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Genetic Control of Immunity to *Eimeria tenella*. Interaction of MHC Genes and Non-MHC Linked Genes Influences Levels of Disease Susceptibility in Chickens

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ABSTRACT


The relative importance of MHC genes and background genes in the genetic control of disease susceptibility and the development of protective immunity to *E. tenella* infection was investigated in eight different strains of 1515-B congenic and four inbred chicken strains. RPRL 1515-B congenic chickens that share a common genetic background but express different *B* haplotypes demonstrated wide variations in disease susceptibility and the development of acquired resistance to *E. tenella* infection. Infection of chickens sharing a common *B* haplotype but expressing different genetic backgrounds showed quite contrasting levels of susceptibility to secondary *E. tenella* infection. In all chicken strains examined, infected chickens developed high levels of serum and biliary anti-coccidial antibodies regardless of their *B* haplotypes. Furthermore, no correlation between antibody levels and the phenotypically expressed levels of disease resistance was demonstrated. These findings lend support to the view that interaction of MHC genes and non-MHC genes influences the outcome of host response to *E. tenella* infection.

INTRODUCTION

*Eimeria tenella* is an intestinal protozoan and all strains of chickens tested so far are known to be susceptible to this parasite. In experimental hosts in-
fected per os with *E. tenella*, chickens develop complete, albeit species-specific, protection against subsequent challenge.

Data from previous work indicate that the genetic make-up of individuals plays a significant role in determining susceptibility or resistance to Eimerian infections (Klesius and Hinds, 17979; Rose et al., 1984; Johnson and Edgar, 1986; Martin et al., 1986; Lillehoj and Ruff, 1987). In mammals, multiple genes have been found to influence the protective immune responses against most parasitic helminths (reviewed in Wassom, 1985). Both major histocompatibility complex (MHC) genes and non-MHC linked genes have been shown to influence immunity to *Trichinella spiralis* infection of mice (Wassom et al., 1983; Bell et al., 1984).

In chickens, the *B* locus represents the MHC and, as in mammals, controls immune responses to different antigens (Benedict et al., 1975; Pevzner et al., 1979), disease susceptibility to spontaneous autoimmune thyroiditis (Bacon et al., 1974), regression or progression of the sarcomas induced by Rous sarcoma virus (Collins et al., 1977; Gebriel et al., 1979; Plachy and Benda, 1981), and disease resistance to Marek's disease virus induced tumor (Hansen et al., 1967; Pevzner et al., 1981; Briles et al., 1983).

The relative importance of MHC and background genes in controlling the development of disease resistance to challenge infection in avian coccidiosis has been a topic of controversy (Clare et al., 1985; Johnson and Edgar, 1986; Martin et al., 1986). A previous study in inbred chickens (Clare et al., 1985) suggests that disease susceptibility is determined by genes linked to the *B* complex whereas other workers (Johnson and Edgar, 1986; Martin et al., 1986) suggest that background genes influence disease susceptibility to *E. tenella*. These studies, however, are inconclusive because the inbred strains used were not proven to be different only at the *B* complex genes.

In the present study, we used eight different strains of 15I5-B congenic chickens that are known to differ only with respect to *B* complex genes (Shen et al., 1984; Bacon et al., 1986) to investigate the role of MHC in controlling the development of protective immunity to *E. tenella* infection. The results from our study demonstrate that interaction of MHC genes and background genes influences the outcome of disease susceptibility to coccidiosis following challenge infection.

