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RECOMBINANTS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE CHICKEN

Iowa State University PH.D. 1982

Recombinants of the major histocompatibility complex in the chicken

by

Douglas Keith Palmer

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GLOSSARY

Adjuvant	An agent that enhances the immune response.
Allo	A prefix used to indicate genetic nonidentity among members of the same species.
Alloreactivity	Rejection of cellular allografts from genetically nonidentical members of the same species.
Alloantigen	A molecule that is antigenic when introduced into another member of the same species.
Alloantisera	Antisera which react specifically with antigenic determinants of members of the same species.
Allogeneic	Genetically nonidentical with respect to other members of the species.
Alloimmunization	Immunizations with transplantation antigens within a species.
Cellular immunity	Immune response involving effector T cells and not dependent on antibody production.
Chorioallantoic Membrane test (CAM test)	Lymphocytes are deposited on the chorioallantoic membrane of the developing embryo and produce pocks in response to transplantation alloantigens.
Congenic	Genetic identity with respect to all loci except for a series of closely linked loci or a single locus.
GAT	A polypeptide synthesized from alanine, tyrosine and glutamic acid residues and used as an antigen in studies of immune responsiveness.
Graft-versus-host (GVH) reaction	Response of grafted lymphocytes against the transplantation alloantigens of the host.
Major Histocompatibility Complex (MHC)	A group of closely linked loci exerting a major effect on transplantation immunity. It is divisible into various regions on the basis of crossover analysis. Species designations include: Rat, Ag-B; Chicken, B; Mouse, H-2; Man, HL-A.

Mixed Lymphocyte Reaction (MLR)	Mixed culture of lymphocytes from two donors and resulting in cellular proliferation if nonidentical at the MHC.
Recombinant haplotype	The result of a crossover within the MHC in a heterozygous individual.
Restriction	Ability of a lymphocyte to recognize antigen only when presented by cells bearing autologous cell surface alloantigens.
Serologically Defined (SD)	Blood cell antigens defined by the use of specific antisera.
Splenomegaly	Enlargement of the spleen produced in embryonic poultry by intravenous injections of lymphocytes.
Transplantation immunity	The ability to reject cellular allografts.
Xeno	Prefix meaning between species.
Xenogeneic	Refers to a species difference with respect to genetics.

INTRODUCTION

The major histocompatibility complex (MHC) consists of a series of closely linked genes, first recognized because of their major influence on tissue graft rejection. During the past decade, the major emphasis of immunogenetic research has been on the MHC in all species studied. All mammals, and perhaps all vertebrates, possess an MHC. Although graft rejection is probably only incidental to its true function, the MHC is a formidable barrier to successful transplantation of tissues. The MHC, designated HLA in man, also is associated with certain diseases. Favorable MHC genes confer resistance to specific diseases and unfavorable MHC genes are associated with susceptibility. Studies of the MHC promise to provide new technologies which should help overcome barriers to transplantation and improve the health and well-being of man as well as economically important species of animals.

The natural functions of the MHC have been, until just a few years ago, only speculative. Early investigations, carried out both *in vivo* and *in vitro* in laboratory species, especially in the mouse, were unable to reveal its natural *in vivo* functions. Plausible theories have now been constructed which are amenable to valid experimental testing. Seemingly, an important physiological function of the MHC is to provide for antigen recognition. That is, molecular structures of invading pathogens (antigens) become associated with molecular products coded by the MHC in

the initial physiological processes of the immune response. Only after the host and pathogen molecules are associated are they recognized as foreign.

The rapid progress in immunogenetics research should ultimately lead to the biochemical characterization of molecular products coded by the MHC and, therefore, provide a more complete understanding of their in vivo functions. The identification of genes linked to the murine and to the human MHC is now close at hand: workers are presently sequencing DNA fragments taken from mice and humans. Such information should help to develop a clearer picture of the function of the MHC as related to its structure. Ultimately, the correction of genetic defects in humans may be possible when the defective gene is identified and its dysfunction is determined.

As information on the human HLA system accumulates, catalogues of the immunological properties of the different HLA types are being constructed. Already, more than 50 diseases are known to be associated with the HLA. Refinements in typing techniques should make it possible to determine an individual's exact sequence of HLA alleles. With knowledge of the disease resistances and susceptibilities and autoimmunities conferred by these alleles, it should be possible to tailor a very precise program of preventative medicine for the individual.

Research in avian immunogenetics may have important implications for human medicine. From the point of view of evolutionary origin, the chicken is a more ancient species than man and, therefore, relationships between structure and function in this species may be more easily elucidated.

Information obtained from the study of the chicken MHC, or B complex, should complement that already known of the mammalian MHC. Models for the study of viral oncogenesis in chickens may reveal details necessary for a better understanding of oncogenic processes in humans. Moreover, studies of transplantation barriers in chickens may give clues which will help overcome barriers to organ transplantation in man.

Knowledge of the physiological functions of the molecules coded by the MHC may also have a major impact on the technology bearing on the health and well-being of the chicken. Avian immunogeneticists have long suspected a direct relationship between the B complex and disease. A B complex type, designated B^{21}, has been identified as highly resistant to the virus induced lymphomas of Marek's disease when compared with a number of other commonly occurring haplotypes. This disease occurs naturally in commercial populations of chickens. More recently, a relationship between B complex types and regression of Rous sarcoma virus induced tumors has been established.

Details of the genetic structure of the B complex are only now emerging. MHC recombinants are useful because they provide new MHC types which differ in small but important ways from the original types. They make it possible to determine the specific genes within the MHC which confer resistance or susceptibility to disease. New and favorable genetic recombinants may well be the key to the improvement of both general and specific resistance of chickens to disease.

LITERATURE REVIEW

The Major Histocompatibility Complex (MHC) in Mammals

Structure of the mammalian MHC

The murine H-2 system is the most extensively studied and best understood MHC. The H-2 complex was discovered by Peter Gorer in 1936 using xenoantisera to recognize segregating blood group antigens (Gorer, 1937). George Snell recognized the importance of histocompatibility phenomena in immunogenetics; he developed congenic lines that serve as a powerful tool in H-2 research (Snell, 1948, 1981). The subsequent production of recombinant haplotypes made it possible to analyze the complex genetic structure of the H-2 complex (Amos et al., 1955; Allen, 1955).

Details of the genetic structure of the H-2 complex have emerged gradually. At least 10 loci in the complex are distributed linearly on chromosome 17; K, [A_β, A_α, E_β], J, E_α, [SS, Slp], [D, L]. The order of the loci enclosed in brackets is not known (Klein et al., 1981).

The classical histocompatibility antigens, genetically coded by genes at the K, D and L loci, are designated Class I. They are of major importance in the control of allograft rejection. A and E antigens, coded by genes of the I region, are designated Class II. The A molecules are dimers of A_α and A_β polypeptides, and the E molecules are dimers of E_α and E_β polypeptides. Evidence indicates that immune response genes code for

the A and E antigens (Benacerraf, 1981). SS and Slp antigens of the S region, designated Class III, code for the C4 component of complement. The J antigens are expressed on suppressor T cells.

The three classes of genes described above are closely linked within the MHC of those species which have been examined sufficiently (Göetze, 1977). Details of the rat and guinea pig MHC, now emerging, suggest far less polymorphism of their MHC products compared with the mouse (Snell, 1981). The three classes of antigens have also been found in the human HLA system. Biochemically, these antigens show close homology among all species studied.

Function of the mammalian MHC

An MHC has been found in all mammals which have been sufficiently investigated and perhaps they exist in all vertebrates (Snell, 1981). Although its function has been speculative for many years, plausible theories can now be tested experimentally (Benacerraf, 1981). The discovery that immune response genes in the mouse were linked to the MHC provided the first experimental evidence for an MHC function (McDevitt and Chinitz, 1969). Subsequently, immune response genes have been found linked to the MHC of other mammals including man.

Evidence indicates that helper T cells detect antigen (carrier) on the surface of "antigen-presenting cells" with morphology corresponding to macrophages. The antigen-presenting cells must bear autologous Ia antigens to stimulate helper T cell proliferation. In turn, the proliferating T cells stimulate immunoglobulin production of hapten-specific

B cells with antigen bound to their immunoglobulin receptors. The B cells, too, must bear autologous Ia antigens. Antigen-presenting cells with autologous Ia antigens also stimulate T cells to proliferate and differentiate into delayed hypersensitivity T cells. The necessity for autologous Ia antigens is called restriction (Benacerraf, 1981).

