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Serum haemolytic complement activities in 11 different MHC (B) typed chicken lines

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Abstract

To study the relation between serum complement levels and the chicken MHC (B) complex, complement haemolytic activity was measured in sera from hens from seven pure-bred B-typed White and one Brown Leghorn lines, and three ISA-Warren lines that had been divergently selected for antibody responses to sheep red blood cells (SRBC). Significant differences occurred in the serum haemolytic complement activities, both belonging to the classic (CPW) and the alternative (APW) pathways, among the 11 different haplotyped chicken lines. Hens with high CPW and high APW titres predominantly displayed the B2 or B21 haplotypes. Chickens with low CPW and APW were found in B14 and B15 haplotypes. Haplotype B14 appears to be different in complement levels when present into the pure-bred lines or into the ISA-Warren line selected for low antibody responses to SRBC. Otherwise, the presence of B21 in ISA-Warren line selected for high antibody responses to SRBC does not differ with the B21 in the inbred lines (except in the NL-line for CPW values). In general the haplotypes B2 and B21 are found in chicken lines with enhanced disease resistance, and the B15 haplotype has been connected with enhanced disease susceptibility. Our results suggest that levels of haemolytic complement activity, either from the classical or from the alternative pathways, may underlie part of the immunocompetence ascribed to the MHC (B) complex in chickens.
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1. Introduction

Identification and breeding of animals with higher genetic resistance to infection have been recognised as a priority by international and national organisations. Resistance to infectious diseases is generally polygenic of nature (Lamont, 1998a) and a large part is under immunological control (Zekarias et al., 2002). The best known gene complex that has been studied with respect to disease resistance and immune responses in chickens is the major histocompatibility or B complex, located on chromosome 16. The chicken B complex is clearly defined by serological, histogenetic, biochemical and molecular methods

Abbreviations: APW, alternative (Ca-independent) pathway; CPW, classical (Ca-dependent) pathway; MHC, major histocompatibility (B) complex; SRBC, sheep red blood cells

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(Plachy et al., 1992) and consists of several clusters of highly polymorphic classical class I and II genes, next to nonclassical class IV loci, some of which are associated with disease resistance (Lamont, 1998b). The chicken B complex is compact and simple, lacking many genes that are present in the mammalian MHC, e.g. most class III region genes (Kaufman et al., 1999a). The small size and its simplicity have been proposed as an explanation for the striking MHC dependent disease resistance in poultry (Kaufman et al., 1999b; Kaufman, 2000).

Complement components and complement receptors belonging to the classical (CPW) and alternative pathways (APW), that both precede the common terminal (lytic) pathway, are major constituents of the innate immune resistance, including anaphylaxis. In addition, complement components and receptors are also important for the initiation of specific antibody responses, antigen trapping in lymphoid germinal centres, development and maintenance of memory cells and switch of IgM into IgG isotypes (Thorbecke et al., 1994). Recently, a third pathway of complement activation, the Mannan-binding lectin (MBL) pathway was described in mammals and in chickens (Laursen and Nielsen, 2000). In mammals, important components of both the classical (C2, C4) and the alternative pathways (factor B) are encoded by genes situated within the MHC class III region, near other loci related to innate immunity, e.g. TNF_{α} and -β (White, 1989). The association between the B complex and complement-genes in poultry is unclear. In pure-bred chicken lines, haemolytic serum complement activity was shown to be controlled by genes, within or located near the B complex, and B-haplotypes influenced the level of complement components. Class IV-haplotypes B2, B17, B18 and B21 were related with increased complement titres, whereas B12, B13 and B15 haplotypes, respectively, showed decreased complement titres (Chanh et al., 1976; Skeeles et al., 1979; Shen et al., 1984). However, the identity of the dominant gene(s) which controls complement activity associated with the B complex remained to be shown.

Previously, we described different initial haemolytic complement activity, C3 and factor B consumption in serum from chickens divergently selected for antibody responses to SRBC, which suggested a genetic or functional relationship between complement components and levels of specific antibodies in chickens (Parmentier et al., 2002). Apart from levels of specific antibodies, these lines also differ in B haplotype distribution, the high (H) line being B21, whereas the low (L) line is predominantly of the B14 haplotype. In the present study we compared initial haemolytic complement levels in sera of these lines and in sera from the economically important (Leghorn) pure-breeds that from the early eighties have become standard reference stocks for B-haplotyping.

