

ASSOCIATION OF SERUM HEMOLYTIC COMPLEMENT
LEVELS WITH THE MAJOR
HISTOCOMPATIBILITY COMPLEX IN CHICKENS*

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The *B* blood group locus in the chicken (1) represents the avian counterpart of the major histocompatibility complex (MHC) of mammals. It is associated with allograft rejection (2), the graft-vs.-host reaction (3, 4), the mixed leukocyte reaction (5), and immune responsiveness to synthetic polypeptide antigens (6, 7). As yet, none of these functions can be assigned to any particular region within the *B* complex as has been done with the human (HL-A) and mouse (*H-2*) MHC (8). Therefore, to determine the genetic relationships of the *B* complex-associated functions, we were motivated to search for additional immunologic functions controlled by genes associated with the chicken MHC.

Also linked to HL-A in man (9) and to the *Ss* locus in the *S* region of the *H-2* complex (10, 11) are genes which influence serum complement (C) levels. In this report we present the first evidence that the total hemolytic C level in chickens is controlled by a gene(s) associated with the MHC, thus providing further evidence that the *B* blood group locus is the avian homologue of the mammalian MHC.

Materials and Methods

Chickens. The genetic analysis was based on three highly inbred lines of White Leghorn chickens developed at the University of California, Davis, Calif., under continued full-sib mating with inbreeding coefficients in excess of 0.98 (7). The genotypes of lines 2, 3, and 7 are B^6B^6 , B^2B^2 , and B^4B^4 , respectively, in accordance with the nomenclature of Briles (see reference 7). A segregating F_3 generation for the B^4 allele was produced based on original crosses of lines 2 \times 7 in the case of F_3 -141, and 3 \times 7 for F_3 -142. A third F_3 type (F_3 -104) was obtained by crossing unlike F_2 types; namely, F_2 -141 \times F_2 -142. The three inbred lines, as well as first crosses, were repeated concurrently with the F_3 generation, and concurrent F_1 crosses are designated as F_1 -241 and F_1 -242, respectively. Matings for the production of F_3 generations were planned in such a way as to obtain B^4B^4 homozygotes with corresponding heterozygotes carrying the B^4 allele. A genetically unrelated commercial strain of White Leghorn chickens and 1-day-old cockerels obtained from a Honolulu hatchery also were used.

Sera. For determination of C levels, sera were obtained by allowing blood to clot at room temperature for 1-2 h followed by refrigeration at 4°C for 1-2 h. The sera were adsorbed five times with packed sheep erythrocytes at 4°C and stored at -70°C. For preparation of hemolysin, outbred

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TABLE I
Serum Hemolytic C Levels in Inbred and Outbred Chickens

Chickens		CH ₅₀ U/ml
Line	No.	
2	10	249.8 ± 17.0*
3	10	272.4 ± 18.3
7	10	193.7 ± 10.5
Commercial outbreds	5	244.6 ± 14.6

* Mean ± SD.

chickens were immunized with sheep erythrocytes. The chicken anti-sheep erythrocyte antisera were inactivated at 56°C for 30 min.

Complement Assay. The method of Gewurz et al. (12) was used with some modifications. Tris buffer, pH 7.4, was used throughout (13). Sheep erythrocytes standardized to 2×10^8 cells/ml were sensitized with an equal volume of a dilution of hemolysin which gave optimal sensitization. Different volumes of appropriate dilutions of test sera in duplicate were added to 0.2 ml of sensitized sheep erythrocytes and the reaction volumes were adjusted to 1.2 ml. After incubation at 37°C for 1 h, the reaction was stopped by addition of 2.0 ml of cold physiological saline. After centrifugation at 4°C, the hemoglobin content was determined in a Beckman Spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) at 412 nm, and the CH₅₀ units were calculated according to the method given by Kabat and Mayer (14).