Restriction is imposed on effector T cells by cell surface molecules coded by the K, D and L loci of the H-2 complex. Cytolytic T cells will lyse only those virus-infected target cells with K, D or L identity. Because T cell immune responses detect "self" by monitoring the cell surface, the function of the K, D or L molecules may be to indicate self. Modification of these molecules by viral antigens may be the signal of "non-self" that results in cytolysis. Alloreactivity can be explained as the recognition of allotypic K, D or L as antigen modified self molecules (Benacerraf, 1981).

In summary, it seems the immune response genes code for the Ia antigens and allow for the recognition of antigen on the surface of antigen-presenting cells by helper T cells. Ia antigens expressed on the surface of B cells with antigen bound to their immunoglobulin receptors allows for stimulation of immunoglobulin production in B cells by helper T cells. Stated more simply, it seems that the primary purpose of the cell surface alloantigens controlled by the MHC is to provide a context for antigen recognition.

The Major Histocompatibility Complex (MHC) of the Chicken

Variants controlled by the chicken MHC

The chicken MHC (B complex) codes for homologues of the Class I and Class II antigens found in mammals. B-F antigens in the chicken, designated Class I, are recognized serologically by hemagglutination. They are glycoproteins with molecular weights of 40,000 to 43,000 daltons and are noncovalently associated with a β-2 microglobulin-like molecule (Ziegler and Pink, 1975, 1976). B-L antigens, designated Class II, can also be detected with alloantisera. They are dimers of molecules with molecular weights of 32,000 and 27,000 daltons and correspond to the α and β chains of the murine H-2 complex, respectively. They are distributed on both lymphocytes and macrophages (Kvist et al., 1978; Brogren and Bisati, 1980). Recent evidence points to two populations of molecules in the chicken that are homologues of the mammalian Class II antigens (Crone et al., 1981).

Class III antigens have not been recognized in the chicken but their existence is inferred because the B complex controls total complement activity (Chanh et al., 1976). A cell surface antigen designated B-G with no known mammalian homologue has been found in the chicken; these are glycoproteins with a molecular weight of 43,000 daltons and are present on the surface of erythrocytes only.

In addition to the control of alloantigens, the B complex controls histocompatibility. Skin transplantation, mixed lymphocyte reaction,

graft-versus-host splenomegaly and graft-versus-host chorioallantoic membrane pocks are all histocompatibility attributes controlled by the B complex. Total serum hemolytic complement levels are also controlled by the B complex (Chanh et al., 1976).

An important function attributed to the B complex is control of the immune response. The B^1 haplotype, identified with low immune response to GAT is characterized by low viability and low serum IgG levels (Pevzner et al., 1975; Rees and Nordskog, 1981). Susceptibility to Rous sarcoma virus (Collins et al., 1977; Schierman et al., 1977) and Marek's disease resistance (Hanson et al., 1967; Pazderka et al., 1975) are controlled by the B complex. The development of spontaneous autoimmune thyroiditis is also controlled by the B complex (Bacon and Rose, 1979).

Genetic structure of the B complex

Details of the linear order of genes in the B complex are meager. The first B recombinant, reported by Schierman and McBride (1969), provided evidence for multiple B complex loci coding for erythrocyte alloantigens. Hàla et al. (1976) discovered a single recombinant among 1,206 chicks tested by hemagglutination. Briles and Briles (1977) detected a serological recombinant during routine blood typing and obtained two additional recombinants from its progeny. Analysis of the recombinant obtained by Hàla et al. (1976) indicated that the crossover occurred between loci coding for the B-G antigens and B-F antigens. Loci of the B-F region control both the B-F and B-L antigens. The function of the B-G region is yet unknown (Vilhelmovà et al., 1977; Ziegler and Pink, 1976).

Briles and Briles (1980) provided evidence for multiple B-G loci within the B complex.

Pevzner et al. (1978) reported a recombinant with a chromosome breakage between loci coding for serologically defined (SD) erythrocyte alloantigens and the Ir-GAT locus. In the B^1 parental haplotype, genes controlling B^1 SD erythrocyte alloantigens were linked to Ir-GAT-Lo, a low responder allele. In the B^{19} parental haplotype, genes coding for B^{19} SD erythrocyte alloantigens are linked to the Ir-GAT-Hi allele, a high responder allele. In the recombinant B^1 SD erythrocyte alloantigens are linked to the Ir-GAT-Hi allele. Thus, one recombinant haplotype is a high responder to GAT. Another recombinant links B^{19} SD erythrocyte alloantigens and the Ir-GAT-Lo allele (Lee and Nordskog, 1981). The I region genes of the mouse were first identified as immune response genes; Ia antigens were discovered in an attempt to detect immune response gene products serologically (Shreffler and David, 1974). The question of whether the Ir-GAT locus controls homologues of the murine Ia antigens remains unresolved until the gene products of the Ir-GAT locus are sequenced. An alternative possibility is that the Ir-GAT locus codes for the T cell receptor for antigen (Benacerraf, 1981).

Lee and Nordskog (1981) found that the B complex region coding for SD erythrocyte alloantigens and the region containing the Ir-GAT locus both contribute to the graft-versus-host splenomegaly reaction. The former region seemed to be of greater relative importance. If the graft-versus-host reaction in the chicken is controlled by a homologue(s) of the murine Ia antigen, then at least one locus closely linked to the B-F

and B-G loci, and separate from the Ir-GAT locus, codes for an Ia homologue. Biochemical evidence, also, points to the presence of two loci coding for Ia-like molecules in the chicken (Crone et al., 1981).

Schierman et al. (1977) provided evidence for a crossover between SD erythrocyte alloantigens and a locus controlling resistance to tumors induced by the Schmidt-Ruppin strain of Rous sarcoma virus. However, incomplete penetrance of dominant genes for regression was not ruled out. Gebriel et al. (1979) showed that the Ir-GAT locus is a distinct entity but closely linked to a locus controlling resistance to tumors induced by the Bryan strain of Rous sarcoma virus.

The genetic structure of the H-2 complex has been defined exclusively in terms of serological tests. Ideally, the chicken B complex should also be defined this way because genotypes cannot be determined definitively using tests which measure phenotypes quantitatively. The paucity of B complex loci with serologically detectable gene products has made mapping of the B complex difficult. The production of Ia alloantisera and monoclonal typing fluids specific for B complex alloantigens promises to speed the mapping of the B complex (Longenecker et al., 1979).

Function of the B complex

So far, little direct experimental evidence has been collected relating the B complex to a physiologically important function. In mammals, MHC-coded cell surface alloantigens provide for antigen recognition (Benacerraf, 1981; Klein et al., 1981). Partial amino acid sequences have already shown homology of the B complex histocompatibility antigens

with murine (H-2) and human (HLA) histocompatibility antigens (Vitetta et al., 1977; Huser et al., 1978). Future work should lead to the biochemical characterization of those molecules coded by the B complex. Because the B complex codes for Class I and Class II molecules, and because the B complex controls immune responsiveness, the primary physiological function of the cell surface molecules coded by the B complex should be closely homologous to that of the mammalian MHC. Future work using the chicken B complex, of a basic nature, should verify basic functions established in other species. The fact that the chicken is more ancient on the evolutionary scale makes it a potentially valuable species for further experimentation and especially for studies on evolutionary homology.

MATERIALS AND METHODS

Stocks

Several lines of chickens, both inbred and outbred, are maintained at Iowa State University. Outbred line S1, originating from a cross of two commercial lines (Nordskog et al., 1973), segregates for three parental B haplotypes; B^1-Ir-GAT-Lo, B^2-Ir-GAT-Intermediate, and B^{19}-Ir-GAT-Hi. B^1-Ir-GAT-Lo homozygotes are low in both viability and humoral immune response to certain antigens (Pevzner et al., 1975). Low viability and low humoral immune response have been linked to B^{19} SD erythrocyte antigens in a recombinant haplotype designated B^{19}-Ir-GAT-Lo. In addition, normal viability and immune response have been linked to B^1 SD erythrocyte antigens in a recombinant haplotype designated B^1-Ir-GAT-Hi (Pevzner et al., 1978; Lee and Nordskog, 1981).

