS *et al.* 1968), was diluted to contain ca. 0.5 billion bacteria in a single injection of 0.5 ml. To each inoculation dose, 17.5 mg. of BSA was added. Blood samples were collected on the 7th, 14th and 21st days post-inoculation, counting from the first injection. Anti-*S. pullorum* agglutination titers were measured on the sera using a macroscopic plate test. The anti-BSA antibodies were titrated by passive hemagglutination with tanned sheep erythrocytes (BOYDEN 1951).

In 1973, 15 hens of each genotype, *B*¹*B*¹ and *B*¹*B*², were inoculated again with *S. pullorum* and with 7.5 mg. of the iron-containing protein, ferritin, added to each inoculation dose. Blood samples were collected on the 4th, 7th, 10th and 14th day post-inoculation. The anti-ferritin antibodies were titrated by passive hemagglutination with BDB-coupled erythrocytes (DAVIS *et al.* 1968).

In 1973, tests were made also for *B* blood group differences in antibody response to a viral antigen. Fifteen adult hens of each genotype, *B*¹*B*¹ and *B*¹*B*², were inoculated with a commercial parainfluenza-3 (PI-3) vaccine¹. Each pellet was injected intramuscularly in both thighs with 2 doses of ¾ ml. each. Blood samples were collected on the 7th, 10th, 14th and 21st days post-inoculation. Serum samples were titrated for anti-PI-3 antibodies with a hemagglutination inhibition (HI) test. Tests were carried out on microtiter plates using bovine erythrocytes. Titers were expressed as hemagglutination inhibition units (DAVIS *et al.* 1968).

Analyses of variance were calculated on log-transformed antibody titers.

RESULTS

Antibody titers to the various antigens at different days post-inoculation are presented in Table 1. The immune response curves of the homozygous and heterozygous *B* genotypes to *S. pullorum*, combining the two years of data, are presented in Figure 1. Single experiments for BSA, ferritin and PI-3 are presented in Figure 2.

Significantly higher antibody response of the *B*¹ heterozygotes to *S. pullorum* was observed in each year. In 1972 the average titer of the *B*¹ homozygous pullets was only 25% as high as that of *B*¹ heterozygotes in the first week and 60% in the

¹ Bar-3. Elanco Products Company, Indianapolis, Indiana.

FIGURE 1.—Immune response of genotypes B^1B^1 , B^1B^2 and B^1B^{19} to *S. pullorum*. Curves are visually fitted.

second week. The difference between the two heterozygous genotypes, B^1B^2 and B^1B^{19} , was not significant. In 1973, only the B^1B^1 and B^1B^2 genotypes were tested. The average titers of the B^1B^1 pullets were 24%, 54%, 41% and 71% as high as the B^1B^2 pullets on the 4th, 7th, 10th and 14th days post-inoculation, respectively.

An analysis of variance of response to *S. pullorum* is presented in Table 2. The mean squares for differences in genotypes were statistically significant in both years. Also, bleeding periods differed as expected. Typically, after the injection

TABLE 2

Analysis of variance of immune response to S. pullorum ($\log_{10}$ of immune titers)

Source	1972		1973	
	df	M.S.	df	M.S.
Genotypes	2	3.953*	1	7.736**
B^1B^1 vs. $(B^1B^2 + B^1B^{19})/2$	1	7.885**	—	—
B^1B^2 vs. B^1B^{19}	1	0.021	—	—
Pullets/genotype	57	0.899	28	0.528
Period	2	32.321**	3	10.472**
G×P	4	0.201	3	0.502*
Error	113	0.172	84	0.159

* $P < 0.05$

** $P < 0.01$

FIGURE 2.—Immune response of genotypes B^1B^1 , B^1B^2 , and B^1B^{19} to ferritin, bovine serum albumin, and parainfluenza 3 virus.

of antigen, the antibody level in the serum gradually increased to a peak and then declined (Figures 1 and 2). The significant genotype $\times$ bleeding period interaction in 1973 indicates that the curves of antibody production with time differ by genotypes.

The heterozygotes were consistently higher in response to the soluble antigens, BSA and ferritin. Likewise, the heterozygotes gave a higher response to para-influenza-3 in three of four periods but in no instance were the differences statistically significant.

Pullet laying-house mortality of the S1 population under study was recorded from 20 to 63 weeks of age in both 1971 and 1972. Those dying of natural causes were necropsied in an attempt to find the cause of death. About 1/3 of the B^1B^1 pullets died, compared with about 1/10 of the heterozygotes (B^1B^2 and B^1B^{19}). Differences between homo- and heterozygotes were statistically significant, but not between B^1B^2 and B^1B^{19} . The major cause of death was lymphoid leukosis and accounted for 20.4% of the mortality in B^1B^1 and 17.7% in the heterozygote.

In earlier necropsy studies carried out in 1968 and 1969, the B^1 homozygotes again had higher mortality than did the B^1 heterozygotes; lymphoid leukosis

accounted for 53% of the deaths of the former and nearly 70% of the latter group. Nevertheless, the combined four years of necropsy data failed to pinpoint any specific disease responsible for the higher mortality of $B'B'$ pullets.

DISCUSSION

The lower ability of the $B'B'$ genotype to produce antibodies to certain antigens may be a part of the genetic mechanism accounting for the higher mortality of this genotype. We hypothesize that the hyporesponsiveness as well as the higher adult mortality of $B'B'$ genotype may be the effect of an immune response gene linked to the B locus. The latter is the major histocompatibility gene in chickens, corresponding to the $H-2$ complex in mice.

The $B'B'$ genotype, which has had consistently high adult mortality over eight generations, seems to be improving slightly generation by generation. NORDSKOG, RISHELL and BRIGGS (1973) suggested that the B gene is neutral but linked to a "fitness" gene. From the results reported here we now think the latter might very well be an Ir gene. Because the proportions of B locus genotypes in the S1 line breeding population were intentionally kept constant each generation, the $B'B'$ genotype is expected to improve in viability as recombination takes place. Improvement would progress as linkages between the B and Ir break up, with natural selection discarding the unfavorable Ir alleles whether or not they are linked to the B' allele. On the other hand, if the rate of improvement in viability is as high as first reported by NORDSKOG, RISHELL and BRIGGS (1973), i.e., 4% per generation, the Ir gene in question must be outside the bounds of the B histocompatibility complex assuming that the B complex is equivalent to the $H-2$ complex in map distance. Conversely, if the Ir gene is within the B complex, the rate of improvement in viability of $B'B'$ by natural selection would seemingly not be detectable by a statistical test of regression over a limited number of generations because the recombination frequency would be very low.

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