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Resistance, Susceptibility, and Immunity to Cecal Coccidiosis: Effects of B Complex and Alloantigen System L

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ABSTRACT This study examined alloantigen system L effects on resistance to initial infection and acquired immunity to Eimeria tenella infection in three B complex genotypes. Experimental progeny segregating for B and L genotypes were produced from pedigree matings of B2B5L1L3 sires and dams. Chicks were weighed and inoculated with 30,000 E. tenella oocysts at 6 wk of age to evaluate resistance in four trials (n = 262). Immunity was studied in four additional trials (n = 244) by immunizing progeny with 500 E. tenella oocysts per day for 5 d beginning at 5 wk of age. Two weeks after the last immunization dose, the birds were weighed and challenged with 30,000 E. tenella oocysts. All birds were weighed again and scored for cecal lesion 6 d after the 30,000 oocyst dose challenge. Weight gain was not affected among immunized birds. Immunity was evaluated by ANOVA. Major histocompatibility (B) complex genotypes B2B5 and B2B5 did not affect resistance to initial challenge with E. tenella based on lesion score and weight gain. However, after immunization, the B2B5 and B2B5 genotypes had significantly lower cecal scores than the B2B5 genotype when the birds were rechallenged. Weight gain was not affected among immunized birds. No significant L system effects with or without immunization were detected. These results are consistent with previous research demonstrating B complex effects on immunity to cecal coccidiosis.

(Key words: major histocompatibility (B) complex, L system, alloantigen, Eimeria tenella, cecal coccidiosis)

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INTRODUCTION

Cecal coccidiosis is a disease in chickens caused by the intracellular protozoan parasite Eimeria tenella. Morbidity due to coccidiosis manifests itself as reduced BW, decreased feed efficiency, and in some cases, mortality. Disease severity is judged by criteria including weight loss, cecal lesions, fecal oocyst content, and mortality. These symptoms need to be considered individually because they usually show little or no correlation (Long et al., 1980; Clare et al., 1985). Long et al. (1980) suggested that there are at least three stages of immunity to cecal coccidiosis characterized by 1) complete resistance to the parasite, 2) discharge of oocysts but no lesion occurrence, or 3) resistance to the clinical effects of the disease despite the presence of severe lesions.

Due to increasing parasite resistance to anticoCCidial compounds currently used in commercial poultry production, there has been active research into the possibility of utilizing the chicken immune response as a method of disease control. One tool to counteract coccidiosis is a live vaccine consisting of low levels of all pathogenic coccidial species, which relies on repeated low dose exposures to the parasite to increase immunity (Johnson et al., 1979). Another measure that has been explored involves the identification of genetic factors related to immunity, which may affect the outcome of the disease. The frequency of these beneficial alleles can then be increased in commercial populations through selective breeding (Johnson and Edgar, 1982).

The B complex was one genetic region examined for its effects (Clare et al., 1985; Lillehoj et al., 1989; Ruff and Bacon, 1989; Caron et al., 1997) on resistance to initial E. tenella infection and immunity to the parasite. Studies that utilized inbred lines, inbred line crosses, and congenic lines found B complex effects on resistance, immunity, or both. However, no B genotype had a consistent response of greater resistance to cecal coccidiosis or greater immunity to the disease among these investigations. This variable response among B haplotypes pointed to genes other than the MHC as potential factors influencing the response to E. tenella infection. Lillehoj et al. (1989) found wide variations in innate resistance to infection and acquired immunity to cecal coccidiosis among birds sharing a common genetic background but differing at their MHC as well as birds sharing a common B haplotype but differing in genetic backgrounds. Other studies iden-
tified some of the non-MHC genes, including the A-E (Johnson and Edgar, 1984), I (Martin et al., 1986), and C (Johnson and Edgar, 1986) alloantigen systems.