Line 19, an inbred White Leghorn line, segregates for three B haplotypes with serological markers B^J, B^L and B^1; White Leghorn line HN, an inbred derived from a Heisdorf-Nelson importation, has been maintained as a population segregating for B^E, B^F and B^1. Line M, an inbred Fayoumi line, segregates for B^Y, B^Z and B^1. The letter superscripts are temporary designations and will eventually be replaced by numbers as information accumulates. Serological information suggests the following identities: $B^J=B^{13}$, $B^L=B^{15.1}$, $B^E=B^{12}$, $B^F=B^{15}$, $B^Y=B^{15.2}$, and $B^Z=B^5$.

Blood Typing Reagents

B system alloantisera were produced by various protocols. Injection of 1-2 ml of freshly collected whole citrated blood on days 1 and 7, followed by collection of plasma in heparin on day 13, proved to be an effective scheme. The minimal amount of stimulation produced highly specific reagents; absorptions were generally not used.

Skin Grafting Procedure

Several layers of collodion were applied to the back skin of each bird and allowed to dry. Full thickness pieces of skin, 1 cm square, were then cut out to supply donor grafts and to form recipient transplantation beds. Donor skin was kept on filter paper moistened with phosphate buffered saline (PBS) until transplanted to a recipient. Rotating the graft 180° prior to transplantation aided in recognition of successful grafts by noting the reversed direction of feather growth. Grafts were held intact with adhesive bandages which were removed on day 7 post-grafting.

Arbitrary scores (Table 1) were assigned to the grafts to indicate graft condition (Polley et al., 1960). Grafts were first scored on day 7 posttransplantation followed by several consecutive scoring days, but with decreasing frequency after this initial period.

Table 1. Scoring system to judge allograft condition

Score	Appearance of Graft
5	Healthy
4	Slight evidence of inflammation
3	Erythematous and slightly shrunken
2	Brownish and erythematous or shrunken
1	Black and shrunken or detached from host integument
0	Graft sloughed

Graft-Versus-Host Splenomegaly

A 1 cm square area was outlined on the shell over a large straight vein of the allantoic membrane of a day 13 embryonated egg. On day 14, donor blood was collected aseptically; the lymphocytes were isolated by the slow spin method or by the ficoll-hypaque gradient technique. The lymphocytes were washed three times in Hank's balanced salt solution and the concentration standardized to 2×10^7 cells/ml. The 1 cm square area of day 14 embryos was swabbed with iodine, the shell was cut along the outline with a moto tool (Dremel Mfg., Racine, Wis.), and the shell fragment was carefully removed leaving the shell membrane intact. Each embryo was injected intravenously with .1 ml of donor blood using a 27 gauge needle. The shell hole was covered with masking tape and sealed with melted paraffin. On day 19 of incubation, the spleen was dissected from the embryo and weighed on an analytical balance.

Mixed Lymphocyte Reaction

Blood was collected aseptically in heparin from adult chickens. Lymphocytes were separated by the slow spin method and washed three times in culture media. The lymphocytes were adjusted to a concentration of 1.5×10^7 cells/ml in Iscove's modified Dulbecco's media (430-2200, Gibco) supplemented with 7.5×10^{-5}M monothioglycerol, 500 µg/ml gentamycin, 4 µg/ml bovine serum albumen (Cohn fraction V, Sigma), 1 µg/ml human transferrin, 110 mg/l sodium pyruvate, and 1 mg/l reduced gluthathione.

Twenty µl aliquots of cell suspension were mixed in the wells of round bottom tissue culture plates (76-013-05, Linbro). Because the

volumes were small, the plates were sealed with tape to minimize evaporation; a small opening allowed gas exchange. The final volume in each well was 40 µl containing 3×10^6 cells.

Plates were incubated at 41.5° C for 72 hours in a 5% CO_2 in air atmosphere. After addition of .5 µCi ^{3}H-thymidine to each culture in 20 µl media, they were incubated for an additional five hours. The cells were harvested on glass fiber filters in a cell harvester (CH-103, Dynatech); the filters were dried at 130° C for one hour and counted in a Betacounter in Aquasol-2 (New England Nuclear) for one minute.

Hemagglutination Assay

B blood group alloantisera were added to the wells of round bottom plates (76-311-05, Linbro) in 50 µl aliquots. Two percent suspensions prepared from erythrocyte samples were added in 50 µl volumes to the wells containing the appropriate reagents. The plates were shaken for five minutes on a Microshaker II (Dynatech) and allowed to stand for one hour at room temperature. Agglutination reactions were assigned scores as indicated in Table 2.

Table 2. Scoring system of hemagglutination reactions produced with B blood group alloantisera

Score	Pattern
4	Mat, no sedimentation
3	Mat, slight sedimentation
2	Mat, moderate sedimentation
1	Pellet, irregular edges of agglutinates
0	Pellet, round, no agglutinates

Triple Hemagglutination Test

The B complex contains at least two loci coding for erythrocyte cell surface antigens, B-G and B-F. Gene products of these loci are polymorphic and detectable in a hemagglutination assay. To obtain recombinants of B-G and B-F alleles, B heterozygotes were mated to homozygotes of a third B allele, and the progeny were tested by hemagglutination with B system alloantisera. The assumption is that each antiserum recognizes both a B-G and a B-F region gene product of a parental haplotype. Recombinants of B-G and B-F region alleles should then react with antisera specific for each of the three alleles in the mating that produced it (Table 3).

Table 3. Triple hemagglutination test for the detection of recombinants

Haplotypes	Alleles G,F/G,F	Reagents		
		Anti-B-1 (G1,F1)	Anti-B-2 (G2,F2)	Anti-B-19 (G19,F19)
Parental				
$B^{1}B^{2}$	1,1/2,2	+	+	
$B^{2}B^{19}$	2,2/19,19		+	+
$B^{1}B^{19}$	1,1/19,19	+		+
Heterozygous recombinant				
$B^{1-19}B^{2}$	1,19/2,2 19,1/2,2	+	+	+
$B^{1-2}B^{19}$	1,2/19,19 2,1/19,19	+	+	+
$B^{2-19}B^{1}$	2,19/1,1 19,2/1,1	+	+	+

Immunofluorescence

Lymphocytes, collected from fresh heparinized blood by the slow spin method, were suspended in PBS containing .1% sodium azide and 2% fetal calf serum (FCS) at a concentration of 5×10^7 cells/ml. Ten µl of B blood group alloantisera mixed with 10 µl of cell suspension were incubated on ice for 20 minutes. The cells were washed twice with the PBS-azide-FCS, suspended in 20 µl of fluorescein labelled rabbit-anti-chicken IgG, and incubated on ice for 20 minutes. The cells were again washed twice in PBS-azide-FCS and suspended in 20 µl of 70% glycerol. The cells were observed for fluorescence with a Leitz Ortholux II microscope fitted with a 54x or 63x objective.

GAT Response Assay

Chickens were immunized intramuscularly with 1 ml of an emulsion of complete Freund's adjuvant and PBS (pH 7.2) containing 100 µg GAT (L-glutamic acid^{60}, L-alanine^{30} and L-tyrosine^{10}, Miles, Elkhart, Ind.) and boosted with 100 µg GAT in 1 ml of PBS on day 21. Serum samples were collected on days 14 and 28. Twenty µl samples of test or control serum were mixed with 180 µl of PBS containing 1% normal chicken serum. After addition of 25 µl of a dilution of iodinated GAT to each sample, they were kept at 4° C for one hour. Fifty µl of rabbit-anti-chicken IgG were then added to each sample. After one hour of incubation at 4° C the samples were centrifuged at 6500g for 10 minutes. Fifty µl of supernatant were then withdrawn from each sample and counted in a gamma counter (Tracor Analytic, Model 1197) for two minutes. The percent antigen bound was determined as

$$100\ (1 - \frac{\text{test sample CPM}}{\text{control sample CPM}}).$$

RESULTS

Production of B Complex Recombinants

Matings to produce serological recombinants in 1980 are described in Table 4. Three thousand, five hundred ninety-two progeny were produced from these matings. Based on a triple hemagglutination test, four of these were designated putative recombinants (Table 5) including: (a) a chicken of line 19 which proved to be an intersex, (b) two line HN males and (c) one line S1 female. Backcross matings to test for recombinant haplotypes are described in Table 6.