Allotypes. Lines 2, 3, and 7 have heavy (H)-chain allotypic phenotypes CS-1.1.4, CS-1.1.3, and CS-1.2 (15), and their genotypes are referred to as *a*, *b*, and *c*, respectively (16). CS-1.1 and CS-1.2 are found on the Fd portion of the H chain and CS-1.3 and CS-1.4 are associated with papain-sensitive regions of the H chain. Based on segregation analysis among F₂ hybrids, CS-1.1.4 and CS-1.1.3 are stable phenogroups (15). These specificities among F₃ generation birds were assayed by a sensitive radioimmunoassay (16). A lipoprotein allotype (L₁), reported by Ivanyi (17), was found only in line 3. This allotype was determined by double-diffusion precipitin analysis with antisera generously supplied by Dr. J. Ivanyi, Department of Experimental Immunobiology, Wellcome Research Laboratories, Beckenham, Kent, England.

Results

Adult birds from lines 2, 3, and 7 were selected at random without regard to age or sex and their sera were assayed for total hemolytic C. The sera of line 7 birds had 193.7 ± 10.5 CH₅₀ U/ml, whereas the sera of lines 2 and 3 had 249.8 ± 17.0 and 272.5 ± 18.3 CH₅₀ U/ml, respectively (Table I). There was no relationship between C levels and sex.

To determine whether the reduced C level of line 7 birds was related to age, the C levels of outbred cockerels and inbred cockerels and hens from ages 1 day through 7 wk were determined. As shown in Fig. 1, at 3 days of age the C levels of line 7 birds were significantly lower than those of lines 2 and 3; however, the mean level of the outbreds at this time was only slightly higher. By 2 wk, the C levels of the outbred birds were the same as line 2 and 3 and significantly higher than the line 7 levels. By 5 wk, the C levels for all lines leveled off so that by 6 wk the concentrations for all birds were about the same as those found in adult (1-yr-old) birds.

Sera from adult F₁ hybrids, produced by crossing line 7 with line 2 and with line 3, were analyzed. The C levels were similar to those of lines 2 and 3 (255.7 ± 13.5 and 259.1 ± 13.0 CH₅₀ U/ml, respectively) (Fig. 1). Second and third generation birds were obtained from crosses of the three inbred lines, and birds were selected on the basis of their homozygosity and heterozygosity for the *B*

