Resistance, Susceptibility, and Immunity to *Eimeria tenella* in Major Histocompatibility (B) Complex Congenic Lines

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**ABSTRACT** The major histocompatibility (B) complex influence on resistance, susceptibility, and immunity to *Eimeria tenella* was examined in UCD B complex congenic chicken lines. In Experiment 1, 6-wk-old chicks from 12 UCD congenic lines were weighed and assigned to either challenge or control groups. The challenge group received a dose of 10,000 *E. tenella* oocysts. Response to challenge was evaluated by body weight gain and cecal lesion scores. Cecal lesion scores in B<sup>3B3</sup> chickens were significantly lower than those of all other genotypes. Genotype B<sup>2B2</sup> had the highest lesion scores, which were significantly different from the lesion scores calculated for B<sup>3B3</sup>, B<sup>18B18</sup>, and B<sup>21B21</sup> chickens but were not significantly different from B<sup>14B14</sup>, B<sup>15B15</sup>, B<sup>17B17</sup>, B<sup>19B19</sup>, B<sup>24B24</sup>, B<sup>BCB</sup>, B<sup>IBI</sup>, and B<sup>QBO</sup> genotypes. The B<sup>21B21</sup> chickens had significantly lower lesion scores than B<sup>2B2</sup>, B<sup>14B14</sup>, and B<sup>BCB</sup> chickens. No other significant lesion score differences were found among the remaining lines. The highest weight gain found in B<sup>19B19</sup> chickens was significantly different from that of B<sup>3B3</sup>, B<sup>14B14</sup>, B<sup>15B15</sup>, B<sup>17B17</sup>, B<sup>18B18</sup>, B<sup>24B24</sup>, and B<sup>BCB</sup> chickens. The B<sup>15B15</sup> chickens had the lowest weight gain, which was significantly different from that of B<sup>2B2</sup>, B<sup>19B19</sup>, B<sup>21B21</sup>, B<sup>24B24</sup>, B<sup>IBI</sup>, and B<sup>QBO</sup> chickens.

(Key words: major histocompatibility complex, parasite immunity)

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**INTRODUCTION**

Coccidiosis, a disease caused by obligate intracellular protozoan parasites of the genus *Eimeria*, constitutes a significant economic impact in poultry. Lower weight gain, reduced feed efficiency, mortality, and prophylactic medication are important cost factors (Danforth and Augustine, 1985). Anticoccidial compounds provide control despite increased drug resistance and decreased development of new medications. Host resistance or increased immune response represent potential alternative control methods.

Genes of the MHC (B complex) influence the response to many diseases in the chicken. Diseases caused by oncogenic and nononcogenic viruses, bacteria, and parasites are affected by the host MHC (Dietert et al., 1991). Briles and coworkers (1977) demonstrated that the B<sup>21</sup> haplotype of the chicken MHC is responsible for strong resistance to Marek’s disease, whereas B<sup>19</sup> is associated with a high degree of susceptibility. Collins and coworkers (1977) determined that B<sup>2B2</sup> chickens regressed Rous sarcoma virus-induced tumors and B<sup>5B5</sup> birds progressed these tumors. The B<sup>5</sup> haplotype provided a more effective response against *Eimeria tenella* than did B<sup>2</sup> (Clare et al., 1985). These results are opposite to that observed for Marek’s disease, lymphoid leukosis, and Rous sarcomas, in which the B<sup>2</sup> haplotype exhibited superior responses (Plachy et al., 1992).

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TABLE 1. Major histocompatibility (B) complex congenic lines of chickens obtained from the University of California-Davis (Abplanalp, 1992)