A bird from Leghorn line 19 giving triple reacting erythrocytes (wing band number 8537) was first recorded as a female but later developed features characteristic of a male. At maturity, this bird had well-developed spurs and pointed hackle and saddle feathers; yet its vocalizations were typically female. It neither produced semen nor laid eggs. Possibly an unbalanced chromosome constitution, such as polysomy or polyploidy, is the cause of both the red cell triple reactivity and the sterility of this bird. It has been judged not to be a recombinant.

The erythrocytes of a line HN male (wing band number 7333) gave strong reactivity with anti-B-E and anti-B-F and weak reactivity with anti-B-1 reagents. Because he transmitted the B^E and B^F haplotypes, but not B^1 to his progeny, this bird was deduced to be a $B^E B^F$ heterozygote. Weak cross reactions of the anti-B-1 reagents rather than recombination

Table 4. Matings to produce serological recombinants and the number of putative recombinants obtained from each single male mating (1980 hatching season)

Line	Male Blood Type	Female Blood Type	Number Progeny Typed	Number Putative Recombinants (Wing Band Number)	Putative Genotype
M	$B^{1}B^{Z}$	$B^{Y}B^{Y}$	80		
	$B^{Y}B^{Y}$	$B^{1}B^{Z}$	211		
	$B^{1}B^{Y}$	$B^{Z}B^{Z}$	29		
	$B^{Z}B^{Z}$	$B^{1}B^{Y}$	82		
			402		
19	$B^{J}B^{J}$	$B^{1}B^{L}$	35		
	$B^{1}B^{J}$	$B^{L}B^{L}$	273		
	$B^{J}B^{L}$	$B^{1}B^{1}$	203	1(8537)	$B^{J-L}B^{1}$
	$B^{1}B^{1}$	$B^{J}B^{L}$	129		
			640		
HN	$B^{E}B^{F}$	$B^{1}B^{1}$	109		
	$B^{1}B^{E}$	$B^{F}B^{F}$	418	1(7333)	$B^{1-E}B^{F}$
	$B^{1}B^{F}$	$B^{E}B^{E}$	110	1(3878)	$B^{1-F}B^{E}$
	$B^{1}B^{1}$	$B^{E}B^{F}$	193		
			830		
S1	$B^{1}B^{1}$	$B^{2}B^{19}$	267		
	$B^{2}B^{2}$	$B^{1}B^{19}$	218		
	$B^{19}B^{19}$	$B^{1}B^{2}$	234		
	$B^{1}B^{2}$	$B^{19}B^{19}$	8		
	$B^{1}B^{2}$	$B^{19}B^{19}$	205		
	$B^{1}B^{19}$	$B^{2}B^{2}$	381	1(13777)	$B^{1-19}B^{2}$
	$B^{1}B^{19}$	$B^{2}B^{2}$	176		
	$B^{2}B^{19}$	$B^{1}B^{1}$	231		
			1,720		
TOTAL			3,592		

are the most likely explanation for his erythrocytes to react with all three test antisera. An alternative possibility is that his blood cells, but not his gonads, were chimeric.

Table 5. Triple hemagglutination test pattern of red cells from putative recombinants (1980 hatching season)

Line	Cross	Wing Band Number	Agglutination Score--First Typing
19	$B^{J}B^{L} \times B^{1}B^{1}$	8537	Anti-B- $\frac{J}{4}\ \frac{J}{4}\ \frac{J}{4}\ \frac{L}{3}\ \frac{L}{3}\ \frac{L}{3}\ \frac{1}{4}\ \frac{1}{4}$
HN	$B^{1}B^{E} \times B^{F}B^{F}$	7333	Anti-B- $\frac{E}{2}\ \frac{E}{4}\ \frac{E}{4}\ \frac{F}{4}\ \frac{F}{4}\ \frac{1}{1}\ \frac{1}{1}\ \frac{1}{1}$
HN	$B^{1}B^{F} \times B^{E}B^{E}$	3878	Anti-B- $\frac{E}{4}\ \frac{E}{4}\ \frac{F}{2}\ \frac{F}{2}\ \frac{F}{2}\ \frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}$
S1	$B^{1}B^{19} \times B^{2}B^{2}$	13777	Anti-B- $\frac{1}{2}\ \frac{1}{2}\ \frac{1}{2}\ \frac{2}{4}\ \frac{2}{4}\ \frac{19}{0}\ \frac{19}{2}\ \frac{19}{1}$

A male of line HN (wing band 3878) produced 274 progeny. The data indicated he repeatedly transmitted three alleles, as judged by progeny testing with anti-B-E, anti-B-F and anti-B-1 reagents. Because this male transmitted normal B alleles to its progeny, the likelihood that he carried a recombinant haplotype seemed nil. Silver staining of the B-linked nucleolar organizer region, kindly performed by courtesy of Dr. S. E. Bloom of Cornell University, proved that this bird was disomic and not trisomic. Very likely, the red cells and gonads of this bird were chimeric, possessing two populations of cells with distinct genotypes. Such a chimera has been previously reported in chickens by Halå et al. (1978),

Table 6. Backcross tests of four putative recombinants produced (1980 hatching season)

Line	Wing Band Number	Sex	Putative Genotype	Crossed With	Progeny	
					Blood Type	Number of Progeny
HN	7333	Male	$B^{1\text{-}E}B^{F}$	$B^{E}B^{E}$	$B^{E}B^{E}$	3
					$B^{E}B^{F}$	1
				$B^{F}B^{F}$	$B^{F}B^{F}$	19
					$B^{E}B^{F}$	14
				$B^{1}B^{1}$	$B^{1}B^{E}$	4
					$B^{1}B^{F}$	8
				$B^{1}B^{E}$	$B^{E}B^{E}$	5
					$B^{E}B^{F}$	6
					$B^{1}B^{E}$	8
					$B^{1}B^{F}$	10
						78
HN	3878	Male	$B^{1\text{-}F}B^{E}$	$B^{F}B^{F}$	$B^{E}B^{F}$	73
					$B^{F}B^{F}$	39
					$B^{1}B^{F}$	34
					$B^{1}B^{E}$	0
					$B^{E}B^{E}$	1
				$B^{1}B^{E}$	$B^{E}B^{E}$	26
					$B^{1}B^{E}$	27
					$B^{1}B^{F}$	18

					$B^{E}B^{F}$	16
					$B^{1}B^{1}$	13
					$B^{F}B^{F}$	1
				$B^{1}B^{1}$	$B^{1}B^{E}$	15
					$B^{1}B^{F}$	2
					$B^{1}B^{1}$	6
				$B^{E}B^{E}$	$B^{E}B^{E}$	2
					$B^{1}B^{E}$	1
						274
19	8537	Inter-sex	$B^{J-L}B^{1}$	$B^{1}B^{L}$	--	0
				$B^{1}B^{J}$	--	0
S1	13777	Female	$B^{1-19}B^{2}$ (B^{19rl})	$B^{2}B^{2}$	$B^{1-19}B^{2}$	36
					$B^{2}B^{2}$	26
					$B^{1}B^{2}$	1
					$B^{2}B^{19}$	1
						64

who described a male with red cells of two distinct B genotypes and which transmitted three B haplotypes to its progeny.

S1 female 13777, mated to a B^2B^2 male, produced 64 progeny classified either as B^2B^2 or $B^{1-19}B^2$ (Table 3). The χ^2 test of a two-allele hypothesis for a 1:1 segregating ratio gave a satisfactory fit to the data at the 5% significance level, confirming that a recombinant of the B^1 and B^{19} haplotypes had been produced. However, two progeny with unexpected blood types, B^1B^2 and B^2B^{19}, were identified in this group. Because a female penned with 13777 had been producing B^2B^{19} chicks, and because a farm worker may have erred as to the parentage of eggs laid before the hens were accustomed to the use of trap nests, the B^2B^{19} chick was judged a pedigree error. In a later hatch, a chick typed as B^1B^2 may also have been a pedigree error.

In 1981, further matings were set up between birds in the S1 line in order to produce additional serological recombinants. The design of these matings is given in Table 7. Of 1,992 progeny tested, 4 were positive using the triple hemagglutination test; these were classified as putative recombinants (Table 8). All were produced from matings of B^2B^2 males to B^1B^{19} heterozygous females. The putative $B^{1-19}B^2$ birds were mated to B^2B^2 homozygotes in a backcross test (Table 9). A χ^2 test of the hypothesis for two-alleles segregating in a 1:1 ratio gave a satisfactory fit to the data at the 5% level of significance for each of the four birds tested.

