MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) AND MHC-LIKE GENES IN CHICKENS: A LESSON FROM BROILERS

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INTRODUCTION

The major histocompatibility complex (MHC) is one of the most intensively studied genetic regions of the vertebrate genome. Its involvement in organ transplantation, antigen processing and presentation to T lymphocytes during the immune response, autoimmunity and other disease associations has made the MHC an active area of research. Three classes of molecules are encoded in what is commonly known as the classical MHC or MHC proper. Classes I and II present antigenic peptides to CD8+ and CD4+ T lymphocytes, respectively during the immune response. The Class I and II molecules are expressed at high levels; they are genetically diverse (one to three functional loci per haplotype, with numerous alleles at each locus); and they share common structural motifs related to their function (Salter-Cid and Flajnik, 1995; Trowsdale, 1995; Kasahara et al., 1996). The human HLA-A, -B, -C and the mouse H-2-K, -D, -L loci encode the Class I MHC molecules, whereas the HLA-D and H-2-I loci encode Class II molecules. In contrast, the Class III genes encode a range of molecules, including complement factors C2, C4 and Bf, as well as tumor necrosis factor and the heat shock 70 protein (Salter-Cid and Flajnik, 1995). Other immune-related genes, including Lmp (low molecular weight protein) and TAP (transporter associated with antigen processing), as well as genes with no immune function, such as G7a (valyl-tRNA synthetase), GI (calmodulin-like) and RING3 (serine-threonine kinase) are also found in the MHC proper (Arnaiz-Villena, 1993; Salter-Cid and Flajnik, 1995). Several genes with unknown function are also scattered throughout the mouse and human MHCs.

To further complicate matters, novel families of MHC-like or non-classical MHC genes have also been described in humans and mice, as well as bony fishes and amphibians (Shawar et al., 1994; Salter-Cid and Flajnik, 1995). The majority of the non-classical genes are related to the Class I MHC genes; however, Class II-related genes have also been described. In the mouse, there are over 58 non-classical Class I genes, pseudogenes and gene fragments that have been described (Powis and Geraghty, 1995). Some of the Class I-like genes and their products include MIC (MHC Class I chain-related genes), the CD1 family, Zn-α2-glycoprotein and the neonatal Fc receptor (Bahramp et al., 1994; Hashimoto et al., 1995; Kasahara et al., 1996). These non-classical or “Class Ib” molecules are generally monomorphic, with limited tissue expression, as compared to the classical or “Class Ia” molecules (Nei et al., 1997). The non-classical MHC genes are structurally similar to their classical MHC homologs; however, the homology between the classical and
non-classical genes is very low. For example, there is only 23-34% amino acid homology between the mouse Fc receptor and the Class Ia MHC proteins of several vertebrate species (Ahous et al., 1993) and an average of 25% amino acid homology between the MICA protein and the Class Ia proteins of humans (Bahram et al., 1994). In several instances, the non-classical Class Ib gene products are also associated with β2-microglobulin, suggesting that this binding region is conserved between classical and non-classical Class I genes (Salter-Cid and Flajnik, 1995). Many of the Class Ib genes (mouse H-2Q, T and M loci; human HLA-E, F, G, H, I and J loci) are located adjacent to classical MHC regions; however, some of them, i.e., CD1, Zn-α2-glycoprotein and the IgG Fc receptor are located outside the MHC-bearing chromosome (Salter-Cid and Flajnik, 1995; Kasahara et al., 1996). Like the classical MHC molecules, the non-classical MHC products have been reported to participate in immune functions and they are recognized by T lymphocytes (reviewed by Shawar et al., 1994; Salter-Cid and Flajnik, 1995). The Class Ib molecules exhibit unique peptide-binding activities, especially for bacterial antigens (Kurlander and Nataraj, 1997); they affect maternal immune tolerance (Rouas-Freiss et al., 1997); and they either inhibit or activate NK cell activity (Rolstad et al., 1997). For example, the mouse H2-M3 molecule binds N-formylated peptides and it has been suggested to play an important role in bacterial and mitochondrial peptide presentation to cytotoxic T cells (Shawar et al., 1991). Similarly, the CD1 molecules present non-peptide, lipid-containing epitopes to T cells and they are also suggested to have an important role in bacterial antigen presentation (Porcelli et al., 1998). Although the majority of the non-classical genes are Class I-related, Class II-like genes have also been described in humans. The non-classical Class II genes include HLA-DM and -DO, and they are reported to have chaperone-like activity during antigen processing (vogt et al., 1997; Kropshofer et al., 1998).

Therefore, in summary, the term “MHC” has expanded to include the classical MHC or MHC proper, in addition to the novel MHC-like genes, often referred to as non-classical MHC genes (summarized in Table 1). As the genetic organization, structure, diversity and function of the MHC and its related genes is realized, it is becoming apparent that the MHC is one of the most complex and intriguing regions of the vertebrate genome.