<table>
<thead>
<tr>
<th>Line</th>
<th>B Haplotype1,2</th>
<th>Source line</th>
</tr>
</thead>
<tbody>
<tr>
<td>004</td>
<td>B17</td>
<td>UCD 003 Inbred full-sib (1956) White Leghorn</td>
</tr>
<tr>
<td>104</td>
<td>B0</td>
<td>UCD 500 Ceylonese Jungle Fowl × Red Jungle Fowl</td>
</tr>
<tr>
<td>253</td>
<td>B18</td>
<td>UCD 002 Inbred White Leghorn</td>
</tr>
<tr>
<td>254</td>
<td>B15</td>
<td>UCD 007 Inbred White Leghorn</td>
</tr>
<tr>
<td>312</td>
<td>B24</td>
<td>UCD 200 NH Inbred</td>
</tr>
<tr>
<td>313</td>
<td>B3</td>
<td>UCD 071 Inbred White Leghorn</td>
</tr>
<tr>
<td>316</td>
<td>B14</td>
<td>UCD 400 NH Wisconsin Inbred</td>
</tr>
<tr>
<td>330</td>
<td>B21</td>
<td>UCD 100 Inbred Australorp</td>
</tr>
<tr>
<td>331</td>
<td>B2</td>
<td>Hy-Line Dwarf White Leghorn</td>
</tr>
<tr>
<td>333</td>
<td>B1</td>
<td>UCD 001 Inbred Red Jungle Fowl</td>
</tr>
<tr>
<td>335</td>
<td>B19</td>
<td>UCD 159 Mount Hope Commercial Richardson</td>
</tr>
<tr>
<td>336</td>
<td>B2</td>
<td>UCD 001 Inbred Red Jungle Fowl</td>
</tr>
<tr>
<td>342</td>
<td>B1</td>
<td>UCD 500 Ceylonese Jungle Fowl × Red Jungle Fowl</td>
</tr>
</tbody>
</table>

1Haplotypes with numerical superscripts are in accordance with international nomenclature as described by Briles and Briles (1982).

2Haplotypes with as yet undefined status are designated with capital superscripts.

Resistance to coccidiosis is very complex involving several host factors, which include age, immune competence, and genetic composition (Lillehoj, 1988). Chickens having increased resistance or susceptibility to coccidiosis have been produced through selective breeding (Edgar et al., 1951; Champion, 1954; Rosenberg et al., 1954; Johnson and Edgar, 1982). The overall mechanisms responsible for the differences in resistance to infection among different lines involve both MHC and non-MHC genes (Lillehoj et al., 1986; Lillehoj, 1988; Clare and Danforth, 1989; Bumstead and Millard, 1992).

Development of congenic lines having a common highly inbred genetic background and differing in their MHC (Abplanalp, 1992) has created an opportunity to compare the degree of disease resistance conferred by specific B complex haplotypes. In the present study, resistance, susceptibility, and immunity to E. tenella were tested in UCD 003 B complex congenic lines. The first experiment examined the genetic differences between resistance and susceptibility. A second study investigated the congenic lines’ response to immunization with small doses of oocysts prior to challenge.

MATERIALS AND METHODS

Stocks

The B congenic lines used in this study are shown in Table 1. These lines were produced by crossing B haplotypes from different sources into the genetic background of Line UCD 003 (B17B17) followed by five backcrosses (Abplanalp, 1992). Heterozygotes were then mated inter se to produce progeny that were homozygous for different B haplotypes. Fertile eggs were shipped from the University of California-Davis to the University of New Hampshire Poultry Research Farm, where they were incubated and hatched. Chicks were vaccinated against Marek’s disease and Newcastle-bronchitis at 1 and 10 d, respectively. Birds were housed in isolation, free from any possible coccidial exposure, in wire floor cages with free access to antibiotic-free feed and water.

Coccidial Cultures

Fresh cultures of the Lilly 65 strain of E. tenella oocysts were obtained from a stock culture held at the University of New Hampshire. The stock culture was propagated in susceptible 3- to 5-wk-old chickens inoculated with 6 to 7 × 104 sporulated oocysts per bird. After 7 d, the chickens were euthanatized and oocysts harvested directly from the cecal pouches. Following peptic digestion (Rikimaru et al., 1961), sporulation of the oocysts was enhanced by bubbling with air in 0.5% potassium dichromate at room temperature, then sterilized using a 50% chlorine bleach solution (Wagenbach and Burns, 1969). The culture was held up to 3 mo at 4 C until used.