Table 7. Matings to produce serological recombinants and the number of putative recombinants produced in each single-male mating (1981 hatching season)

Male Blood Type	Female Blood Type	Number of Progeny Typed	Number of Putative Recombinants
B^2B^2	B^1B^{19}	348	1
B^2B^2	B^1B^{19}	318	1
B^2B^2	B^1B^{19}	245	0
B^2B^2	B^1B^{19}	341	0
B^2B^2	B^1B^{19}	306	2
B^1B^2	$B^{19}B^{19}$	41	0
B^1B^2	$B^{19}B^{19}$	32	0
B^2B^{19}	B^1B^1	329	0
B^2B^{19}	B^1B^1	32	0
Total		1,992	4

Table 8. Triple hemagglutination test pattern of red cells from four S1 putative recombinants produced from B^1B^{19} x B^2B^2 matings (1981 hatching season)

Wing Band Number	Sex	Agglutination Score-First Typing					
		Anti-B-	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{2}{4}$	$\frac{19}{4}$	$\frac{19}{4}$
1547	Male		4	4	4	1	4
1779	Male		4	4	3	0	4
6093	Male		4	4	4	4	4
12610	Female		4	4	4	4	4

Immunofluorescent Analysis of the Recombinant Haplotypes

The tissue distribution of the B^1 and B^{19} antigens on the recombinant haplotypes was determined by immunofluorescence. Results are given in Table 10. The anti-B-1 serum stained red cells only and the anti-B-19 serum stained both red cells and lymphocytes of the recombinant-B^2 heterozygous birds. Because B-G antigen is found on red cell membranes but not lymphocyte membranes, the evidence indicates that the recombinant haplotypes each must have derived their B-G region from the B^1 parental haplotype. On the other hand, because the B-F antigen is found on both red cell and lymphocyte membranes, the recombinant haplotypes must have derived their B-F region from the B^{19} parental haplotype. Accordingly, the new recombinant haplotypes were designated B^{19r1}, B^{19r2}, B^{19r3}, B^{19r4}, and B^{19r5} (Tables 6 and 9). Because the anti-B-2 reagents stained red cells and not lymphocytes, they apparently recognized the B-G antigen only.

Table 9. Backcross tests ($B^{1-19}B^2 \times B^2B^2$) of four S1 putative recombinants produced (1981 hatching season)

Wing Band Number	Progeny		Revised Haplotype Designation
	Blood Type	Number	
1547	$B^{1-19}B^2$	65	B^{19r2}
	B^2B^2	62	
1779	$B^{1-19}B^2$	40	B^{19r3}
	B^2B^2	33	
6093	$B^{1-19}B^2$	60	B^{19r4}
	B^2B^2	53	
12610	$B^{1-19}B^2$	4	B^{19r5}
	B^2B^2	3	

Table 10. Immunofluorescent staining of red cells and lymphocytes from birds carrying the haplotypes B^{19r1}, B^{19r2}, B^{19r3}, B^{19r4}, and B^{19r5}

Test Cells of Bird Number	Genotype	Type of Cells	Fluorescence		
			Anti-B-1	Anti-B-19	Anti-B-2
12139	B^1B^2	WBC	+	-	-
12092	B^1B^2	WBC	+	-	-
12046	B^2B^{19}	WBC	-	+	-
11817	B^2B^{19}	WBC	-	+	-
6535	$B^{19r1}B^2$	WBC	-	+	-
6529	$B^{19r1}B^2$	WBC	-	+	-
6993	$B^{19r1}B^2$	WBC	-	+	-
6531	$B^{19r1}B^2$	WBC	-	+	-
1547	$B^{19r2}B^2$	WBC	-	+	-
1779	$B^{19r3}B^2$	WBC	-	+	-
6093	$B^{19r4}B^2$	WBC	-	+	-
12610	$B^{19r5}B^2$	WBC	-	+	+
12092	B^1B^2	RBC	+	-	+
11817	B^2B^{19}	RBC	-	+	+
6529	$B^{19r1}B^2$	RBC	-	+	+
6531	$B^{19r1}B^2$	RBC	-	+	+
1547	$B^{19r2}B^2$	RBC	-	+	+
1779	$B^{19r3}B^2$	RBC	-	+	+
6093	$B^{19r4}B^2$	RBC	-	+	+
12610	$B^{19r5}B^2$	RBC	-	+	+

GAT Response Analysis of the B^{19rl} Haplotype

Results of testing B^{19rl} homozygous birds for immune response to GAT are given in Table 11. Sera were collected on day 14 (primary) and day 28 (secondary) after the primary immunization. In both the primary and secondary responses, the B^{19rl} homozygotes were high responders to GAT. Several days prior to testing the B^{19rl} homozygotes, the same batch of GAT was used to measure the immune response of another group of chickens. The average response of 96 birds classified as Ir-GAT-Hi responders was 36.4. Seventy-four birds classified as Ir-GAT-Lo responders gave responses near zero. Because the B^{19rl} homozygotes consistently gave a high immune response to GAT, the conclusion was that this haplotype contains the Ir-GAT-Hi gene.

Table 11. Test of B^{19rl} homozygous birds for immune response to the synthetic polypeptide GAT

Genotype	Primary Response (Number)	Secondary Response (Number)
B^{19rl}-B^{19rl}	74.4(14)	51.4(14)

Skin Transplantation Analysis of the B^{19rl} Haplotype

The results of two skin grafting experiments using $B^{19rl}B^{2}$ backcross progeny are given in Table 9. Typically B-incompatible grafts are rejected and B-compatible grafts are intact at 10 days post-grafting using 14-day old chicks. Graft exchanges among 6- and 10-week old chicks in

Table 12 gave earlier rejection than expected. For example, only one of seven B^1B^1, control grafts on B^1B^2 recipients and three of nine B^2B^2 control grafts on B^1B^2 recipients were intact 10 days post-grafting. The use of older birds for grafting in this experiment probably accounts for the earlier rejections. Exchanges among two-week old birds, presented in Table 13, are more in accord with expectation. That is, B-compatible control grafts were largely intact and B-incompatible control grafts were mostly rejected at 10 days post-grafting.

Of special interest were graft exchanges involving the B^{19rl} haploytype. As given in Tables 12 and 13, $B^{19rl}B^2$ grafts on B^1B^2 recipients were rejected (zero of nine and zero of six, respectively) at 10 days post-grafting. Most B^1B^1 and B^1B^2 grafts on $B^{19rl}B^2$ recipients were rejected (seven of eight and five of five, respectively) and the majority of $B^{19}B^{19}$ and B^2B^{19} grafts on $B^{19rl}B^2$ recipients were accepted (six of ten and six of six, respectively) at 10 days post-grafting. These results demonstrate a strong histocompatibility difference between the B^{19rl} and B^1 haplotypes. On the other hand, no evidence for a histocompatibility difference could be detected between the B^{19rl} and B^{19} haplotypes.

Graft-Versus-Host Analysis of the B^{19rl} Haplotype

Results of three replicates of a graft-versus-host splenomegaly test are given in Table 14. $B^{19rl}B^2$ lymphocytes injected into B^1B^{19} embryos produced spleen enlargement significantly greater than controls but when $B^{19rl}B^2$ lymphocytes were injected into B^2B^{19} embryos no spleen enlargement

Table 12. Survival of skin grafts exchanged to determine whether major histocompatibility differences exist between the B^{19rl} haplotype and the B^{1} and B^{19} parental haplotypes (Experiment 1)

Time	Donor Genotype	Recipient Genotype			
		$B^{19rl}B^{2}$	$B^{1}B^{2}$	$B^{2}B^{19}$	$B^{2}B^{2}$
		(Accepted/Total)			
10 days	$B^{19rl}B^{2}$	5/6	0/9	6/8	0/8
	$B^{1}B^{1}$	1/8	1/7	0/8	1/9
	$B^{2}B^{2}$	4/7	3/9	8/10	7/9
	$B^{19}B^{19}$	6/10	0/9	7/9	0/10
25 days	$B^{19rl}B^{2}$	2/6	0/9	3/8	0/8
	$B^{1}B^{1}$	0/8	0/7	0/8	0/9
	$B^{2}B^{2}$	4/7	3/9	4/10	3/9
	$B^{19}B^{19}$	0/10	0/9	3/9	0/10