2. Materials and methods

2.1. Chickens

Blood was obtained from eight pure-inbred lines maintained at the Houghton Poultry Research Station (Huntingdon, Cambs, England) in a specified pathogen-free (SPF) environment since 1972. These lines have been extensively studied for resistance to *Eimeria* spp (Bumstead and Millard, 1987, 1992; Bumstead et al., 1995); *Salmonella* spp (Bumstead and Barrow, 1988, 1993); infectious bronchitis virus and *Escherichia coli* (Bumstead et al., 1989; Cook et al., 1990) and infectious bursal disease virus (Bumstead et al., 1993). B haplotypes and genetic background of these lines are shown in Table 1. From each line, nine hens were sampled.

Hens originating from an ISA Brown (Warren) cross (medium heavy layers) were divergently selected for 12 generations for a high (H) or low (L) primary agglutinating antibody response at 5 days after intramuscular immunisation with SRBC at 37 days of age, or from a randombred control (C) line originating from the same parental line (Van der Zijpp and Nieuwland, 1986). These lines are held in non-SPF normal husbandry (battery) conditions and were extensively studied for responsiveness to SRBC (Kreukniet et al., 1992; Van der Zijpp et al., 1992; Parmentier et al., 1993, 1996; Pinard et al., 1993a; Pinard and Van der Zijpp, 1993); phagocyte and lymphocytes responsiveness (Kreukniet et al., 1994; Parmentier et al., 1995) and complement levels (Parmentier et al., 2002). Chickens had received routine vaccinations to Marek', Newcastle disease, infectious bronchitis and infectious bursal disease at earlier ages. MHC(B) haplotype distribution and genetic background of the three lines are shown in Table 1. From each line, 39 hens were sampled.

Table 1
Source and type of eight pure-inbred chicken lines and three selection lines each containing 9 hens (pure lines) or 39 hens (selection lines)

	MHC(B) haplotype	Background
Pure lines		
7_2	B2 B2	White Leghorn birds, Marek's disease susceptible
6_1	B2 B2	White Leghorn birds, Marek's disease resistant
NL	B21 B21	White Leghorn birds, Marek's disease resistant
ZERO	B21 B21	White Leghorn birds, free from all *ev*-loci
15I	B15 B15	White Leghorn birds homozygous
WL	B14 B14	Wellcome B14 line
CL	B4 B12	Reaseheath C line
BLG	B19 B20	Brown Leghorn line
Selection lines		
H-line	B21	ISA-Warren, high antibody responder to SRBC, more than 96% B21, some B19
L-line	B14	ISA-Warren, low antibody responder to SRBC, more than 96% B14, some B24
C-line	B14 B19 B21 B24	ISA-Warren, control group of SRBC selected chickens, various combinations of B14, B19, B21 and B24

2.2. Blood collection

Blood was collected from birds (on average 6 months of age) at location via vein puncture and instantly put on ice for about 1 h to impair spontaneous complement activation between the first and last collecting of blood. After 1 h of coagulation at room temperature, blood was centrifuged at $1000 \times g$ for 10 min at 4 °C. Sera were transported to Antwerp on dry ice and stored at −80 °C until use.

2.3. Complement

Complement activity (CH50 U/ml) was determined with a haemolytic technique as described previously (Demey et al., 1993; Parmentier et al., 2002) using an adapted light-scattering method. In brief, sera were diluted serially in appropriate buffers in flat-bottomed 96-well microtitre plates and incubated with sensitised (Haemolysin, Biomerieux, ref. no. 72202) sheep erythrocytes to measure CPW or rabbit erythrocytes to measure APW, respectively. Plates were shaken in a Titertek (Flow Laboratories) every 30 min during the period of incubation. The results (the amount of light scattering by erythrocytes upon lysis) were read at 655 nm in a microtitre reader (BioRad model 3550). Readings were transformed by log–log equation (Von Krogh, 1916) and the haemolytic titre was expressed as the titre that lysed 50% of the erythrocytes (CH50 U/ml).

2.4. Statistics

Statistical analyses were carried out in Stata 7 (StataCorp, 2001). Mean CPW and APW values were compared using negative binomial regression. Probability levels were Bonferroni adjusted to account for the large number of comparisons made. Clustering and dendrogram construction was done using Euclidean distance and hierarchical agglomerative average linkage.