A study on resistance to *E. tenella* infection as a function of eight alloantigen systems found a significant *L* system effect on lesion scores within a defined MHC background (Taylor and Briles, 2000). $B^2B^1L^1L^1$ chickens had higher lesion scores than $B^2B^1L^1L^2$ and $B^2B^1L^2L^2$ chickens. No *L* effects were evident in a $B^2B^5$ genotypic background. Alloantigen system *L* has demonstrated effects on immune functions including monocyte phagocytosis (Qureshi et al., 2000), antibody response to SRBC and *Brucella abortus* (Medarova et al., 2003), and bursa size (Scott et al., 1988). Two studies have reported a significant *L* effect on Rous sarcoma outcome (LePage et al., 2000; Medarova et al., 2002). DeSilva (1965) also found that the *L* system influenced fertility.

We sought to characterize how *L* effects on resistance to infection and acquired immunity to cecal coccidiosis in chickens having fully segregating combinations of two *B* and two *L* alleles. The chickens were produced by the same mating types used to study the role of the *L* system in Rous sarcoma outcome (Medarova et al., 2002a) as well as antibody responses to SRBC and *Brucella abortus* (Medarova et al., 2002b). Because of genetic segregation within families, these matings minimize the effect of other background genes, reveal possible interrelationships between the influence of *L* and *B* systems, and facilitate discrimination between the effects on resistance to *E. tenella* infection and those on immunity to the disease.

**MATERIALS AND METHODS**

**Stock**

Modified Wisconsin Line 3 Ancona × Line NIU 4 sires ($B^2B^1L^1L^2$) White Leghorns were crossed with inbred Line 6.15-5 dams ($B^2B^1L^1L^1$) to produce the parental stock consisting of 50% inbred Line 6.15-5 having the genotype $B^2B^1L^1L^2$ (Medarova et al., 2002a). Pedigree matings of four $B^2B^5L^1L^2$ sires to five $B^2B^5L^1L^2$ dams per sire produced experimental progeny that segregated for all possible combinations of *B* and *L* alleles. The chicks were hatched at the University of New Hampshire Poultry Research Farm and were wing-banded for identification. Vaccinations against Marek's disease and Newcastle-bronchitis were administered at hatch and 10 d, respectively. The chicks were housed in isolation, free from coccidial exposure, in wire floor cages with free access to antibiotic-free water and feed. The chickens were typed for *B* and *L* systems in agglutination assays utilizing antisera specific for the haplotypes of the parental stocks (Briles and Briles, 1982) as described by LePage et al. (2000).

**Coccidial Cultures**

Cultures of the Lilly 65 strain of *E. tenella*³ oocysts were used in the third passage from a stock culture held at the University of New Hampshire. In vivo propagation of the stock culture involved inoculation of 3-to-5-wk-old birds with 50,000 sporulated oocysts per bird. Seven days postinoculation, oocysts were harvested from the cecal pouches. The cecal contents were subjected to peptic digestion (Rikimaru et al., 1961). Oocyst sporulation was induced by bubbling with air in 0.5% potassium dichromate at room temperature. The sporulated oocyst mix was sterilized in a 50% chlorine bleach solution (Wagenbach and Burns, 1969). Inocula were counted using a hemocytometer and administered per os to the crop using an inoculation tube and a syringe.

**Resistance on Initial Exposure to *E. tenella***

Four hatches having 262 experimental progeny were used to evaluate resistance to *E. tenella* infection. Six-week-old birds were weighed and inoculated with a single dose of 30,000 *E. tenella* oocysts. The birds were weighed again and cecal lesions were scored 6 d after challenge. The inoculated birds were compared to an unchallenged control group (n = 32) from the same parental matings that produced the experimental chicks.

**Immunity to *E. tenella***

Progeny from four hatches having a total of 244 chicks were used to study immunity to *E. tenella*. Birds were immunized with 500 *E. tenella* oocysts per day for 5 d beginning at 5 wk of age as described (Clare et al., 1985, 1989). Two weeks after the last immunization dose, the birds were weighed and challenged with 30,000 *E. tenella* oocysts. Six days after challenge, the birds were weighed again and cecal lesion scores were assessed. The immunized birds were compared to an uninfected control (n = 12) and unimmunized, challenged (n = 27) control groups consisting of birds from the same parental matings.