Table 13. Survival of skin grafts exchanged to determine whether major histocompatibility differences exist between the B^{19rl} haplotype and the B^{1} and B^{19} parental haplotypes (Experiment 2)

Time	Donor Genotype	Recipient Genotype			
		$B^{19rl}B^{2}$	$B^{1}B^{2}$	$B^{2}B^{19}$	$B^{1}B^{19}$
		(Accepted/Total)			
10 days	$B^{19rl}B^{2}$	5/5	0/6	6/6	0/5
	$B^{1}B^{2}$	0/5	5/6	0/6	0/6
	$B^{2}B^{19}$	6/6	0/6	6/6	0/5
	$B^{1}B^{19}$	0/6	1/6	0/6	2/6
25 days	$B^{19rl}B^{2}$	4/5	0/6	5/6	0/5
	$B^{1}B^{2}$	0/5	3/6	0/6	0/6
	$B^{2}B^{19}$	5/6	0/6	6/6	0/5
	$B^{1}B^{19}$	0/6	0/6	0/6	2/6

could be detected. Again, major histocompatibility differences between the B^{19rl} and B^{1} haplotypes were indicated. $B^{2}B^{19}$ lymphocytes injected into $B^{1}B^{2}$ embryos produced significantly greater splenomegaly than $B^{19rl}B^{2}$ lymphocytes injected into $B^{1}B^{2}$ embryos. This suggests that both "ends" of the chromosome segment, representing the B complex, contribute to the graft-versus-host reaction. Interestingly, Lee and Nordskog (1981) observed that $B^{1}B^{1}$ lymphocytes cause greater enlargement of $B^{19}B^{19}$ spleens than $B^{19}B^{19}$ lymphocytes in $B^{1}B^{1}$ spleens. In the present experiment, enlargement of $B^{2}B^{19}$ spleens produced by $B^{1}B^{2}$ lymphocytes was significantly greater ($P < .01$) than the reciprocal test.

Table 14. Mean spleen weight (mg) produced by graft-versus-host splenomegaly reactions involving the B^{19rl} haplotype

Donor Genotype	Recipient Genotype		
	$B^{1}B^{2}$		$B^{2}B^{19}$
$B^{1}B^{2}$	19.0 ± 10.5 (3)	**	85.2 ± 3.6 (25)
	**		**
$B^{2}B^{19}$	51.4 ± 4.3 (18)	**	15.2 ± 3.9 (21)
	*		
$B^{19rl}B^{2}$	40.3 ± 3.7 (24)	**	16.1 ± 2.5 (52)

* Significant at the .05 level.

** Significant at the .01 level.

Mixed Lymphocyte Reaction Analysis of the B^{19rl} Haplotype

Results of a two-way mixed lymphocyte reaction test are given in Table 15. Such a test, using lymphocytes of $B^{19rl}B^{2}$ and $B^{2}B^{19}$ individuals, gave no increase in counts per minute over the negative (B-compatible) control, but for a test using lymphocytes of $B^{19rl}B^{2}$ and $B^{1}B^{2}$, a highly significant increase in counts per minute was found relative to the negative control based on the Student's t test. Because the mixed lymphocyte reaction is mediated by Ia antigens in the mouse, this suggests a major difference in Ia-like antigens between the B^{19rl} and B^{1} haplotypes in the chicken.

Table 15. Results of two-way mixed lymphocyte reactions between the B^{19rl} haploytype and its two parental haplotypes, B^{1} and B^{19}

Cells	Counts Per Minute	Number of Birds, Wells
$B^{1}B^{2} \cdot B^{2}B^{19}$	1,651 ± 101	8, 48
$B^{19rl}B^{2} \cdot B^{1}B^{2}$	1,418 ± 101 **	8, 48
$B^{19rl}B^{2} \cdot B^{2}B^{19}$	183 ± 50	8, 48
$B^{19rl}B^{2} \cdot B^{19rl}B^{2}$	138 ± 50	4, 12
$B^{1}B^{2} \cdot B^{1}B^{2}$	218 ± 50	4, 12
$B^{2}B^{19} \cdot B^{2}B^{19}$	197 ± 50	4, 12

** Significant at the .01 level.

Each lymphocyte suspension was paired with both a B-compatible and a B-incompatible suspension. The ratio of counts per minute for each B-incompatible pair to the average count per minute of the two corresponding B-compatible pairs was then calculated. An analysis of variance was made on the log-transformed values. The assumption was that test samples and plates were random effects and sets and duplicate pairs were fixed effects. The analysis of variance, presented in Table 16, revealed that four factors significantly affect the mixed lymphocyte reaction: (1) experiment, (2) replication, (3) genotype pair (Set), and (4) duplicate pairs. Estimated variance components and percentages were also calculated (Table 16). These results demonstrate that most of the variation is associated with experiment and replication differences.

Table 16. Analysis of variance for the mixed lymphocyte reaction experiments

Source of Variation	D.F.	S.S.	M.S.	E.M.S.	Variance Component	Variance Component (%)
EXPERIMENT (E)	1	52.69	52.69**	$\sigma_e^2 + 2\sigma_E^2$	26.004	82.0
RELICATE (R)/E	2	32.85	16.43**	$\sigma_e^2 + 4\sigma_R^2$	3.937	12.4
SET (S)/R/E	8	49.59	6.20**	$\sigma_e^2 + 12\sigma_S^2$	0.460	1.5
DUPLICATE (D)/S/R/E	12	192.63	16.05**	$\sigma_e^2 + 24\sigma_D^2$	0.641	2.0
Error	120	81.42	0.68	σ_e^2	0.678	2.1
Total (corrected)	143	409.18				

** Significant at the .01 level.

Skin Transplantation Analysis of the B^{19r2}, B^{19r3} and B^{19r4} Haplotypes

Results of two replicate tests of a skin transplantation experiment between individuals representing B^{19r2}, B^{19r3} and B^{19r4} haplotypes are given in Table 17. All birds with recombinant haplotypes were B^2 heterozygotes. At 10 days post-grafting, B^2B^2 birds rejected all grafts from birds bearing the recombinant haplotypes. In contrast, recipient recombinant-B^2 heterozygotes did not reject B^2B^2 grafts except for a few exceptions. Grafts exchanged within and between the different recombinant haplotypes were largely intact at 10 days post-grafting suggesting that the three recombinant haplotypes bear identical major histocompatibility alleles.

Table 17. Survival of skin grafts exchanged to determine whether major histocompatibility differences exist between the B^{19r2}, B^{19r3} and B^{19r4} haplotypes

Time	Donor Genotype	Recipient Genotype			
		$B^{19r2}B^2$	$B^{19r3}B^2$	$B^{19r4}B^2$	B^2B^2
10 days	$B^{19r2}B^2$	15/16	13/16	15/16	0/13
	$B^{19r3}B^2$	14/16	14/14	16/16	0/15
	$B^{19r4}B^2$	15/16	14/16	16/16	0/16
	B^2B^2	15/16	14/14	16/16	15/16
25 days	$B^{19r2}B^2$	15/16	11/16	15/16	0/13
	$B^{19r3}B^2$	14/16	13/14	16/16	0/15
	$B^{19r4}B^2$	14/16	11/16	16/16	0/16
	B^2B^2	15/16	13/14	16/16	14/16

Recombination Frequency Within the B Complex

Observed recombination frequencies between the B-G and B-F loci are given in Table 18. The recombination frequencies did not differ significantly between experiments when tested with the χ^2 test for the Poisson distribution (Snedecor and Cochran, 1967). Because B antigens may not have reached adult levels, certain otherwise detectable recombinants may not have been detected in these experiments. Experiment 1 chicks were typed at four weeks of age and Experiment 2 chicks were typed at six weeks of age. Possibly the typing of birds at an early age in Experiment 1 may account for the lower estimate of frequency of recombination. All recombinants detected involved $B^{1}B^{19}$ heterozygotes although other heterozygote combinations were tested. Also, the reagents used to detect recombinants did not necessarily recognize both the B-G and B-F loci.