THE CHICKEN MHC (B) AND RFP-Y SYSTEM

The chicken MHC was originally identified by Briles and coworkers as the B blood group locus (Briles et al., 1950). A decade later, it was observed that B blood group differences were responsible for skin graft rejections and therefore, it was concluded that the “B locus” was the chicken MHC (Schierman and Nordskog, 1961). Soon after this discovery, other immunological phenomena such as the mixed lymphocyte reaction and the graft-versus-host reaction were also associated with the chicken B blood group locus (Jaffe and McDermid, 1962; Miggiano et al., 1974). These findings suggested that the red blood cell antigens of the B system were similar to the histocompatibility antigens of the skin and to the membrane proteins on lymphocytes. It was the classical experiments of Zeigler and Pink (1976), who used B-specific antisera to purify the B blood group antigens and discovered that more than one B antigen existed on white blood cell membranes. These molecules were designated B-I’ for the histocompatibility antigen (Class I) and B-L for the...
immune-associated antigen (Class II). Evidence for a third type of MHC molecule soon followed. Recombinational events had occurred in two birds which caused the red blood cells from each of these birds to react with 2 different B-typing reagents (Hala et al., 1976). One of the proteins was expressed on both red and white blood cells (B-F), and the other was expressed only on red blood cells and it was designated B-G (Class IV). Therefore, it was concluded that the chicken B locus was actually a complex of genes that encoded at least three distinct groups of MHC proteins; Classes I (B-F), II (B-L) and IV (B-G) (Pink et al., 1977). Each of these MHC proteins can be differentiated from the others by their molecular structure, tissue specificity and biological function (reviewed by Guillemot and Auffray, 1989; Plachy et al., 1992). The Class I or B-F proteins are composed of a single polypeptide chain (α chain), in association with β2-microglobulin and they are expressed on the surface of all cell types in the body, including erythrocytes (Crone et al., 1985). In contrast, the Class II or B-L proteins are heterodimeric (α and β chains) and their expression is restricted to cells (B cells, specialized epithelial cells, macrophages, activated T cells) of the immune system (Crone et al., 1981). The B-F and B-L molecules function in antigen presentation to T lymphocytes, similar to their mammalian Class I and II homologs, respectively. The Class IV or B-G proteins are unique to chickens and they were originally reported to exist in homodimeric and heterodimeric forms on the red blood cell membrane (Kline et al., 1988). However, the Class IV proteins are also expressed on thrombocytes, thymocytes, bursal B cells, peripheral B and T cells, stromal cells of the thymus, bursa and cecal tonsil and epithelial cells of the small intestine, ceca and liver (reviewed by Kaufman et al., 1991). Although the exact function of the B-G molecule remains unknown, it has been found to exert an adjuvant effect during antibody responses to other blood group antigens, ex. B-F molecules (Hala et al., 1981). The B-G molecule is also speculated to have either an antigen presentation function similar to that of Class I and II molecules; to bind antigen similar to that of B cells; or to participate in the development of the B cell repertoire (Kaufman and Salomonsen, 1992).

It has been well over two decades since Pink and colleagues (1977) described the three locus model of the chicken B complex. With the advent of molecular genetic techniques, rapid progress has been made in characterizing the molecular structure and genetic diversity of the chicken MHC. The genomic organization of the chicken MHC is somewhat different from that of mammals (Guillemot and Auffray, 1989; Trowsdale, 1995; reviewed by Kaufman et al., 1995). In general, the chicken MHC is compact (due to shorter introns within the chicken MHC genes); it does not contain discrete Class I, II and III subregions; and there is a low rate of recombination between the MHC genes. An extensive genomic map of the chicken MHC was originally developed by Guillemot and coworkers (1988). In total, 20 genes were mapped within 4 cosmid clusters, and at least 11 of them were MHC genes. There were 5 B-Lβ genes (B-Lβ1 to B-Lβ5) and 6 B-F genes (B-F1 to B-F6) and they were found to be intermingled among each other within 250 Kb of DNA. Interestingly, the B-Lβ genes could be separated into two isotypic families based on their differential hybridization to a 3' untranslated specific oligonucleotide probe. This finding has proven to be of significance because one of the isotypic families has since been mapped to a second MHC system in chickens, namely the Rfp-Y system (Miller et al., 1994). In addition, Guillemot and coworkers (1988) identified one B-G gene that was 12 kb upstream of the B-F and B-L genes, as well as several non-MHC genes whose functions
were not characterized. Since the original finding, at least one of these genes was reported to encode a polypeptide which shares 22% amino acid homology with the β subunit of the human guanine nucleotide-binding proteins and bovine transducin (Guillemot et al., 1989), and a second gene (17.5) encodes a product that is 16-23% homologous to the C-type animal lectin proteins (Bernot et al., 1994). Although it has been demonstrated that complement (Class III) levels are associated with the chicken MHC (Chan et al., 1976), the specific complement genes that are involved were not determined. In fact, for a long time, it was thought that the complement genes (C4, C2, etc.) were located elsewhere in the genome due to the small size of the chicken MHC. However, direct evidence for Class III genes in the B region was provided by Spike and Lamont (1995) and Kaufman et al., 1999.