3. Results

The pure-bred lines from Leghorn origin showed in general lower CPW titres and in general higher APW titres than outbred chickens from ISA-Warren origin (Table 2). A significant correlation ($r = 0.55$) between the (Ca-dependent) CPW and the (Ca-independent) APW titres were found within the lines. In the pure-bred lines, highest CPW and APW titres were found in the B19B20 haplotype (line BLG). Genotypes bearing haplotype B2 and B21 of the pure-bred lines possess high CPW and APW titers (lines ZERO, 7_2, 6_1 and NL). The lowest CPW and APW were found in the B15 genotype (line 15I). In the (out-bred, ISA-Warren) selection lines, highest CPW and APW titres were found in the H-line, which is predominantly of the B21 genotype. Hens belonging to the H-line did not differ significantly for CPW titers with the pure-bred lines,

Table 2
Haplotyped hens and their mean complement titres (CH50 U/ml ± S.E.M.)

Line	Haplotype	Ca-dependent pathway (CPW)	Line	Ca-independent pathway (APW)	Haplotype
15I	B15 B15	579 ± 33^{a}	L-line	364 ± 12^{a}	B14 B14 (B24)
CL	B4 B12	667 ± 25^{ab}	15I	407 ± 23^{ab}	B15 B15
WL	B14 B14	679 ± 48^{abc}	C-line	412 ± 23^{ab}	B14, B19, B21, B24
NL	B21 B21	695 ± 31^{abc}	WL	483 ± 36^{bc}	B14 B14
6_1	B2 B2	736 ± 42^{abcd}	H-line	515 ± 18^{bc}	B21 B21 (B19)
7_2	B2 B2	785 ± 27^{abcd}	CL	584 ± 26^{cd}	B4 B12
ZERO	B21 B21	785 ± 68^{abcd}	7_2	610 ± 23^{cd}	B2 B2
BLG	B19 B20	871 ± 41^{bcd}	6_1	613 ± 21^{cd}	B2 B2
C-line	B14, B19, B21, B24	894 ± 39^{cd}	NL	633 ± 58^{cd}	B21 B21
L-line	B14 B14 (B24)	941 ± 39^{d}	ZERO	646 ± 37^{cd}	B21 B21
H-line	B21 B21 (B19)	958 ± 37^{d}	BLG	726 ± 48^{d}	B19 B20

Values with no common superscripts (a, b, c, d) differ significantly ($P < 0.05$) between the respective averages. (B19), (B24): at most 4% of the haplotypes in H and L line, respectively.

bearing the B21 haplotype (except line NL), nor in APW titers. There was a significant difference for CPW and APW between the inbred WL-line and the L-line of the outbred SRBC-selection lines in which haplotype B14 is present.

Euclidean distances between the complement titres of the eight pure-bred chicken lines (White Leghorn) and the three SRBC-selection lines (ISA-Warren) are demonstrated in Fig. 1. The closest distances in complement titres are between the Zero, 7_2, and NL, 6_1