Table 18. Estimates of recombination frequencies from Experiments 1 and 2

Group	Number Recombinants/ Number Tested	Frequency of Recombination (%)
Experiment 1	1/3592	.028
Experiment 2	4/1992	.201
Experiment 1 and 2 average	5/5584	.090
Heterozygous Females	4/2927	.137
Heterozygous Males	1/2657	.038
$B^{1}B^{19}$ Heterozygotes	5/2333	.214
All Other Heterozygotes	0/3251	.000

DISCUSSION

Linear Order of Genes

The primary purpose of this study was to identify and characterize recombinants of B complex genes which code for serologically detectable cell surface alloantigens. Recombinants are especially useful in determining the gene complexity and ultimately the linear order of genes on a chromosome segment. Five birds, seemingly bearing recombinant haplotypes as determined by a triple hemagglutination assay, were verified as true recombinants in backcross tests. One of these, B^{19rl}, has been further studied with respect to alloimmune properties and immune responsiveness. The others hopefully will be analyzed further at a later date.

The B complex has been associated with a chromosome in the size range of 15-18 (Bloom and Cole, 1978). The current model of the B complex, proposed by Vilhelmová et al. (1977), is given in Figure 1. The order of the loci and their position relative to the centromere has not been determined. The B-G and B-F regions were separated by a crossing over event and the resulting recombinant was detected by hemagglutination. The B-G region, coding for an erythrocyte alloantigen, has no known function. The B-F region, coding for a protein homologous to the murine K and D antigens, controls histocompatibility. The B-L antigens, dimers of polypeptides believed homologous to the α and β chains of murine Ia molecules, are coded by the B-F region.

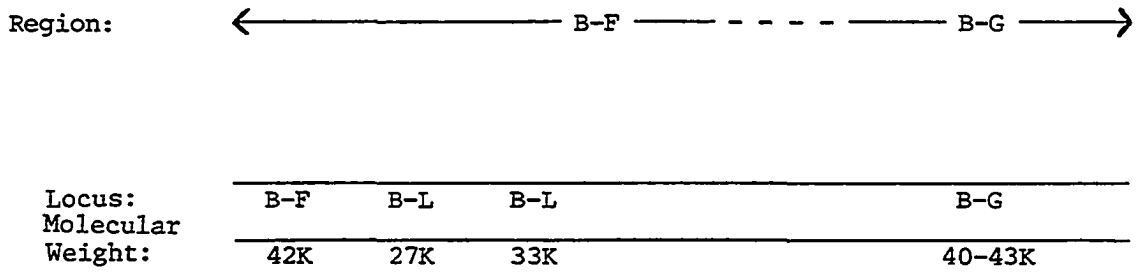

Figure 1. Model of the B complex proposed by Vilhelmová et al. (1977). Dashed line indicates region of crossing over. The B-L molecules are dimers of polypeptides presumably coded by distinct structural genes. Molecular weights are determined by Ziegler and Pink (1975, 1976), Kvist et al. (1978) and Brogren and Bisati (1980)

A chromosome segment containing the B-G and B-F loci has been separated from the Ir-GAT locus and recovered in recombinant form as reported by Pevzner et al. (1978) and Lee and Nordskog (1981). Their studies suggest that the Ir-GAT locus occupies an end position of the B complex. In the present study, the B-G and B-F genes were separated in each of five independent recombination events. The first recombinant, B^{19rl}, has now been produced in homozygous form. B^{19rl} homozygotes have proved to be high responders to the synthetic polypeptide GAT; therefore, this haplotype is deduced to contain the Ir-GAT-Hi gene of the B^{19} parental haplotype. Immunofluorescent testing indicates that the B-G gene was derived from the B^{1} parental haplotype and the B-F gene from the B^{19} parental haplotype. Therefore, the B-F and Ir-GAT genes both must be contained in a chromosome segment derived from the B^{19} parental haplotype. Evidently, the B-G locus also occupies an end position of the B complex. The combined results suggest that the order of the genes is B-G, B-F and Ir-GAT.

In the mouse, experiments have provided evidence that the immune response gene products are the Ia antigens themselves (Benacerraf, 1981). The arguments for this are: (a) Ia antigens and immune response genes map in the same subregions of the H-2 complex, (b) monoclonal antibodies to Ia antigens, when bound to antigen-presenting cells, specifically block in vitro responses to antigen and (c) studies of complementation by A_α and A_β and E_α and E_β polypeptides reveal a close relationship between chain structure and the genetic control of immune responsiveness to synthetic polypeptides.

Others in this laboratory, working with B complex parental and recombinant haplotypes involving loci coding for SD erythrocyte alloantigens and the Ir-GAT locus, have provided data showing that the immune response region containing the Ir-GAT locus controls the production of antigens homologous to the murine Ia antigens as regards both tissue distribution and molecular weight (R. C. Birkmeyer and A. W. Nordskog, Department of Animal Science, Iowa State University, unpublished). These antigens almost certainly are equivalent to the B-L antigens identified by Ziegler and Pink (1976). The functional homologies of the chicken B complex to the murine H-2 complex lead to the conclusion that the Ir-GAT locus is identical to a B-L locus. First, the Ir-GAT locus controls immune responsiveness. Secondly, a B-L locus controls the production of molecules homologous to murine Ia antigens in molecular weight, ontogeny of cellular expression and in tissue distribution (Ewert and Cooper, 1982). Thirdly, immune response genes (Ir-GAT) and genes

coding for molecules homologous to the murine Ia antigens (B-L) have not been separated by crossing over. Therefore, identity of the Ir-GAT locus and a B-L locus must be assumed, which leads to the proposal of a new model for the B complex (Figure 2). Blocking of *in vitro* responses to GAT by binding specific monoclonal antibody to the B-L antigens on antigen-presenting cells would provide final evidence for the common identity of the Ir-GAT locus and a B-L locus.

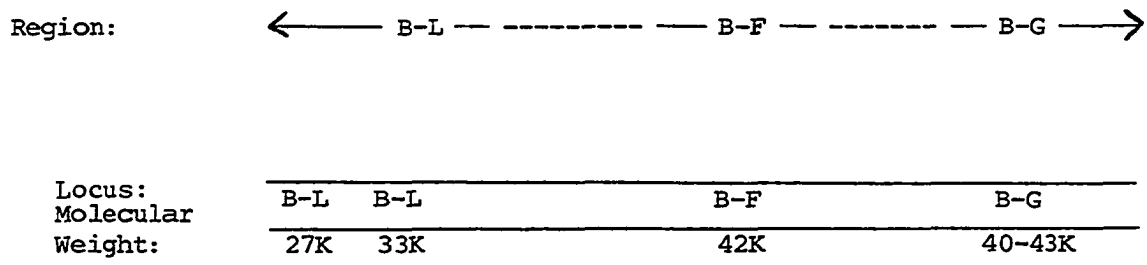

Figure 2. Proposed order of loci for the B complex. Dashed lines indicate regions of crossing over. The B-L molecules are dimers of polypeptides presumably coded by distinct structural genes

Recent biochemical evidence now reveals two populations of molecules which are homologues of murine Ia molecules (Crone *et al.*, 1981). The location within the B complex of genes coding for these molecules can be inferred. One population of molecules is likely coded by the Ir-GAT locus or by a locus linked closely to it. Because the graft-versus-host reaction is controlled by Ia antigens in the mouse, and because the chromosome segment containing the B-G and B-F genes exerts a major influence on graft-versus-host reactivity, a second population is likely coded by a gene closely linked to B-F. In addition, the B complex controls

total hemolytic complement activity (Chanh et al., 1976). This is assumed to involve a locus (loci) controlling variant forms of a complement component(s) within the B complex. In addition, Schierman et al. (1977), Collins et al. (1977) and Gebriel et al. (1979) all provided evidence for a locus with alleles conferring resistance (Rs) and susceptibility (rs) to tumors induced by the Bryan strain of Rous sarcoma virus. Taking these findings into account, a theoretically more complete model of the B complex is proposed (Figure 3).

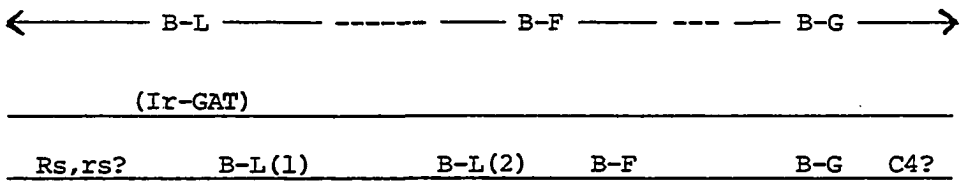

Figure 3. Theoretical model of the B complex. Dashed line indicates region of crossing over. The existence of B-L(2) is inferred because of biochemical evidence for two populations of Ia-like molecules and because the B-F region as defined by studies in this laboratory controls graft-versus-host reactivity. B-L(1) and B-L(2) each represent two structural genes coding for polypeptides that form dimers

Several problems arise in the interpretation of the immune response data. In the mouse, Ia antigens are dimers of molecules coded by two distinct genes. At least four structural genes have been identified: A_α, A_β, E_α and E_β. The A molecules are dimers of A_α and A_β and the E molecules are dimers of E_α and E_β polypeptides. Low immune response could

result from the lack of expression of one or both of the A or E molecules (Klein et al., 1981).