**MATERIALS AND METHODS**

**Chickens**

Fertile 15I5-B congenic chicken eggs were produced at the Regional Poultry Research Laboratory (RPRL), and shipped to the Animal Parasitology Institute for incubation. Development and the histocompatibility status of RPRL 15I5-B congenic chickens have been previously described (Shen et al., 1984;
Bacon et al., 1986). The RPRL-15I5 inbred line of White Leghorn chickens served as the background line for the development of the B congenic chickens. The inbred background line RPRL-15I5 contributed B15 and inbred lines RPRL-6, -7, -15I4, and Reaseheath line C contributed the B2, B2, B5, and B12 haplotypes, respectively. B13 and B19 haplotypes were derived from line JM-P. To designate a line, the donating line is followed by a period, then a hyphen, and the introduced B haplotype. Serology, skin grafting, and mixed lymphocyte response have verified both the B homozygosity within each line and the line differences for the histocompatibility region of the B complex (Bacon et al., 1986). GHS B13 and GHS B6 congenic eggs were obtained from the Dept. of Animal Science, Iowa State University, IA. GHS chickens differ genetically at the MHC, with GHS B13 and GHS B6 birds having B13/B13 and B6/B6 genotypes, respectively. SC and FP chicken eggs were purchased from Hy-line International Production Center, Dallas Center, IA. All chicken eggs were hatched at the Animal Parasitology Institute and housed in clean wire-floored cages and provided feed and water ad libitum. Four-to-five-week-old birds were used in all experiments.

Parasites

The wild type strain of E. tenella (Lab strain #24) used in the present study was developed from a single oocyst isolation and maintained at the Animal Parasitology Institute, Beltsville, MD. We have also used the WisF125 strain, a precocious strain of E. tenella, in order to investigate the immunogenicity of mutant parasites. The WisF125 strain of E. tenella was provided by one of us (TKJ). Development and characteristics of WisF125 have been previously described (Jeffers, 1975; Johnson et al., 1979).

Initial and challenge infections of chickens

Two independent experiments comprised of 6–12 chickens per group were carried out in order to compare the disease susceptibility and the development of protective immunity to E. tenella infection in eight different strains of 15I5-B congenic and in four inbred chickens. Disease susceptibility following initial infection was assessed on the basis of lesion score (LS), packed cell volume (PCV) and oocyst production. LS and PCV were determined at 5 days post initial inoculation (PPI) with 1×10^5 or 5×10^4 sporulated oocysts of E. tenella (LS #24). Oocyst production was determined on individual chickens by collecting droppings for 4 days starting at 5 days post initial inoculation with 1×10^4 sporulated E. tenella.

Development of protective immunity to coccidiosis in B congenic and inbred chickens was assessed by comparing the oocyst numbers obtained following challenge infection with LS #24 E. tenella. Challenge infection was carried
out at 3 weeks post primary inoculation by giving $1 \times 10^4$ sporulated oocysts (LS #24) per os. In some experiments, groups of chickens were inoculated twice, 1 week apart, with $5 \times 10^4$ oocysts of the precocious strain of *E. tenella* (WisF125). Chickens inoculated with the WisF125 strain were challenged at 5 weeks post primary inoculation with $1 \times 10^4$ *E. tenella* (LS #24) to determine whether the initial inoculation with the WisF125 elicited protective immunity. Oocyst counts were made on fecal samples that were collected for 4 days starting at 6 days post challenge inoculation. Multiple counts of oocysts present in each fecal sample were made using a McMaster's counting chamber.

*Lesion score determinations*

Caeca from chickens inoculated with *E. tenella* were scored for lesion at 5 days post primary inoculation using the previously described method (Johnson and Reid, 1970).

*Packed cell volume (PCV) determinations*

PCV levels were determined at 5 days post primary inoculation in chickens inoculated with $1 \times 10^5$ *E. tenella*. Heparinized microhematocrit tubes were filled to the 100% level directly from the punctured wing vein. The tubes were sealed and spun at 13 000g for 5 min in an International microcapillary centrifuge (International Equipment Co., Boston, MA). PCV levels were read from a standard scale designed for use with microhematocrit tubes.