More recently, Milne and coworkers (Genbank Accession # AL023516; submitted May, 1998) have provided B-region DNA sequence for cosmid clones cB12, c4.5 and cBF23 that were originally isolated by Guillemot et al. (1988). This data is a landmark in chicken MHC research. Not only have these authors identified Class I (B-F) and II (B-Lβ) genes in these cosmids, but also several other MHC-linked genes including TAP, RING3, tapasin (TAP binding protein) and complement C4. In addition, some non-MHC genes include a histone H3-like gene, a Leu-tRNA gene, a lectin-like natural killer cell surface receptor gene, a C-type animal lectin gene and a G (zipper protein)-like gene. The tapasin gene (involved in assembly of the Class I molecules with β2-microglobulin in the endoplasmic reticulum) has only recently been published (Frangoulis et al., 1999).

The B-G region is comprised of numerous loci (at least 18) and it is in linkage disequilibrium with the classical B region genes (reviewed by Kaufman et al., 1991; 1995). At least four of the B-G loci are transcribed; the translated product is a single immunoglobulin-like polypeptide with cytoplasmic tails that vary in length (Miller et al., 1991). The length variation is due to differences in the number of heptad repeat units associated with the cytoplasmic tails (Kaufman et al., 1990). Interestingly, the myelin-oligodendrocyte glycoprotein (MOG) gene which is suspected to influence susceptibility to multiple sclerosis and the butyrophilin gene which is expressed in the bovine mammary gland are both related to the chicken B-G genes (Trowsdale, 1995).

As indicated earlier, the chicken MHC genes are grouped into two separate systems, the B region and the Rfp-Y system. Over the past few years, restriction fragment length polymorphism (RFLP) analyses of B-F and B-Lβ genes in pedigreed families have revealed diverse MHC-like restriction fragments which segregated independently of the serologically-defined MHC (B-region) haplotypes (Chaussé et al., 1989; Tilanus et al., 1989; Juul-Madsen et al., 1993). These unexplained restriction fragments led Briles and coworkers (1993) to identify a second group of MHC genes in chickens, termed Rfp-Y. Interestingly, the Rfp-Y system maps to the same microchromosome (no. 16) as the classical MHC (B region); however, the genes within each system appear to segregate independently (Miller et al., 1996). Lack of genetic linkage of the B and Rfp-Y systems is hypothesized to be due to a high frequency of recombination within the nucleolar organizing region (NOR) which may separate the two systems. The Rfp-Y system is composed of two Class I and two Class II loci; cosmid clusters II and IV from Guillemot and coworkers (1988) have since been proven to originate from the Rfp-Y system (Miller et al., 1994). Non-MHC genes have
also been identified in the \textit{Rfp}-Y system; for example, the \textit{17.5} gene described earlier (Bernot et al., 1994). The physiological functions of the \textit{Rfp}-Y gene products are not known; however, a report indicates that \textit{Rfp}-Y (B-LβIII) transcripts are present in the spleen (Zoorob et al., 1993). Controversy exists in the role of the \textit{Rfp}-Y genes in Marek’s disease (MD) resistance or susceptibility (Bacon et al., 1996; Wakenell et al., 1996). The \textit{Rfp}-Y genes had no significant effect on MD tumors or viremia in Cornell lines N (MD-resistant) and P (MD-susceptible) and their backcross; nor in Lines 6 (MD-resistant) and 7 (MD-susceptible) and their F$_2$ progeny (Bacon et al., 1996). In contrast, the \textit{Y$^3$} haplotype was associated with a higher incidence of Marek’s disease tumors in an MHC-congenic (Ancona background; Briles et al., 1993) chicken line (Wakenell et al., 1996). A relationship of the \textit{Rfp}-Y gene products to minor histocompatibility antigens has also been demonstrated by Pharr and coworkers (1996). In this case, \textit{Rfp}-Y incompatibility caused rejection of skin grafts at a more frequent and faster rate than \textit{Rfp}-Y compatibility. However, the \textit{Rfp}-Y effects were less than that observed with classical B-incompatibility.