Criteria of Evaluation

Cecal lesion scores were used to measure severity of infection in Experiment 1 and degree of immune response in Experiment 2. Scoring followed the procedure of Johnson and Reid (1970) where 0 = no gross lesions; 1 = very few scattered petechiae on the cecal wall; no thickening of the cecal walls; normal cecal contents; 2 = lesions more numerous with noticeable blood in the cecal contents; cecal wall is somewhat thickened; normal cecal contents; 2 = lesions more numerous with noticeable blood in the cecal contents; cecal wall is somewhat thickened; normal cecal contents present; 3 = large amounts of blood or cecal cores present; cecal walls greatly thickened; little, if any, fecal contents in the ceca; and 4 = cecal wall greatly distended with blood or large caseous cores, fecal debris lacking or included in cores. Dead birds were scored as 4.

Birds were weighed on Day 1 prior to inoculation with oocysts, and again on Day 6. Weight gain was calculated by subtracting the initial weight from the weight on Day 6. Mortality after inoculation was also determined for all groups.
Experiment 1. Susceptibility

Chicks from 12 B complex congenic lines having genotypes: B2B2, B3B3, B4B14, B15B15, B17B17, B18B18, B19B19, B21B21, B24B24, BCBC, BIBC, BOBO, were used. At 6 wk of age, all chicks were weighed and assigned to either challenge or control groups. The control group received no inoculation. The challenge group received a dose of 10,000 Lilly 65 strain E. tenella oocysts. Inocula were counted using a hemocytometer and were administered per os to the crop using an inoculation tube and syringe. Each line was represented by four to five chicks in control as well as challenge groups in three hatches constituting a total of 351 individuals. Cecal lesion scores of the challenge group are shown in Figure 1. Genotype

Statistical Analysis

Mean cecal lesion scores and body weight gain were evaluated by analysis of variance. In Experiment 1, the challenge group was analyzed with hatch and line as main effects. Hatch, line, and treatment were the main effects in the analysis of Experiment 2 to determine the efficacy of the immunization regimen. The immune group was analyzed for differences among lines in their response to immunization. Significant means for both experiments were separated by Fisher’s protected Least Significant Difference at P < 0.05.

RESULTS

Experiment 1 compared resistance and susceptibility among 12 UCD B complex congenic lines inoculated with 10,000 E. tenella oocysts. The control group received no inoculation and produced no cecal lesions. The mean cecal lesion scores of the challenge group are shown in Figure 1. Lesion scores in B3B3 chickens were significantly lower than the scores of every other genotype tested. The B21B21 genotype had the next lowest lesion score, which was significantly different from scores of the B2B2, B14B14, and BCBC genotypes. Chickens of the B2B2 genotype had the highest cecal lesion scores, which were significantly greater than the lesion scores found in B3B3, B18B18, and B21B21 lines. Lesion scores of B2B2 chickens of the genotype were not statistically significant relative to scores of B14B14, B15B15, B17B17, B19B19, B24B24, BCBC, BIBC, and BOBO genotypes. Based on lesion scores after primary inoculation, B3B3 chickens were more resistant to E. tenella than any other line tested. No single line showed increased susceptibility although B2B2 chickens were more susceptible than were B3B3, B18B18, and B21B21 chickens.

Mean weight gains 6 d after 10,000 E. tenella oocyst inoculation are shown in Figure 2. Genotype B19B19 had
the highest weight gain, which was significantly different from $B^3B^3$, $B^{14}B^{14}$, $B^{15}B^{15}$, $B^{17}B^{17}$, $B^{18}B^{18}$, $B^{24}B^{24}$, and $B^C^B^C$. The lowest weight gain, exhibited by $B^{15}B^{15}$ chickens, was significantly lower than $B^B^B$, $B^{19}B^{19}$, $B^{21}B^{21}$, $B^B^B$, and $B^Q^B^Q$. The $B^3B^3$ chickens, which had the lowest cecal lesion score, had the second lowest weight gain. The correlation coefficient for lesion score and weight gain was 0.126 indicating little relationship between the two criteria for the 351 chicks in Experiment 1.

In Experiment 2, immunity was evaluated after an immunizing regimen consisting of daily inoculations of 500 $E. tenella$ oocysts for 5 d followed by challenge with 10,000 oocysts 14 d after the final immunizing dose. Immunization was successful because the three groups were significantly different from each other (Figure 3). The control group was not exposed to coccidia and had no cecal lesions. The challenge group, which had no exposure to coccidia prior to challenge, had the highest lesions (3.13 ± 0.1), whereas the immune group, which had been previously immunized, had an intermediate value (2.14 ± 0.1).