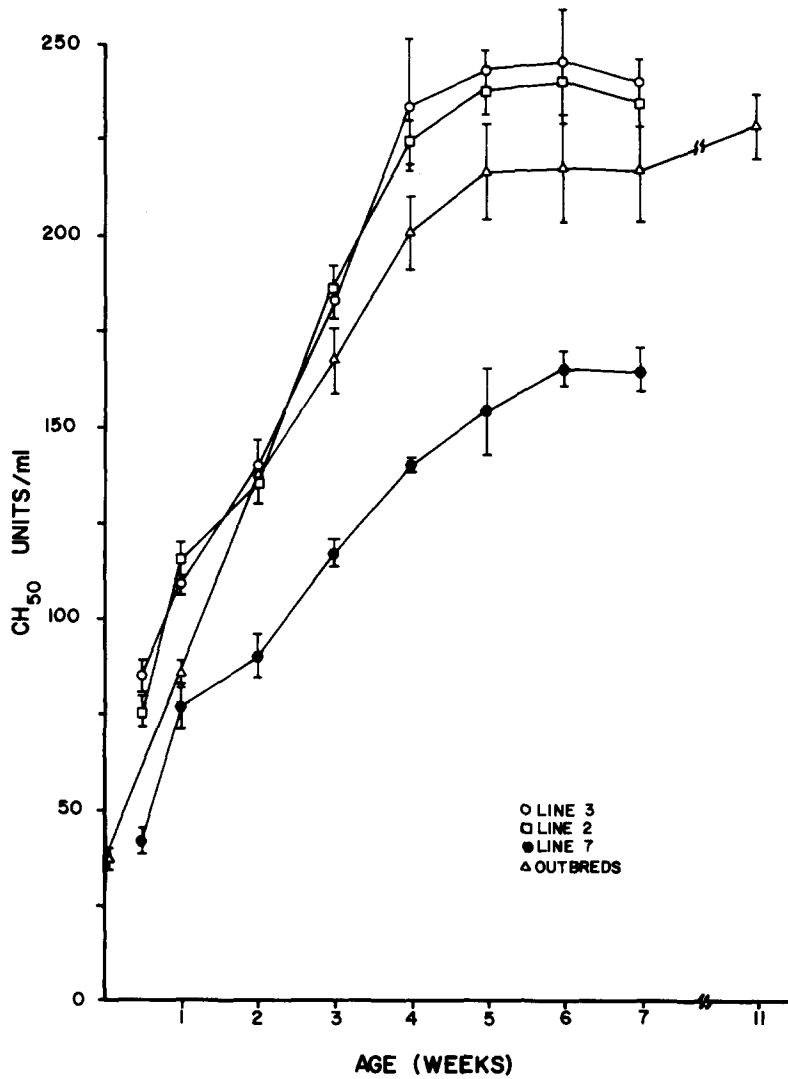


FIG. 1. The relationship between serum hemolytic C and age of inbred and outbred chickens. Each point is the mean \pm SD of 3-10 chicks.

locus, as shown in Fig. 2. All hybrids which were homozygous for the line 7 *B* allele (B^4B^4) had low C levels (174.1 ± 6.8 and 171.0 ± 8.9) regardless of whether they were derived from crosses between lines 7 and 2 or between lines 7 and 3. All sera from birds with the homozygous *B* allele of line 3 (B^2B^2) and heterozygous at the *B* allele (B^6B^4 and B^2B^4) had levels of C comparable to the levels in parental lines 2 and 3. There was no association of C levels with the CS-1 7S immunoglobulin and lipoprotein allotypes (Table II).

Discussion

All line 7 birds assayed were found to have about 70% of the serum hemolytic C of lines 2 and 3 and outbred production line birds. Line 7 birds also are low

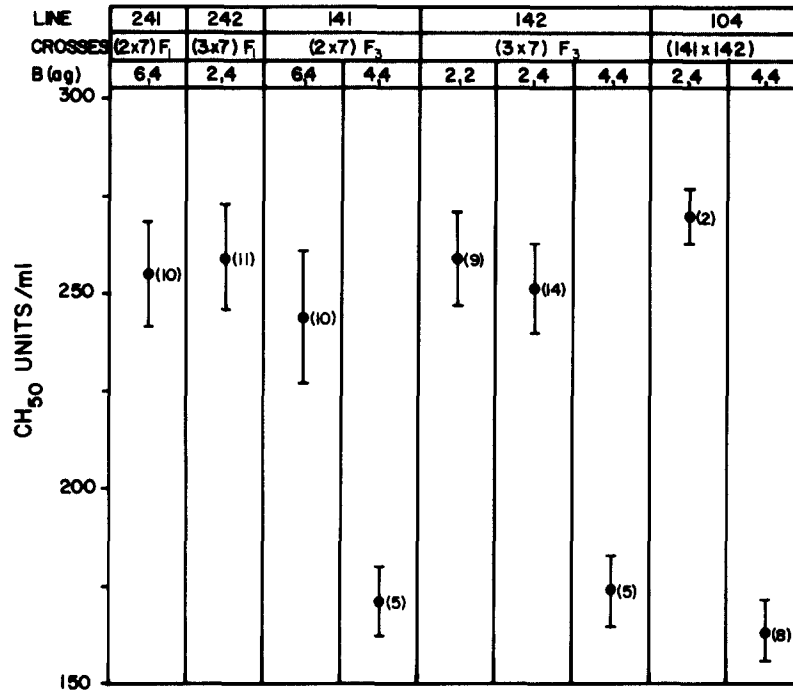


FIG. 2. Serum hemolytic C levels of F₁ chickens and their *inter se* (F₃) crosses. The F₃ hybrids were divided into subgroups according to their major histocompatibility genotypes. Each point is the mean of the number of chickens given in the brackets \pm SD.