Therefore, two groups of MHC genes are located on chromosome 16 in the chicken and each group consists of both classes I and II (Miller et al., 1994; FIGURE 1). The classical \textit{B} system contains \textit{B-FI}, \textit{B-FIV}, \textit{B-LβI} and \textit{B-LβII} loci whereas the \textit{Rfp}-Y system consists of \textit{Y-FIV}, \textit{Y-FVI}, \textit{Y-LβIII}, \textit{Y-LβIV} and \textit{Y-LβV} loci. The classical \textit{B} system is linked to the Class IV genes, and both the \textit{B} and \textit{Rfp}-Y systems are suggested to be separated by the NOR. Whether the \textit{Rfp}-Y genes are classical or non-classical MHC genes is not clear. Due to the polymorphic nature of the \textit{Rfp}-Y genes and their minor differences from \textit{B} region genes (82% homology between the \textit{B-Lβ} and \textit{Y-Lβ} families; Zoorob et al., 1993), the \textit{Rfp}-Y genes probably represent a distinct isotypic family that is part of the classical chicken MHC repertoire. Interestingly, Zoorob and coworkers (1993) identified a third isotypic family of \textit{B-Lβ} genes, \textit{B-LβVI}. Whether the \textit{B-LβVI} genes are expressed and from which system (\textit{B} or \textit{Rfp}-Y) they are derived are not known.

Lastly, there is a strong linkage disequilibrium of the chicken MHC genes (Simonsen et al., 1980). That is, certain alleles within the \textit{B} region tend to segregate as a unit, rather than independently. This phenomenon is due to the low frequency of recombination among the loci at the MHC. For instance, there have been no identifiable recombination events between the \textit{B-F} and \textit{B-L} subregions, whereas the frequency of recombination between the \textit{B-G} and \textit{B-F/B-L} subregions is estimated to be less than .05% (map distance is less than .56 centimorgans) (Koch et al., 1983; Hala et al., 1988). Because there have been very few recombination events between MHC subregions, the term “haplotype” is used to refer to the combination of alleles at all loci within the \textit{B} region on a single chromosome. In the chicken, there are over 30 B-haplotypes, thus indicating the polymorphic nature of this complex (Briles et al., 1982; Heller et al., 1991). Similarly, \textit{Rfp}-Y haplotypes are based on their Class I and II composition. Currently, there are at least 11 \textit{Rfp}-Y haplotypes that have been reported in chickens (Briles et al., 1993; Wakenell et al., 1996; Juul-Madsen et al., 1997).
BROILER MHC GENES

Although analysis of chicken MHC haplotypes in White Leghorns and experimental chicken lines has progressed steadily, little research emphasis has been placed on the MHC haplotypes which segregate in meat-type or broiler chickens. Because broiler chickens are mostly derived from the White Plymouth Rock and White Cornish breeds, it is expected that broiler chickens would have additional unique MHC haplotypes. In Denmark, Simonsen (1987) attempted to identify MHC haplotypes in a White Cornish flock and other broiler populations using hemagglutinating reagents which were developed for standard White Leghorn haplotypes. However, these attempts were reported as futile, with many of the broiler chickens reacting non-specifically with one or more of the reagents. The only exception was some B15 typing reagents, where the "B13" haplotype was found segregating at low frequency in the flock of White Cornish chickens. Simonsen (1987) also identified B21-like haplotypes which were provisionally designated as B130-B133. Both the B130 and B131 haplotypes were indistinguishable from B21 based on the graft-versus-host reaction (GVHR); however, each of these haplotypes only reacted with the B-F21 reagent, but not the B-G21 reagent. In addition, the B130 and B131 haplotypes are serologically distinct from each other. In contrast, B132 was serologically similar to B130, but yet a GVHR could be induced between these two haplotypes. Whether the B130 and B131 haplotypes confer Marek's disease resistance similar to that of their B21 homologue in White Leghorns was not determined.

In another study, Heller and coworkers (1991) examined the MHC haplotypes in broiler chickens that were selected for either early high or early low antibody responses to Escherichia coli and Newcastle disease virus (NDV). Using a panel of standard blood typing reagents developed in White Leghorns, they identified 12 antisera out of 27 that displayed differential hemagglutination reactions in the broiler chickens. Once again, "provisional" haplotypes were designated for the MHC specificities because it was not known whether the typing reagents reacted with antigenic epitopes fully identical to those in White Leghorns. However, based on the hemagglutination patterns, at least five MHC specificities (B5, B13, B15, B19 and B5) were identified in this broiler population. Interestingly, the B0 haplotype was identified by its non-reactivity with the White Leghorn typing reagents. Of importance, the B5 haplotype was associated with early high antibody response to E. coli and NDV, whereas the B15 and B19 haplotypes were markers for early low antibody responsiveness. At least 40% of the early high antibody responders were of the B0 haplotype. Because the B0 haplotype did not react with any of the White Leghorn typing reagents, it may represent one or more undescribed broiler chicken MHC haplotypes that are associated with high antibody response (Heller et al., 1991).