The identification of immune response gene products using simple serological tests is of obvious usefulness. In the chicken, at least two populations of B-L molecules have been reported (Crone et al., 1981). Low immune response to GAT could be attributed to the lack of expression of one or both of the structural genes for a dimer. If neither structural gene coding for a dimer conferring responsiveness is expressed, three types of nonresponder recombinant haplotypes could be produced from the original nonresponder haplotype. This can theoretically occur because recombinant haplotypes will be nonresponders whether they contain either one or both of the genes which are not expressed.

Alloimmune Properties

According to Vilhelmová et al. (1977), the mixed lymphocyte reaction, skin graft rejection and graft-versus-host reactivity are controlled by the B-F region of the B complex. Because the B-F region, as defined in their studies, is presumed to contain both the B-F and B-L loci, the determination of the properties controlled separately by the B-F locus and by the B-L locus has, so far, not been reported.

For recombinants reported more recently (Pevzner et al., 1978; Lee and Nordskog, 1981), the Ir-GAT gene, presumed to be a B-L gene, has been separated from a chromosome segment containing the B-G and B-F genes. The Ir-GAT locus seemed not to influence skin graft rejection and had only a minor influence on the graft-versus-host reaction when compared with

the region containing the B-G and B-F genes. Because the graft-versus-host reaction is controlled by Ia antigens in the mouse, and because the chromosome segment containing the B-G and B-F genes exerts a major influence on graft-versus-host reactivity, it is yet unclear whether the chromosome segment containing the B-G and B-F genes contains an unidentified B-L gene(s). If so, it must be linked closely to B-F between the B-G and Ir-GAT genes.

The relatively minor influence of the Ir-GAT locus on the graft-versus-host reaction contrasts with the strong influence of the H-2 immune response genes on this phenomenon. A possible explanation is that the different gene products of this locus are structurally very similar or that Ir-GAT-Lo responders result from the lack of expression of a structural gene. Lee and Nordskog (1981) pointed out that the relative contribution of the B-F and Ir-GAT regions may depend on the position of the chromosome break point. Their interpretation assumes that the B-F region, as defined in their study, contained more loci than the Ir-GAT region and that the additional allelic differences resulted in the increased graft-versus-host reactivity. Additional studies are needed to determine which interpretation, if any, is correct.

In the present study, the mixed lymphocyte reaction, skin graft rejection and the graft-versus-host reaction were all controlled by the chromosome segment within the B^{19rl} haplotype containing the B-F and Ir-GAT genes. These functions appeared not to be controlled by the B-G locus. In summary, the information discussed here supports the conclusion

that the Ir-GAT region has a minor influence on graft-versus-host reaction; the B-F region controls the mixed lymphocyte reaction, graft-versus-host reaction, and skin graft rejection; and the B-G region controls none of these. Additional recombinants must be produced before these functions can be assigned to the Ir-GAT and B-F genes. Because the number and nature of genes contained within the regions defined in this study and previous studies are unknown, the mammalian model has been used to allow inferences as to which properties are controlled by the different classes of genes in the B complex. In particular, the assumption is that B-F genes control skin graft rejection and B-L genes control mixed lymphocyte reaction and graft-versus-host reaction. Functional properties of the B-G locus cannot be inferred because no mammalian homologue has been discovered.

Recombination Frequency

The combined estimate of the recombination frequency of B-G and B-F from Experiments 1 and 2 is 5/5,584. This may be an underestimate because the chicks may have been typed before the B-G and B-F antigens reached mature levels. Also, whether each test reagent was capable of detecting both the B-G and B-F antigens is unknown. Because all recombinants were obtained from $B^{1}B^{19}$ heterozygotes, this particular heterozygote combination may have been unique in permitting the detection of both the B-G and B-F antigens. In the mouse, certain heterozygous combinations are known to have higher recombination frequencies than others. The average percentage recombination rate between K and D is given as 0.329 but certain

heterozygous combinations have yielded recombination frequencies near zero (Klein, 1975). That the recombination frequency within the chicken B complex may not be far different from that in the mouse H-2 complex is not surprising.

Future Studies

Future studies of recombinant haplcytypes might focus on one or more of several different areas. The antigen coded by the Ir-GAT locus of the B^{19rl} haplotype might be immunoprecipitated with the appropriate antisera to determine whether it codes for the Ir-GAT-Lo or Ir-GAT-Hi gene product. Such an experiment should confirm the results of the immune response studies. Failure of Ir-GAT-Hi lymphocytes to produce one-way mixed lymphocyte reactions with Ir-GAT-Lo target cells would suggest that Ir-GAT-Lo responders might have resulted from failure to express a structural gene. As suggested earlier, specifically blocking *in vitro* responses to GAT by binding specific monoclonal antibody to the B-L antigens on antigen-presenting cells would provide evidence for the identity of the Ir-GAT locus and a B-L locus.

Another area of interest in chickens would be the possible determination of the genetic mechanism of the restriction phenomena already extensively studied in mice. Wainberg *et al.* (1974) have shown that chicken spleen cells preferentially kill self neoplastic target cells rather than allogeneic target cells. If the B complex restricts killing by cytotoxic T cells, then certain recombinants might be useful in determining the degree to which the B-G and B-F loci influence restriction.

Perhaps such studies would aid in elucidating the functional properties of the B-G locus. In mice, it seems that the primary purpose of the cell surface alloantigens is to provide a context for antigen recognition (Benacerraf, 1981). Studies of restriction phenomena would help determine whether the same were true in chickens.

Reciprocal immunizations between birds bearing different recombinant haplotypes produced in this study could be utilized to determine whether all have the same sequence of genes coding for cell surface alloantigens. If multiple B-G, B-F or B-L genes exist then the recombinants likely would have serologically detectable differences. Briles and Briles (1980) have already provided genetic evidence for multiple B-G loci; biochemical studies now lend evidence for multiple B-F and B-L loci (Brogren and Bisati et al., 1979; Crone et al., 1981). The production of additional recombinants for more refined analysis of the B complex should help to better establish the number of loci and their functional properties.

SUMMARY

The study consisted of the production and analysis of genetic recombinants of the chicken B complex, the major histocompatibility complex of the chicken. The purpose was to provide data complementing that of the mammalian major histocompatibility complex. By mating $B^{1}B^{19}$ heterozygotes to $B^{2}B^{2}$ homozygotes, 5 recombinants were identified in 5,584 chicks tested by hemagglutination. The results indicate that the recombination frequency within the chicken MHC is comparable to that recorded in the mouse where the frequency of recombination has been estimated within the range of near zero for certain heterozygous combinations to an average value of .329 of 1 percent.

Analysis of the data indicates that the B^{1} parental haplotype contributed the B-G gene and the B^{19} parental haplotype contributed the B-F gene to the recombinant haplotypes. The new recombinants have been designated B^{19r1}, B^{19r2}, B^{19r3}, B^{19r4}, and B^{19r5}. The B-F region of the B^{19r1} haplotype controls allograft rejection, mixed lymphocyte reaction, and graft-versus-host splenomegaly. No functional properties could be assigned to the B-G genes.

Recombination between a locus controlling immune response to the amino acid polymer GAT and a chromosome segment containing the B-G and B-F genes has previously been reported from Iowa State University studies. Because the B^{19r1} haplotype confers high immune response to the amino acid

polymer GAT, it was likely produced from a crossover between the B-G locus and a chromosome segment containing the B-F and Ir-GAT-Hi genes.

The region controlling immune response to GAT very probably is identical to the previously described B-L region. The putative order of these regions is, therefore, B-G, B-F and B-L. The recombinants B^{19r2}, B^{19r3} and B^{19r4} are all identical for those B complex loci which control histocompatibility. Additional studies with these recombinants are needed to determine whether they all contain the same linear sequence of genes.

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