**Evaluation Criteria**

Cecal lesion scoring followed the procedure outlined by 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 somewhat thickened, normal cecal contents; 3 = large amounts of blood or cecal cores present, cecal walls greatly thickened, little or no fecal contents in the ceca; and 4 = cecal wall greatly distended with blood or large caseous cores, and fecal debris lacking or included in cores. Dead birds were given a score of 4. Weight gain was calculated by subtracting the initial weight obtained at challenge from the weight obtained 6 d following challenge.

**Statistical Analysis**

Weight gain and mean cecal lesion scores were analyzed by least squares ANOVA with hatch, sex, sire, dams

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³Eli Lilly and Co., Greenfield, IN.
within sires, B genotype, L genotype, and B × L interaction as main effects. This procedure was used to evaluate the resistance study and the immune study. In an additional analysis for immunity, weight gain and cecal lesion scores of immunized birds were compared with the unimmunized, challenged group to assess the efficacy of the immunization protocol. Significant means were separated by Fisher’s protected least significant difference at P < 0.05.

RESULTS

Experimental progeny had B and L genes segregating independently of each other. Six-week-old birds were studied to insure their immunocompetence for resistance to infection as well as immunity. Neither the B nor the L system affected resistance significantly based on weight gain or lesion score. The $B^2B^2$ genotype had the highest cecal lesion scores (3.00 ± 0.09), whereas the $B^2B^5$ genotype had the lowest lesion scores (2.74 ± 0.10). However, these differences were not statistically significant. The $L^1L^1$, $L^1L^2$, and $L^2L^2$ genotypes had cecal lesion scores of 2.85 ± 0.10, 2.83 ± 0.08, and 2.84 ± 0.10, respectively, that were not statistically different (Figure 1).

The $B^2B^2$ birds that had the highest lesion scores also had the lowest weight gain (26.49 ± 6.44 g), and the $B^1B^5$ birds had the lowest lesion scores but the highest weight gain (32.16 ± 4.73 g; Figure 2). These cecal lesion scores and weight gains were negatively correlated (r = −0.989). Weight gain did not differ significantly among the three L genotypes. The lowest weight gain (27.29 ± 5.66 g) occurred in $L^1L^1$ birds, and the highest weight gain (31.75 ± 3.81 g) was found in the $L^1L^2$ birds (Figure 2).

In the immune study, differences in cecal lesion score ($P < 0.0001$) and weight gain ($P < 0.0001$; data not shown) were found between the immune group and the unimmunized, challenge control group. The unimmunized, unchallenged control group had no cecal lesions. Together, these data indicated that the immunization protocol used was successful. The B genotype had an effect on lesion score ($P < 0.007$) in immunized progeny. The $B^2B^2$ genotype had significantly higher lesion scores (1.61 ± 0.14) than either the $B^2B^5$ (1.21 ± 0.09) or $B^5B^5$ (1.00 ± 0.14) genotypes (Figure 3). Weight gain was not affected significantly by B genotype (Figure 4) as the $B^2B^2$ birds gained 22.80 ± 4.12 g and the $B^2B^5$ birds gained 30.74 ± 4.72 g. The correlation coefficient between weight gain and lesion scores as a function of B genotype was lower than in the resistance study (r = −0.561).

The L genotype had no significant effect on lesion scores (Figure 5) or weight gain (Figure 4). There were no significant lesion score differences among the $L^1L^1$ (1.23 ± 0.15), $L^1L^2$ (1.29 ± 0.09), and $L^2L^2$ (1.25 ± 0.13) genotypes. The weight gains (g) according to the L genotypes were $L^1L^1$ (24.87 ± 4.12), $L^1L^2$ (21.95 ± 2.95), and $L^2L^2$ (23.59 ± 3.81). These values did not differ significantly.

DISCUSSION

The current study found no B or L system effects on resistance to initial E. tenella infection. B complex effects on resistance have been examined in B complex congenic lines that have different MHC genotypes on a common inbred background. Resistance to E. tenella varied among the 15.B congenic lines (Ruff and Bacon, 1989; Lillegred et
al., 1989) as well as the UCD B congenic lines (Caron et al., 1997). On the other hand, contrasting oocyst production among MHC identical lines with different genetic backgrounds suggested a role for non-MHC genes in innate resistance to infection with *E. tenella* (Lillehoj et al., 1989).