Among immune birds, $B^3B^3$ and $B^Q^B^Q$ chickens had lesion scores, which were significantly lower than those of the $B^{19}B^{19}$, $B^{24}B^{24}$, $B^{14}B^{14}$, and $B^B^B$ chickens (Figure 4). Lesion scores found in the $B^{19}B^{19}$ and $B^{24}B^{24}$ chickens were significantly higher than $B^3B^3$, $B^{15}B^{15}$, $B^C^B^C$, and $B^Q^B^Q$ chickens, suggesting that the former two genotypes were not well-immunized compared to the latter four genotypes. The $B^3B^3$ genotype had the lowest numerical lesion scores postimmunization as well as the lowest numerical lesion scores postprimary challenge in Experiment 1. No significant differences in weight gain and no correlation between lesion score and weight gain were found in immune birds (data not shown).

**DISCUSSION**

In Experiment 1, 12 $B$ congeneric lines of chickens having the common UCD genetic background were compared for their resistance to coccidial infection. Some $B$ complex genotypes were more resistant than others following 10,000 $E. tenella$ oocyst challenge. The $B^3B^3$ and $B^{21}B^{21}$ genotypes were more resistant to $E. tenella$ than $B^2B^2$ chickens, which were more susceptible based on cecal lesion scores. The remaining nine genotypes were not significantly different from each other.

The $B^{19}B^{19}$ chickens had the highest weight gain and the $B^{15}B^{15}$ chickens had the lowest weight gain (Figure 2). There was little relationship between lesion score severity and weight gain in the congeneric lines, as detected in other studies (Clare et al., 1985; Martin et al., 1986; Ruff and Bacon, 1989). Genotype $B^{19}B^{19}$ was the most resistant by the weight gain criteria, but these birds had high lesion scores as well. On the other hand, the $B^3B^3$ chickens, which were most resistant as measured by low lesion scores, also had low weight gain. These results reemphasize the dilemma in choosing a single criterion on which to base resistance or susceptibility (Ruff and Bacon, 1989).

Both criteria, lesion scores and weight gain, reflect certain aspects of the response to the parasite, but neither alone can be relied upon to depict the overall immune competence of the individual. Lesion scores represent the parasite’s physical damage to the host’s intestines. Variables such as lymphocyte and parasite numbers, which may affect lesion scores, are related to the immune response. Additional variables affect weight gain, such as feed consumption or nutrient processing efficiency during infection. Birds with severe intestinal lesions have gained weight during a coccidial infection,
and birds showing fewer lesions have gained very little weight.

The immunizing protocol in the Experiment 2 used five consecutive daily doses of 500 E. tenella oocysts. Immunization was successful because immunized birds had lower lesion scores than the uninimmunized, challenged birds. Differential B haplotype effects were seen among the immunized UCD congenic lines. Immunized B3B3 and B2BQ chickens had lower lesion scores than the B19B19, B24B2, B14B14 and B2B2 genotypes. Genotypes B15B15, B17B17, B2BC, and B0BO were intermediate in response.

Previous studies revealed significantly greater immunity to Eimeria following daily low doses of oocysts compared to the same total oocyst number in a single immunizing dose (Joyner and Norton, 1973; Joyner and Norton, 1976). Clare et al. (1986) demonstrated that repeated daily doses of 200 oocysts did not induce detectable immunity, whereas five daily doses of 500 oocysts stimulated protection in progeny segregating for B2 and B5 haplotypes. The higher immunizing dose reached or exceeded the threshold to stimulate immunity (Clare et al., 1986). The immunizing threshold may not have been achieved in the current study’s poor responding lines.