*Isotope-specific anti-coccidial antibody determination*

Serum IgG and biliary secretory IgA (sIgA) antibody levels of chickens were determined using a previously described enzyme-linked immunosorbent assay (Lillehoj and Ruff, 1987). Briefly, individual serum or bile secretion was obtained from chickens inoculated with $1 \times 10^4$ oocysts of *E. tenella* (LS #24) or $5 \times 10^4$ oocysts of WisF125 at 5 days following challenge inoculation. Serum or bile to be tested was diluted in phosphate buffered saline (PBS) supplemented with 0.1% bovine serum albumin (BSA). Antigen-coated plates were prepared by incubating 50 µl of coccidial antigen (crude oocyst extract, 1 µg/well) diluted in a sodium bicarbonate buffer (pH 9.5) in wells of flat-bottomed immunoplate (Thomas Scientific, Philadelphia, PA). The plates were incubated overnight at 4°C and then washed three times with PBS containing 0.05% Tween 20 (washing buffer). Serial dilutions of sera or bile were prepared in PBS containing 0.1% BSA. The antigen-coated plates were blocked with 10% BSA in PBS for 2 h at room temperature to reduce non-specific binding. Sera or biles to be tested were serially diluted two-fold with starting dilution of 1:8. After 2 h of incubation at room temperature, the plates were washed four times
with the washing buffer. A 50-μl sample of rabbit or goat anti-chicken sera (Miles, Elkhart, IN) specific for heavy chains of chicken IgG and IgA was then added, and plates were incubated for 1 h at 37°C. The plates were then washed four times and incubated with 50 μl biotin conjugated anti-rabbit or goat anti-serum (Sigma, St. Louis, MO). After 30 min of incubation at 37°C, the plates were washed and incubated with 50 μl of Streptavidin-peroxidase (Zymed, San Francisco, CA). The enzyme reaction was initiated by the addition of ortho-phenylene-diamine dihydrochloride dissolved in a 0.05 M citrate phosphate buffer containing 0.03% fresh H₂O₂. Absorbance was read at 450 nm with a multi-channel spectrophotometer 30 min after initiating enzymatic reaction (Titertek Multiskan, Flow Laboratories, McLean, VA).

Statistical analysis

The mean and standard deviations were determined for all experiments. Statistical analyses among groups were performed using a one-way analysis of variance (Freund and Littell, 1981) to determine overall significance. Statistical significance was determined at $P < 0.05$. Spearman’s coefficient of rank correlation ($\gamma$) was used to determine whether there was a direct relationship between two variables.

RESULTS

Determination of an optimal inoculating dose for oocyst production

In order to determine an optimal parasite dose to use to compare oocyst production in different strains of chickens, six independent groups of 4-week-old SC chickens were inoculated with different doses of E. tenella ranging from $10^1$ to $10^5$ oocysts. Fig. 1 shows that the number of oocysts produced by chickens varied widely depending upon the parasite dose. Chickens inoculated with $10^3$ or $10^4$ oocysts consistently showed the highest oocyst production whereas chickens inoculated with parasite doses of greater or lesser numbers produced significantly less oocysts ($P < 0.01$).

Strain differences in oocyst production following initial inoculation with E. tenella

In order to compare oocyst production in 15I5-B congenic and inbred chicken strains, 4-week-old chickens of each group were inoculated with E. tenella (LS #24). Since an inoculating dose of $10^4$ oocysts gave optimal oocyst production in SC chickens (Fig. 1), we used this dose for comparing oocyst production in the following experiments. Fig. 2 shows the average oocyst production obtained from two independent experiments. Genetically different chicken strains
Fig. 1. Effect of inoculation parasite dose of *E. tenella* on oocyst production in SC chickens. Six, 4-week-old SC chickens were inoculated per os with different doses of sporulated oocysts of *E. tenella* (LS #24) ranging from $1 \times 10^1$ to $1 \times 10^5$. Oocyst production was assessed in individual chickens from fecal droppings collected for 4 days starting at 5 days PPI. Each bar represents an average oocyst production of six chickens.

<table>
<thead>
<tr>
<th>LINE</th>
<th>N</th>
<th>OOCYST NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-21</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>C-12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>6-2</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>P-13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>7-2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>P-19</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>15-5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>15I5-15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>GHS B13</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>GHS B 6</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Strain differences in oocyst production following initial infection with *E. tenella*. Each strain of chickens was inoculated with $1 \times 10^4$ sporulated oocysts of *E. tenella*. Oocyst counts were made from the individual fecal droppings collected for 4 days beginning at 5 days PPI. Two independent experiments were carried out using two different groups of each chicken strain of the same age hatched separately.
showed wide variations in oocyst production following initial infection. In general, .7-2 and .N-21 chickens produced the least number of oocysts, whereas strains FP (B15B21), .C-12, GHS B6, .P-13, SC, .6-2 and 15I5 produced substantially higher numbers of oocysts compared to other strains of chickens (P<0.05). Strains that share the same B haplotype but express different genetic backgrounds showed significantly different levels of response as seen in .7-2, .6-2 and SC (B2B2) chickens (P<0.05).