Molecular analysis of two broiler MHC haplotypes, designated B^{A4} and B^{A4variant} indicated that some of the MHC genes are shared between broilers and Leghorns (Li et al., 1997). In this study, the B^{A4} haplotype had similar B-F and B-LβIII gene sequences to that of the B^{21} haplotype; however, its B-G genotype was distinct. Although the B^{A4variant} haplotype had the same B-G molecular genotype as B^{A4}, the two haplotypes could be distinguished serologically. This may be due in part to different B-F genes in these haplotypes. Both the B-F and B-Lβ sequences were distinct between these haplotypes and the B^{A4variant} sequences were unique to broilers. More recent research from the same group
focused on additional MHC haplotypes in a broiler chicken line (Li et al., 1999). In this study, they compared B-F and B-Lβ sequences from broilers that were homozygous at the B system. Not only did they identify unique broiler alleles, but they also observed alleles that were common to both broilers and White Leghorns. Interestingly, some of the B-F and B-Lβ sequences that are common to broilers and White Leghorns were found in linkage disequilibrium with different B-G alleles in the broiler line.

In a separate study, the MHC haplotypes in a commercial meat-type grandparent line were characterized by restriction fragment length polymorphism (RFLP) analysis using a Class IV DNA probe (Uni et al., 1992). At least 23 polymorphic PvuII restriction fragments, ranging in size from 1.1 to 5.2 kb were identified in this population. These fragments identified different RFLP haplotypes and each haplotype consisted of between 4 and 16 restriction fragments. Discrimination between homozygous and heterozygous RFLP patterns was not performed in this study. However, in a separate study, this research group further investigated two chicken lines, originally derived from the commercial line and divergently selected for early or late antibody response to E. coli (Landesman et al., 1993; Uni et al., 1993). Fourteen homozygous RFLP haplotypes were identified in these birds, and five of these haplotypes were found either in the low antibody response line or in the high antibody response line. This research provides the first direct evidence that broiler MHC haplotypes also influence the immune response to disease organisms. In addition, a relationship of broiler MHC haplotype and egg production has also been reported (Tarleton et al., 1994). Therefore, characterization of MHC haplotypes will be of value to broiler breeding programs especially where selection for immune response and disease resistance are of importance. Association of certain broiler MHC haplotypes with production traits may also provide a selective advantage.

**Molecular Genotypes in a Commercial Broiler Population**

We are currently characterizing the MHC haplotypes in a commercial broiler chicken population. Our laboratory has obtained three DNA probes which identify chicken MHC Classes I (Dr. Henry Hunt, Avian Disease and Oncology Laboratory, East Lansing, MI), II (Xu et al., 1989) and IV (Goto et al., 1988), respectively. We have used the Class II and IV probes to determine the restriction fragment length polymorphism (RFLP) patterns in pedigreed families of Lines A and B. In this study, DNA samples were digested with the restriction enzyme, PvuII and electrophoresed in a 0.8% agarose gel at 30 volts for 22 hours. The enzyme, PvuII was chosen for RFLP analysis because it gave distinct polymorphisms without loss of resolution in Southern blot analysis. The DNA was transferred to nylon membranes (Hybond N+, Amersham Life Sciences, Inc., Arlington Heights, IL) following the standard Southern blot procedure (Sambrook et al., 1989). Prehybridization, hybridization and stringency washes were described previously (Emara et al., 1992). The RFLP data have been collected from a total of 293 birds. At least four (a-d) Class II (TABLE 2) and seven (1-7) Class IV (FIGURE 3) homozygous molecular genotypes (RFLP patterns) are segregating in Lines A and B. The frequencies of the molecular genotypes in the two broiler lines are presented in FIGURE 2 (Class II) and
FIGURE 4 (Class IV). Genetic variation at the MHC between these two lines is apparent. The Class II “a” allele is in highest frequency in both lines; however, the frequencies of the other Class II alleles are different between the two lines and in fact, the Class II allele “b” was not observed in Line B. In contrast, the Class IV alleles are unique to each broiler line. The Class IV alleles, “3-7” were only observed in Line A, whereas alleles “1-3” were only observed in Line B. Co-segregation of the Class II and Class IV molecular genotypes have resulted in the identification of eight molecular MHC haplotypes in this broiler population.

SEROLOGICAL ANALYSIS OF BROILER MHC HAPLOTYPES

To facilitate rapid identification of broiler MHC haplotypes, a study was initiated to produce broiler chicken alloantisera within this population. Reciprocal full- and half-sibling alloimmunizations (n = 36) were established for the production of alloantisera. Sibling immunizations were used in order to minimize differences at minor blood group antigens. Briefly, 1.5 ml of donor blood was collected in 1.5 ml sodium citrate solution and then, samples were injected into the brachial vein of recipients. Chickens received two injections (once per week for 2 weeks) and blood was collected 5 days after the second immunization for harvesting of serum. Of the 36 recipients, 22 birds responded to alloimmunizations with titers of four or greater. Fifteen of these 22 antisera gave differential hemagglutination reaction patterns among the broiler chickens that were tested. We have identified five of these antisera, in addition to five antisera kindly supplied by Dr. W. Elwood Briles that appear to react with certain Class IV molecular genotypes. Analysis of Class I (B-F) genotypes is in progress and will facilitate further characterization of these alloantisera. Due to the cross-reactivity of the alloantisera with public MHC epitopes, it is apparent that a panel of antisera will be necessary to differentiate the MHC haplotypes in broilers. And, as demonstrated by Li et al. (1999), the presence of B-F and B-G alleles common to broilers and White Leghorns should make it possible to identify pre-existing standard “B” reagents that will be useful in typing at the MHC in broiler chickens. Alternatively, alloantisera produced within the broiler population will also be useful reagents and this research is ongoing.