Our results on the B complex effects on immunity to *E. tenella* agree with two previous findings (Clare et al., 1985; Caron et al., 1997). Repeated low dose immunization lowered lesion scores significantly without weight gain differences in B2B2 and B1B2 birds but not B2B3 birds (Clare et al., 1985). Immunized UCD B congenic lines revealed lower lesion scores in B2B2 and B1B2 chickens compared with other genotypes including B2B3 (Caron et al., 1997). Ruff and Bacon (1989) found higher immunity in immunized congenic Lines 15.6-2 (B2B3) and 15.7-2 (B2B3) than in Line 15.151-5 (B2B3). Differences in genetic backgrounds, immunization protocols, and parasite strains are factors that possibly influenced the variable B complex effects.

Cell-mediated responses play a central role in the induction of protective immunity against *E. tenella*. Antibody responses contribute only marginally to protection. Genetic variation in humoral responses following infection with *Eimeria* has not been observed, whereas such variation in T-cell responses has been demonstrated conclusively (Lillehoj and Trout, 1993; Bumstead et al., 1995). While CD4+ T cells have been implicated for their role during primary infection, CD8+ T cells have been defined as key components during secondary infection (Trout and Lillehoj, 1996; Lillehoj, 1998). In addition, CD8+ T lymphocytes have been proven important as transporters of sporozoites to the epithelial sites where they develop, further complicating the understanding of the contribution of these cells to the anti-coccidial immune response (Trout and Lillehoj, 1993). The complex *Eimeria* life cycle changes the parasite surface antigens through the course of an infection increasing the difficulty of determining the anti-coccidial immune response mechanisms (Lillehoj et al., 1989). Antigen recognition differences by specific MHC haplotypes might favor responses defined predominantly by either CD8+ or CD4+ T cells. Furthermore, particular MHC haplotypes confer better innate resistance to infection whereas others mediate better acquired immunity (Clare et al., 1989).

Divergent selection for resistance or susceptibility to *E. tenella* infection altered MHC and non-MHC alloantigen frequencies. Some B haplotypes were unique to either the resistant or the susceptible lines. The selected groups also had significantly different allele frequencies at the A-E and C loci (Johnson and Edgar, 1984; 1986). Martin et al. (1986) found an alloantigen I effect on natural and controlled exposures to cecal coccidiosis in lines selected for antibody response to sheep erythrocytes. The degree of resistance differed among I alleles depending on the background of the high antibody or low antibody responder lines.

These effects suggested that other alloantigens, such as L, might contribute to non-MHC effects on coccidiosis. The L alloantigen is polymorphic with two segregating alleles, L1 and L2, (Gilmour, 1959; Briles, 1962). Chicken erythrocytes as well as leukocytes have L surface proteins (Kopti and Briles, unpublished data). Gilmour (1959) studied alloantigens following 18 generations of full-sib matings from an outbred line. Only three loci, including L, maintained segregation which suggested a possible survival advantage associated with the preservation of heterozygosity. Alloantigen L affected phagocytosis (Qureshi et al., 2000), antibody responses (Medarova et al., 2003), and Rous sarcoma outcome (LePage et al., 2000; Medarova et al., 2002).

Taylor and Briles (2000) found a significant L system effect on resistance to *E. tenella* infection measured by lesion scores in a B2B2 MHC background. The experimental chickens were 50% Wisconsin Line 3 and 50% Line NIU 4 White Leghorns. In the current study, no L effect was observed in birds with a different genetic composition that had full segregation for the B and L alleles. The dissimilar genetic backgrounds may have contributed to the different results in the two experiments. The lack of observable L system effects on either resistance or acquired immunity to *E. tenella* infection in the chickens of this study means that this alloantigen did not influence cells that generate responses to the parasite.

In conclusion, consistent with a previous study (Clare et al., 1985), the B complex affects immunity but not innate resistance to coccidial infection. These effects could be due to differential genetic mechanisms involved in acquired immunity versus innate resistance to the parasite through dissimilar contributions of unique MHC haplotypes. The L system did not affect either innate resistance or acquired immunity to the disease in this study. Other non-MHC background gene effects, although minimized by the matings used, cannot be excluded completely.

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