Lesion score (LS) and packed cell volume (PCV) determination following initial inoculation with E. tenella

Chickens infected with E. tenella show other clinical signs of coccidiosis such as caecal lesions and loss in PCV that, along with oocyst production, have been used to assess disease susceptibility. To compare LS and PCV levels in congeneric and inbred chickens, we inoculated chickens with either 1×10⁵ (Experiment 1) or 5×10⁴ (Experiment 2) sporulated oocysts of E. tenella. These inoculating doses were predetermined to give measurable clinical signs such as caecal lesions and reductions in PCV (Lillehoj and Ruff, 1987). Table 1 shows average LS and percent reductions in PCV level obtained from two independent experiments. FP chickens showed significantly higher LS compared to the others (P<0.05). In general, .7-2, .N-21, .P-19, GHS B13, GHS B6 and SC chickens showed substantially lower LS whereas FP, .15I-5, .C-12, .P-13 and .6-2 depicted higher LS compared to the others. When PCV levels were compared among different strains tested, .7-2, .N-21, and .P-19 chickens gave minimum reduction in PCV level whereas SC, FP and GHS B6 chickens showed a greater reduction in PCV level compared to the others. In general, wide variations in LS and PCV level were seen among different genetic strains tested and the oocyst numbers did not correlate with LS (r=0.21) or PCV level (r=0.41).

Strain differences in oocyst production following challenge inoculation with E. tenella

In order to compare oocyst production among different strains of chickens following challenge inoculation, two groups of each chicken strain were inoculated with either a precocious strain, WisF125, or wild type strain, LS #24. The two different E. tenella strains were used to compare their immunogenicity. A challenge inoculation with LS #24 E. tenella was given at 3 weeks following initial inoculation since previous work (Lillehoj, 1986) showed that chickens develop good protective immunity starting at 3 weeks following initial inoculation.

Average oocyst numbers obtained from two independent experiments after challenge infection are shown in Fig. 3. In general, all chickens, regardless of
TABLE 1

Lesion score (LS) and packed cell volume (PCV) determinations following initial inoculation with *E. tenella*

<table>
<thead>
<tr>
<th>Chicken strain</th>
<th>Number of chickens</th>
<th>LS* ± SD</th>
<th>PCV* (% reductionb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 1</td>
<td>Exp. 2</td>
<td>Exp. 1</td>
</tr>
<tr>
<td>.7-B²</td>
<td>5</td>
<td>5</td>
<td>1.6 ± 1.1b</td>
</tr>
<tr>
<td>.N-B²¹</td>
<td>4</td>
<td>5</td>
<td>2.4 ± 0.1a,b</td>
</tr>
<tr>
<td>.15I-B⁵</td>
<td>5</td>
<td>5</td>
<td>2.6 ± 0.6a,b</td>
</tr>
<tr>
<td>.C-B¹²</td>
<td>4</td>
<td>5</td>
<td>2.8 ± 0.5a,b</td>
</tr>
<tr>
<td>.P-B¹⁹</td>
<td>7</td>
<td>5</td>
<td>2.6 ± 0.5a,b</td>
</tr>
<tr>
<td>.P-B¹³</td>
<td>5</td>
<td>5</td>
<td>3.0 ± 0.8a,b</td>
</tr>
<tr>
<td>.6-B²</td>
<td>5</td>
<td>5</td>
<td>3.0 ± 1.0a,b</td>
</tr>
<tr>
<td>GHS B⁶</td>
<td>7</td>
<td>5</td>
<td>2.9 ± 1.0a,b</td>
</tr>
<tr>
<td>GHS B¹³</td>
<td>7</td>
<td>5</td>
<td>2.5 ± 0.3a,b</td>
</tr>
<tr>
<td>SC</td>
<td>4</td>
<td>5</td>
<td>2.8 ± 0.5a,b</td>
</tr>
<tr>
<td>FP</td>
<td>4</td>
<td>5</td>
<td>3.2 ± 0.5a</td>
</tr>
</tbody>
</table>