The current results corroborate previous studies (Clare et al., 1985; Lilleshøj et al., 1986; Lilleshøj and Ruff, 1987; Ruff and Bacon, 1989) indicating an important role for the B complex in response to primary infection and in the development of immunity to E. tenella. The poor response exhibited by the B2B2 genotype was found by Clare and colleagues (1986) and Ruff and Bacon (1989). Two congenic lines (15.6-2 and 15.7-2) having the B2 haplotype from different donor lines, both had higher susceptibility to primary E. tenella infection based on weight gain, oocyst production and plasma pigment values compared to lines containing the B5, B12, B13, and B19 haplotypes (Ruff and Bacon, 1989). No lesion score differences were observed. These same two B2B2 congenic lines also were more susceptible to Eimeria acervulina as indicated by weight gain postchallenge.

The B2B2 genotype had less protection following repeated low dose immunization than the B5B5 chickens (Clare et al., 1985). A genetically engineered E. tenella antigen also stimulated less protective immunity in B2B2 congenic chickens than in the B5B5 line (Clare and Danforth, 1989). On the other hand, Ruff and Bacon (1989) found that congenic lines 15.6-2 and 15.7-2 (B2B2) had low immunity to E. tenella after a single 100 oocyst immunization but had 84% protection after four 100 oocyst doses. Congenic line 15.15I-5 (B5B5) had only 23% protection in the same protocol.

Responses of individual B haplotypes may be influenced by non-MHC background genes (Clare et al., 1985; Lilleshøj et al., 1986; Lilleshøj and Ruff, 1987; Ruff and Bacon, 1989). Congenic lines minimize the background genes influence by placing particular B haplotypes on a common genetic background. However, differences in genetic background genes among various chicken lines, alter the context in which particular B haplotypes are expressed. Three studies found differential immune responses between B2 from line 6, and B5 from line 15 (Clare et al., 1985; Clare and Danforth, 1989; Ruff and Bacon, 1989) in two genetic backgrounds: inbred line 6 in the former two studies and inbred line 15 in the latter study. The B2B2 genotype also showed a poor response in the current study, using the UCD 003 background, which supports the low response of the B2 haplotype. Conversely, successful immunization of the B2B2 genotype on the Line 15 background (Ruff and Bacon, 1989) but not in B2B2 on the Line 6 background (Clare et al., 1985; Clare and Danforth, 1989) suggests a non-MHC background gene influence.

Mechanisms for protective immunity to chicken coccidiosis are thought to depend on T cells (Giambrone et al., 1980; Lilleshøj, 1987; Rose and Long, 1971), with the possibility of a secondary role for antibodies (Rose and Hesketh, 1979; Crane et al., 1986; Clare and Danforth, 1989). Therefore, quantitative differences in T cell numbers may influence the qualitative aspects of cellular immunity. Line FP (B15B21) chicks have more splenic T lymphocytes than Line SC (B2B2) chicks at 1 d of age. A single primary inoculation stimulated protective immunity at 1 d of age in Line FP whereas Line SC did not develop equivalent immunity until 4 wk of age (Lilleshøj, 1988).

Recognition of parasite antigens may differ among particular B complex haplotypes. For example, B2B2 and B19B19 genotypes had high lesion scores after primary inoculation and a poor response to immunization, whereas genotypes B15B15 and B0B0 also had high lesion scores, but showed good response to immunization. Congenic lines in the present study may also differ in their immune competence at the time of vaccination as influenced by the number of mature T cells or the amount of processed antigen available to stimulate a T cell response. These effects could be related to MHC genes, background genes or a combination of the two.

The amount of antigen to stimulate immunity may fluctuate based on the number of parasites produced at various life-cycle stages. Quist et al. (1993) showed that cells from a line selected for a sixfold differential resistance to E. tenella had less initial parasite infection and less subsequent asexual stage development than did cells from the susceptible line. These lines differ in their frequencies of particular Ea-A and Ea-E blood group antigens as well (Johnson and Edgar, 1984). Some haplotypes, which had lower lesion scores after primary inoculation in the current study, may not produce sufficient parasites to stimulate immunity resulting in their lower protection after immunization.

Both B complex and background gene effects should be considered in the evaluation of responses to E. tenella infection and the acquisition of immunity. The same background genes present in a series of congenic lines may have variable interaction with a spectrum of B haplotypes. Likewise, responses of similar B complex
haplotypes may differ depending upon the particular genetic background.

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REFERENCES