MAPPING OF AN MHC-LIKE GENETIC SYSTEM TO CHICKEN CHROMOSOME 1

During our molecular genotyping studies in pedigreed broiler families, we identified an MHC Class II-like system that segregates independently of the classical MHC (B region) and Rfp-Y system. Hybridization of PvuII-digested genomic DNA with a B-LβII (B region; Class II) probe revealed two groups of DNA bands, which segregate independently of each other. Group 1 was identified as the classical MHC (B) region genes due to their intense hybridization with the B-LβII probe, whereas group 2 consisted of weakly-hybridizing DNA bands. A B-LβIII probe (Rfp-Y) hybridized to the group 1 (B-region) DNA bands; however, it did not hybridize to the group 2 DNA bands. Therefore, the group 2 DNA bands were considered to be part of a separate system, which we have temporarily named DEL0001. Segregation data for the three Class II regions (B, Rfp-Y and DEL0001) in a pedigreed broiler family are presented in
TABLE 3. It was obvious in the segregation data from pedigreed families that we were identifying an independent system. To date, we have identified three homozygous \textit{DEL0001} molecular genotypes that are based on digestion of chicken genomic DNA with the restriction enzyme, \textit{PvuII} (FIGURE 5).

Based on the conclusion that \textit{DEL0001} was independent of the classical \textit{B} and \textit{Rfp-Y} systems, we conducted two separate mapping studies to localize this MHC-like system in the poultry genome. To determine whether \textit{DEL0001} is located on the MHC- and \textit{Rfp-Y} bearing chromosome (no. 16), genomic DNA samples from the three genotypes segregating in the UCD-Trisomic line were analyzed. Disomic, trisomic and tetrasomic individuals contain 2, 3 and 4 copies, respectively of chromosome 16. This line is ideal for mapping genes to the MHC- and \textit{Rfp-Y}-bearing chromosome in chickens via the detection of a proportional increase in the hybridization intensity of DNA bands. Therefore, genomic DNA from di-, tri- and tetrasomic birds was digested with \textit{PvuII} and analyzed in Southern blots using the B-LJ3II probe. The UCD-Trisomic chicken line was found to be monotypic for allele 1 (2.2 kb \textit{PvuII} fragment). As expected, there was a proportional chromosome 16-related increase in the intensity of classical \textit{B}-region DNA bands (Group 1 DNA bands; 2.8 and 4.3 kb) in di-, tri- and tetrasomic birds, respectively. In contrast, there was no observable difference in the intensity of the \textit{DEL0001} DNA band (2.2 kb) among di-, tri- and tetrasomic birds, respectively. Similarly, there was no observable difference in the intensity of a 3.2 kb \textit{HindIII} \textit{DEL0001} DNA fragment among di-, tri- and tetrasomic individuals. Therefore, it was concluded that \textit{DEL0001} was not located on chromosome 16.

Next, we analyzed DNA samples from the East Lansing reference population in order to map \textit{DEL0001} to a particular chromosome or linkage group in the chicken genome. The East Lansing reference population was derived from a Jungle Fowl (JF) X White Leghorn (WL) cross, and it is described elsewhere (Crittenden \textit{et al.}, 1993). Genomic DNA samples from JF, F1 males, four WL females and 52 backcross (BC1) progeny were used in the linkage mapping studies. Initial RFLP studies with the restriction enzyme, \textit{PvuII} indicated that the JF and WL chickens were monotypic for allele 1. However, subsequent studies revealed that the 3.2 kb \textit{HindIII} fragment of allele 1 was present in the WL females and the 1.9 kb \textit{HindIII} fragment of allele 2 was present in the JF. Therefore, \textit{HindIII} was used in Southern hybridizations with the B-LJ3II probe to genotype the BC1 progeny in the East Lansing reference population. Segregation data that were based on the inheritance of the JF (1.9 kb) and WL (3.2 kb) \textit{DEL0001} alleles in the fifty-two BC1 progeny indicate that \textit{DEL0001} is located on the \textit{p}-arm of chromosome 1 (FIGURE 6).