a LS and PCV were carried out at 5 days post primary infection.
Exp. 1: inoculating dose of 1 x 10⁵ was used.
Exp. 2: inoculating dose of 5 x 10⁴ was used.
b Reduction = 100 × (1 - PCV of inoculated chicken / PCV of uninoculated chicken)
c, d, e Values within columns not sharing a common superscript letter are significantly different by ANOVA (SAS) at P<0.05.

...their B haplotypes, produced substantially fewer oocysts after challenge inoculation than after initial inoculation (P < 0.05). Eight different strains of 15I₅-B congenic chickens that share a common genetic background but express different B haplotypes demonstrated wide variations in oocyst production. Strains FP (B₁⁵B²¹), .15-5, GHS-B¹³ and .P-19 showed minimal or no oocyst production, whereas .7-2, .P-13, .6-2 and 15I⁵ showed a substantially higher number of oocysts when compared to other strains following challenge inoculation with 10⁴ sporulated *E. tenella* (P < 0.05). .N-21, .C-12 and SC (B²²B²) chickens demonstrated an intermediate level of oocyst production.

Interestingly, FP (B₁⁵B²¹), SC(B²²B²) and .C-12 were more resistant than others to challenge infection although they were highly susceptible to the initial infection. In contrast 15I₅, .6-2 and .P-13 were susceptible to both initial and challenge infections.

When oocyst numbers obtained from two different groups of each different strain of chickens that were given initial inoculation of *E. tenella* LS #24 or WisF125 were compared, no substantial difference (one exception was .15I-5...
Fig. 3. Strain differences in oocyst production following challenge infection with *E. tenella*. Results of two independent experiments (exception is 15I3-5 chickens; results of only one experiment are shown). Chickens of different strains were inoculated with 1\(\times\)10^4 oocysts of *E. tenella* (LS #24) or with two, 5\(\times\)10^4 injections of WisF125 given 1 week apart. Challenge infection with LS #24 was given at 3 weeks PPI. Oocyst counts were made on individual samples collected for 4 days starting at 6 days PSI.

chickens) was noted, suggesting that WisF125 strain is as immunogenic as the *E. tenella* LS #24 strain.

Strains of chickens sharing a common *B*-haplotype but expressing different genetic backgrounds (.6-2, .7-2 and SC) showed different levels of oocyst production following challenge infection; .7-2 and .6-2 chickens produced significantly higher number of oocysts when compared to SC chickens (*P* < 0.05). Similarly, GHS-B^13 and .P-13 demonstrated a quite contrasting response to challenge infection (*P* < 0.05).

*Serum IgG and biliary sIgA response in different chicken strains*

In order to compare anti-coccidial antibody responses of different chicken strains, immune serum samples and bile secretions obtained following challenge infection were tested for coccidial specific antibodies. Table 2 shows average antibody titers of different strains of chickens obtained from two independent experiments. Variations in antibody response were demonstrated not only in serum IgG levels but also in biliary sIgA levels. All infected chickens produced high levels of IgG and sIgA antibodies regardless of their *B* haplotypes. Strains .7-2, .N-21, .6-2 and .P-19 inoculated with LS #24 *E. tenella* produced higher IgG levels than other strains. Comparing sIgA levels, .N-21 and .P-13 produced higher levels of antibody than other chickens. The levels
### TABLE 2