TABLE II
Relationship between Hemolytic C Levels and B Blood Group and Allotype Genotypes in Hybrids

Hybrid line	Locus	Genotype	No. chickens	Low C level	High C level
141 = (2 \times 7)F ₃	B	4/4	5	5	0
		6/4	10	0	10
	CS-1*	a/c	13	5	8
		c/c	2	0	2
142 = (3 \times 7)F ₃	B	4/4	5	5	0
		2/4	14	0	14
		2/2	9	0	9
	CS-1	b/c	22	5	17
		c/c	5	0	5
		L ₁ †	+	24	5
		-	3	0	3
104 = (2 \times 7) (3 \times 7)F ₃	B	4/4	8	8	0
		2/4	2	0	2
	L ₁	+	8	8	0
		-	2	1	1

* 7S immunoglobulin allotype.

† Serum lipoprotein allotype (17).

antibody responders to the copolymer poly(L-Glu⁶⁰L-Ala³⁰L-Tyr¹⁰) (GAT), and the response to GAT also is associated with the MHC (7). Lest it be thought that line 7 birds are generally immunologically incompetent, they produce more antibody in response to limiting doses of bovine serum albumin than line 3 birds (18), and they synthesize levels of anti-dinitrophenyl antibodies comparable to outbreds (7). Furthermore, line 7 birds clearly had lower C levels throughout the period of maturation. The F₁ hybrids, obtained by mating line 7 birds with either of the high C lines, had C levels comparable to lines 2 and 3. Therefore, the low C level appears to be a recessive trait.

The results show that the C level in the chicken is associated with the MHC as it is in mammals. All of the low C birds found in F₃ generations had the B genotype of line 7 (B⁴B⁴). There was no overlap in the standard deviations of the CH₅₀ values between these birds and the F₃ birds with high C levels (B genotypes: B²B⁴, B²B², and B⁶B⁴). Furthermore, there was no correlation between C levels in F₃ birds with either the 7S immunoglobulin H-chain allotype linkage groups or a lipoprotein allotype.

In the mouse, the gene(s) responsible for the H-2-dependent differences in C levels is associated with the *Ss-Slp* locus (10, 11), and recently C4 was identified as the Ss protein (19, 20). In addition, C3 levels were somewhat associated with H-2 (21) and in man C2 deficiency was shown to be HL-A linked (9). At present, we do not know which C component(s) deficiency accounts for the reduced C levels in line 7 birds.

Finding the association between C levels and the MHC in the nonmammal strengthens the importance of the relationship between closely linked genes controlling histocompatibility, immune responsiveness, and C activity. It is unlikely that these immunological functions are linked by chance in species as different as chickens, mice, and men; and this linkage favors the view that this region has remained relatively unchanged over millions of years. Perhaps the C gene(s) of the MHC may prove to be the oldest component of the MHC in view of the recently described presence of C3 proactivator in starfish hemolymph (22). We agree with the view of Pazderka et al. (23) that the chicken "is eminently suited" for a species-wide evaluation of the linkage between the MHC-associated functions because of the "many differently selected populations large and stable enough to permit prolonged and repetitive sampling." For these reasons the chicken also is suited for revealing the evolutionary genetic mechanisms affecting immunoglobulin structural genes as indicated by recent studies on allotypes by E. K. Wakeland in our laboratory.

Summary

The total hemolytic complement (C) levels in inbred line 7 chicks and adults were lower than C levels in inbred lines 2 and 3 and in outbred chickens of the same age. In all birds, adult levels of C were obtained in 5- to 6-wk-old chickens. Analysis of F₁ and F₃ generations clearly showed that the C level in chickens was determined by a dominant gene(s) associated with the major histocompatibility complex. Finding this association in a nonmammal strengthens the importance of the relationship between closely linked genes controlling histo-

compatibility, immune responsiveness, mixed leukocyte reaction, and C activity.

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