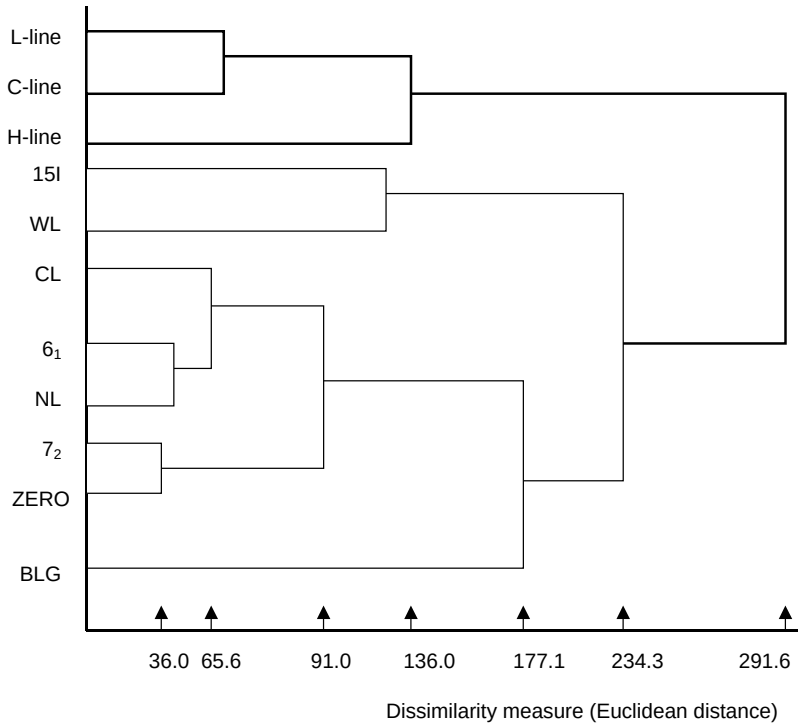

Fig. 1. Dendrogram based on the complement titres of eight pure-bred chicken lines (Compton) and three SRBC-selected lines (Wageningen).

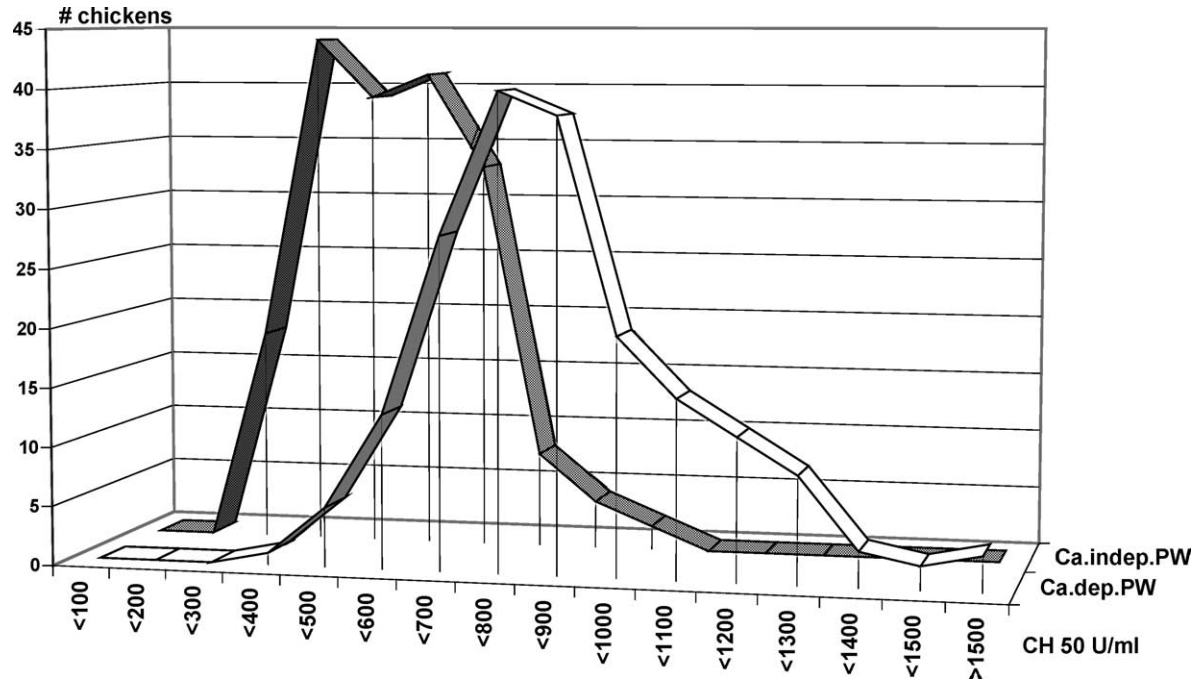

Fig. 2. Distribution of Ca-dep-PW and Ca-indep-PW titers of 189 MHC(B) haplotyped hens.

lines (containing B2- and B21-genotypes). Haplotype B14 and B15 are close related in the pure-bred lines concerning complement levels. The largest Euclidean distance is found between hens from the SRBC-selection lines and the pure-bred lines.

Fig. 2 demonstrates the overall complement distribution of all 189 B haplotyped hens used in this study. Titres of both the CPW and the APW were normally distributed.