Serum and biliary antibody responses following challenge inoculation

<table>
<thead>
<tr>
<th>Chicken strain*</th>
<th>Log₂ titer ± SDb</th>
<th>Bile IgA</th>
<th></th>
<th>Log₂ titer ± SDb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum IgG</td>
<td>WisF125</td>
<td>LS # 24</td>
<td></td>
</tr>
<tr>
<td>N-B21</td>
<td>9.8 ± 1.9bc,c</td>
<td>n.d.</td>
<td>10.0 ± 0.1a</td>
<td>n.d.</td>
</tr>
<tr>
<td>C-B12</td>
<td>7.9 ± 2.5bc,c</td>
<td>7.1 ± 2.7a</td>
<td>10.0 ± 0.7ab,c</td>
<td>10.5 ± 0.7a</td>
</tr>
<tr>
<td>6-B2</td>
<td>8.3 ± 1.7d</td>
<td>8.3 ± 3.1a</td>
<td>7.0 ± 0.2bc</td>
<td>11.0 ± 0.5a</td>
</tr>
<tr>
<td>P-B13</td>
<td>9.3 ± 1.5bc,c</td>
<td>8.6 ± 2.9bc</td>
<td>10.0 ± 0.1b</td>
<td>8.0 ± 1.4a</td>
</tr>
<tr>
<td>7-B2</td>
<td>8.7 ± 2.4ab</td>
<td>8.7 ± 1.7ab</td>
<td>11.0 ± 0.7ab,c</td>
<td>10.0 ± 0.4a</td>
</tr>
<tr>
<td>P-B19</td>
<td>7.8 ± 2.1ab</td>
<td>6.8 ± 2.1ab,c</td>
<td>11.5 ± 1.4ab,c</td>
<td>9.0 ± 2.8a</td>
</tr>
<tr>
<td>15I-B5</td>
<td>7.8 ± 0.8ab</td>
<td>8.8 ± 1.1ab</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>SC</td>
<td>6.2 ± 1.5d,e</td>
<td>7.6 ± 2.0a</td>
<td>8.0 ± 2.8bc,e</td>
<td>7.0 ± 0.4a</td>
</tr>
<tr>
<td>FP</td>
<td>6.8 ± 0.3a</td>
<td>7.8 ± 0.1a</td>
<td>12.5 ± 0.7ab,c</td>
<td>10.5 ± 0.7a</td>
</tr>
<tr>
<td>GHS B51</td>
<td>7.5 ± 2.1bc,c</td>
<td>n.d.</td>
<td>10.0 ± 1.4ab,c</td>
<td>n.d.</td>
</tr>
<tr>
<td>GHS B6</td>
<td>7.8 ± 0.5ab</td>
<td>n.d.</td>
<td>5.5 ± 0.7c</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*Number of animals used in each group is shown in Fig. 2.

bSerum and bile secretions were obtained from chickens at 7–10 days following challenge inoculation. Initial infection was carried out using either LS # 24 or WisF125 strain of *E. tenella*. Challenge infection with LS # 24 was given at 5 weeks PPI. Mean antibody titers of two independent experiments are shown.

cValues within columns not sharing a common superscript letter are significantly different by ANOVA (SAS) at P < 0.05.


of protection against challenge infection did not correlate with either the IgG antibody titers (r = 0.29) or the sIgA antibody titers (r = 0.59). In general, WisF125 and LS # 24 strains of *E. tenella* elicited similar levels of antibody responses in different strains of chickens, again suggesting that these parasite strains have comparable immunogenicity.

### DISCUSSION

The relative importance of MHC genes and background genes in controlling the disease susceptibility to coccidiosis has been a topic of controversy. The results of the present study suggest that both MHC genes and genes within the genetic background control the phenotypically expressed levels of disease susceptibility or resistance characteristic of a given genotype. Eimerian parasites are characterized by complex life cycles and each stage of the *Eimeria* species is known to possess many different antigens (Wisher, 1986; Jenkins and Dame, 1987). Since individual chicken strains may differ genetically in their ability
to respond to each one of these antigens, it is anticipated that multiple genes would influence the host immunity against *Eimeria* infection.