This mapping data has proven to be of significance for two reasons. First, a comparative mapping to the mouse genome with closely linked loci, LDHB and GAPD, indicates that other immune-related genes such as Ly49 (natural killer cell receptor), NK1 (natural killer cell-associated antigen), CD69 (very early activation antigen) and CMV1 (cytomegalovirus resistance 1) are located in this syntenic group [Mouse Genome Database (MGD), Mouse Genome Informatics, 1999] (FIGURE 6). And secondly, in a separate finding, Bumstead and coworkers (1998) mapped a quantitative trait locus (MDV-1) for Marek's disease resistance to the same region of chicken chromosome 1. The
Thus far, the chicken MHC loci are reported to reside in two regions on chromosome 16; the classical B and Rfp-Y regions, both of which contain Classes I and II loci. Our data demonstrate that a third MHC or MHC-like system exists in chickens and it contains at least a Class II-like gene. Fine mapping of the region of homology between the Class II (ccII-7-1; Xu et al., 1989) probe and DEL0001 has revealed that the homology lies in the 5' promoter region of the Class II probe. Thus, the portion of the probe that encodes the Class II protein does not hybridize to DEL0001 and this may explain why the B-LβIII cDNA probe did not identify this system. Interestingly, we have also identified some Class I restriction fragments that segregate with DEL0001. However, in this case, the Class I probe is a cDNA probe and our current hypothesis is that the DEL0001 system may be a Class I-like gene with a promoter region that is conserved with the Class II (B-LβII) gene. Much like that of the mammalian non-classical MHC genes, the chicken DEL0001 system exhibits limited polymorphisms, with only three molecular genotypes that were identified in 10 chicken lines studied. Whether this MHC-like system is functional and whether it contains classical or non-classical MHC genes, remains to be determined. As members of the immunoglobulin supergene family, these MHC-like genes may prove to be important genes in NK cell activity, or perhaps be related to the MDVI locus (responsible for Marek’s disease resistance) mapped by Bumstead and coworkers (1998).

REFERENCES


TABLE 1. CHARACTERISTICS OF CLASSICAL AND NON-CLASSICAL MAJOR HISTOCOMPATIBILITY COMPLEX GENES.

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<th>CLASSICAL MHC GENES</th>
<th>NON-CLASSICAL MHC GENES</th>
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<td>Class I and II</td>
<td>Primarily Class I-like; a few Class II-like</td>
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<td>Several (1-3) functional loci</td>
<td>Exhibit all or none of the classical MHC traits</td>
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<tr>
<td>High levels of expression</td>
<td>Low levels of expression</td>
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<tr>
<td>Broad tissue distribution</td>
<td>Limited polymorphisms</td>
</tr>
<tr>
<td>Conserved structural features</td>
<td>Found within and external to the MHC</td>
</tr>
<tr>
<td>Peptide binding/presentation function</td>
<td></td>
</tr>
<tr>
<td>Highly polymorphic (many alleles/locus)</td>
<td></td>
</tr>
</tbody>
</table>

From Kasahara et al., 1996.

B system

<table>
<thead>
<tr>
<th>B-G gene clusters</th>
<th>Cluster 1</th>
<th>NOR</th>
<th>Rfp-Y system</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-FI &amp; B-FII</td>
<td></td>
<td></td>
<td>Y-FV &amp; Y-FVI</td>
</tr>
<tr>
<td>B-LβI &amp; B-LβII</td>
<td></td>
<td></td>
<td>Y-LβIII to Y-LβV</td>
</tr>
<tr>
<td>Class III (C4)</td>
<td></td>
<td></td>
<td>non-MHC (17.5 gene: C-Type animal lectin)</td>
</tr>
<tr>
<td>non-MHC (TAP 1, TAP2, tapasin, C-Type lectin, lectin-like natural killer cell receptor, histone H3-like gene, RING3, Leu-tRNA gene)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1. Hypothesized model of the chicken B and Rfp-Y systems. (Adapted from Miller et al., 1994; Miller, 1995; and Genbank Accession number AL023516).
**TABLE 2.** RESTRICTION FRAGMENT LENGTH POLYMORPHISMS ASSOCIATED WITH CLASS II MOLECULAR GENOTYPES IN BROILER CHICKENS.

<table>
<thead>
<tr>
<th>Restriction Fragment (kb)</th>
<th>Class II Molecular Genotypes</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.8</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.1</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

**FIGURE 2.** Four Class II molecular genotypes were identified by Southern blot analysis in two commercial broiler lines. The Class II homozygous genotypes (alleles) are designated a, b, c and d, as indicated in Table 2. All alleles were observed in the two broiler lines, except allele "b" which was not observed in Line B.
FIGURE 3. Class IV molecular genotypes in a broiler population. Southern blot analysis was conducted on 35 pedigreed dam families (n=293). At least six homozygous genotypes (alleles) were identified and a seventh genotype was only observed in heterozygous condition.