4. Discussion

Haemolytic complement activity is extremely important in early (innate) host defence. Activated complement neutralises viruses in mammals (Hirsch, 1982), and similarly in aves (Skeeles et al, 1979; Pandit et al., 1997), some forms of parasites (Touray et al., 1994) and underlies resistance to bacteria (Nolan et al., 2002; Patel and Jaiswal, 1994; Holmskov and Jensenius, 1993; Saxena et al., 2000). Apart from various functions in the initiation and as an effector of the innate immune system, complement components and receptors are involved in the initiation and regulation of the specific immune response of mammals (Thorbecke et al., 1994) and probably of aves as well.

At present, we studied statistical associations between CPW and APW titres, and the major histocompatibility (B) complex in eight pure-bred chicken lines and three SRBC-selection lines. Significant differences in complement activities between 72 chickens from eight pure-inbred lines and 117 chickens of the 12th generation of chickens selected for antibody responses to SRBC were found. It is unknown whether these differences may be based on the husbandry (SPF versus conventional) as well, however, within both locations, line/breed differences were found. Chickens with high CPW and high APW were characterised by the B2 and B21 (pure-bred lines) and B21 haplotype (selection lines), respectively. Chickens with low CPW and APW corresponded with the B15 haplotype whereas the lowest APW titres were also determined in B14 haplotypes characterised hens. The B15 is lacking in the selection lines. In general, haplotypes B2 and B21 are found in birds with enhanced general immunity (Bacon and Witter, 1992, 1994a,b; Hepkema et al., 1993). This enhanced disease resistance

might be related with the higher initial complement levels in birds with these haplotypes. The B15 haplotype is often related with a decreased or lower immunocompetence (Bacon and Witter, 1992, 1993). This suggests that lower complement levels are related with lower disease resistance. In addition, the difference in complement activities (Parmentier et al., 2002) between the high and low antibody selection lines further indicated a relationship between levels of complement from both the CPW and the APW and the magnitude of specific (humoral) immunity. In the present study, the relation between B-haplotypes and levels or consumption of complement components after immunisation or infection were, however, not measured.

The complement titres from the SRBC-selection lines on the one hand, and the White Leghorn based pure-bred lines on the other hand showed segregation based on the Euclidean distance complete linkage method. These results indicated (1) a positive (cor)-relation between APW and CPW lines indicating either common genetic or functional regulation and (2) that the variety in complement levels between the pure-bred and the selection lines is also for an important part related with non-MHC 'background' genes, or alternatively due to different husbandry conditions.

Studies of the chicken B complex have resulted in important clues with respect to advantages conferred by certain MHC alleles to specific immune responses. In general, there is an association of the B21- and B2-haplotypes within the Leghorn breeds with enhanced disease resistance and enhanced specific immunity as compared with other haplotypes. Also in the SRBC selection lines, higher resistance to Marek's disease (Pinard et al., 1993b) and *Eimeria* infection (Parmentier et al., 2001) was found in the B21 carrying H line. In the present study it is indicated that these features of the B2 and B21 haplotypes may be related with, or based on the higher initial complement levels in birds carrying these haplotypes. Similarly, birds carrying the B15 haplotype generally are characterised by lower specific immune competence, and lower resistance against infection. Likewise, this might be related with the lower complement levels found in birds with the B15 haplotype.

It has been described that immune responsiveness in mammals depends both on the classical polymorph class I and class II MHC genes in combination with a less but still polymorph class III region (White, 1989; Peelman et al., 1996; Westman et al., 1994) resulting in an MHC 'supratype'. However, in contrast to mammals, a genomic region analogous to the MHC class III region containing various genes encoding levels of both classical (C2, C4), heat shock proteins, and alternative complement components (factor B) next to TNF_{α} and -β have not been positioned in poultry. The structure of the B complex leaves no space for a class III region (Guillemot et al., 1989). Factor B was suggested outside the B locus (Koch, 1986) and also the C4 gene was suggested outside the B complex (Kaufman et al., 1999a). The G9a class III gene, however, may be linked with the chicken MHC (Spike and Lamont, 1995). Taken the important role of complement in the initiation and regulation of specific immunity into account, further studies are urged to unravel the functional and genetic relation between the B complex, complement levels and immune mediated disease resistance in poultry. Identification of the gene(s) encoding complement components should reveal whether differences in disease resistance between poultry stocks is primarily based on either the specific (antigen presentation, classical class I or II alleles) or non-specific innate (complement, TNF) part of the chicken immune system.

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