FIGURE 4. Seven Class IV molecular genotypes were identified by Southern blot analysis in two commercial broiler lines. The Class IV homozygous genotypes (alleles) are designated 1 to 7. Unique Class IV genotypes were observed for each broiler line.
TABLE 3. SEGREGATION OF B (B-LβII), RFP-Y AND DEL0001 ALLELES IN A PEDIGREED BROILER FAMILY.

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>MHC (B-LβII)</th>
<th>RFP-Y</th>
<th>DEL0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIRE</td>
<td>ab</td>
<td>2/5</td>
<td>2/2</td>
</tr>
<tr>
<td>DAM</td>
<td>ab</td>
<td>2/2</td>
<td>1/1</td>
</tr>
<tr>
<td>Progeny 1</td>
<td>aa</td>
<td>2/5</td>
<td>1/2</td>
</tr>
<tr>
<td>Progeny 2</td>
<td>aa</td>
<td>2/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Progeny 3</td>
<td>ab</td>
<td>2/5</td>
<td>1/2</td>
</tr>
<tr>
<td>Progeny 4</td>
<td>ab</td>
<td>2/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Progeny 5</td>
<td>bb</td>
<td>2/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Progeny 6</td>
<td>bb</td>
<td>2/5</td>
<td>1/2</td>
</tr>
</tbody>
</table>

1Class II (B-LβII) genotypes are the same as those described in Table 2.
2Rfp-Y genotypes were kindly assigned by Dr. Marcia M. Miller, City of Hope, Beckman Research Institute, Duarte, CA.
3DEL0001 genotypes are the same as those described in Figure 5.

RESTRICTION FRAGMENTS ASSOCIATED WITH DEL0001

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>PvuII</th>
<th>HindIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEL0001</td>
<td>2.2 kb</td>
<td>3.2 kb</td>
</tr>
<tr>
<td>(allele 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEL0001</td>
<td>2.0 kb</td>
<td>1.9 kb</td>
</tr>
<tr>
<td>(allele 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEL0001</td>
<td>3.0 kb</td>
<td>Not determined</td>
</tr>
<tr>
<td>(allele 3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 5. Molecular genotypes associated with the chromosome 1 MHC-like genetic system, DEL0001. At least three DEL0001 alleles have been identified in chickens.
FIGURE 6. Partial genetic map of chicken chromosome 1. The MHC-like system (DEL0001) was mapped to the p-arm of chromosome 1 with closely-linked loci, glyceraldehyde 3-phosphate dehydrogenase (GAPD) and lactate dehydrogenase (LDHB). Mouse chromosome 6 contains the syntenic or homologous group of genes (GAPD and LDHB). Also located on mouse chromosome 6 include genes involved in NK cell activity (Ly49 complex) and cytomegalovirus resistance (CMV-1). Mapped to chicken chromosome 1 in the same area as DEL0001 is MDV-1, a QTL that confers resistance to Marek’s disease (Bumstead et al., 1998).
DISCUSSION QUESTIONS AND RESPONSES

Larry Bacon: I believe there is further verification of B-LβII types being identified between broilers and White Leghorns that you may want to verify in publication.

Yes. You are probably referring to the paper in immunogenetics that was just released and includes research on broiler MHC haplotypes from Sandra Ewald’s laboratory (Li et al., 1999). These researchers observed both B-F and B-LβII genes that were common to broilers and White Leghorns. They also noted that some of the common B-F and B-LβII genes were in linkage disequilibrium with different broiler B-G genes, when compared to the White Leghorn B-G genes.

Elwood Briles: Your progress is an excellent example of the new discoveries that can result from collaboration between researchers.

Yes, this has been a large group effort with expertise and resources from UC Davis (M. Delany); Iowa State University (S. J. Lamont); USDA, East Lansing (L. D. Bacon, H. Cheng); City of Hope, Beckman Research Institute, Duarte, CA (M. M. Miller); and last, but not least, yourself (Dr. Briles) for contributing antisera for testing of the broiler MHC haplotypes and your endless time and suggestions. This collaboration originated from a couple of the regional projects (NE-60 and NC-168) and demonstrates the importance of these federally-funded projects.

Dominic Eifick: Have you looked for homology with the DEL0001 gene in other species.

We have narrowed the region of homology between the Class II (ccII-7-1) probe and DEL0001 to a 300 base pair region in the promoter region of the Class II gene. We have submitted this sequence to Genbank (Blast) and there really wasn’t any strong homology (except with its own sequence, ccII-7-1). We did see 20-35 base pair matches with some odd genes including the mouse cellular retinoic acid binding protein and fibroblast growth factor. If we consider mouse chromosome 6 that contains the syntenic group (the chromosomal region that contains similar genes found on chicken chromosome 1), then we may want to consider such genes as Ly49, CD69, NK1 or CMV-1 (FIGURE 6). There are some interesting alignments of the 300 base pair fragment with these genes.