Wide variations in host response to *E. tenella* infection were demonstrated when different chicken strains were inoculated with oocysts. We used multiple parameters to assess disease susceptibility including lesion score (LS), packed cell volume (PCV) and oocyst production. Strains consistently showing higher susceptibility than others on the basis of oocyst production were 15I₅, FP, .6-2 and .P-13. Strains .7-2 and .N-21 were least susceptible on the basis of LS and oocyst production. Different parameters used to assess susceptibility did not always correlate with each other. Oocyst production in general gave a consistent result although there were substantial individual variations (data not shown).

Different strains of chickens secondarily challenged with *E. tenella* demonstrated wide variations in levels of oocyst production. In general, all chickens produced less oocysts following challenge infection than the initial infection. This is probably due to the development of protective immunity to coccidiosis. In general, strains showing development of protective immunity after initial infection with LS ≠ 24 strain of *E. tenella* also developed protective immunity against coccidiosis when the WisF125 strain was used in the initial inoculation. This result suggests that WisF125 is as immunogenic as wild type strain. Although WisF125 strain has a defective second generation schizony (Jeffers, 1975), previous studies (Jeffers, 1978; Johnson et al., 1979) and the present study suggest that this mutant strain has intact immunogenicity to elicit protective host responses.

One major impeding factor in studying the role of MHC genes in coccidiosis has been the lack of the availability of genetically well-defined chicken strains. In this study we used 15I₅-B congenic chickens having different B complex genes but expressing the same background genes in order to minimize the effect of genetic background “noise”. In addition, we examined several inbred chicken strains that share the same B haplotypes as some of the 15I₅-B congenic chickens, but express different genetic backgrounds, in order to investigate the role of MHC linked and non-MHC linked genes in controlling disease susceptibility to coccidiosis. Fig. 3 shows that although 15I₅ chickens (background line) were highly susceptible to reinfection, introduction of *B¹⁹* or *B¹²* genes to the 15I₅ genetic background resulted in modification of the level of disease susceptibility. When we compared the disease susceptibility of two chicken strains that share the same B haplotype but express different genetic backgrounds, their responses were quite different. Strain .P-13 chickens were highly susceptible to reinfection whereas GHS *B¹⁵* chickens were not. Similarly .6-2, .7-2 and SC chickens demonstrated different levels of protective immunity. These results strongly suggest that the intricate interaction of MHC genes and non-MHC genes influences the outcome of host response to coccidiosis after a challenge inoculation.
The results of the previous studies on the role of host genes in controlling disease susceptibility to coccidiosis in inbred chickens have suggested an important role for the MHC genes (Clare et al., 1985), as well as the role of background genes (Johnson and Edgar, 1986; Martin et al., 1986) in controlling anti-coccidial responses. The results of the present study suggest that genes within the genetic background play an important role in controlling the development of protective host immune responses to *E. tenella* infection.

Recent studies suggest that resistance to coccidia in subsequent infections is a function of cell-mediated immunity (Rose and Hesketh 1984; Lillehoj, 1987) although a role for humoral factors has not been excluded (Rose, 1974; Abu Ali et al., 1976). When serum and biliary anti-coccidial responses were assessed in infected inbred and congenic chickens, there was no direct correlation between levels of antibody production and the levels of protective immunity. Furthermore, .6-2, .7-2 and .P-13 chickens produced high levels of serum IgG and biliary slgA even though these chickens were highly susceptible to a challenge infection. This result further suggests that antibodies do not play a major role in protective immunity against coccidiosis.

Immunological mechanisms eliciting protective response to *Eimeria* are not well understood at present. Studies of chicken strains such as 15I5, .C-12, .6-2 and .P-13 that are highly susceptible to *E. tenella* infection will be very beneficial because the analysis of immunological determinants of genetic susceptibility will be possible in these chicken strains. In this respect, it is interesting that the peritoneal macrophages of .C-12 and 15I5 chickens have been shown to exhibit very low phagocytic activity and .P-13 macrophages were found to be much less efficient in killing ingested bacteria than other 15I5-B congenic chickens (Qureshi et al., 1986). Preliminary flow cytometric analysis of spleen lymphocytes in these strains of chickens suggests that highly susceptible strains of chickens have fewer cells expressing a mature T cell marker when examined at 1 week of age (Lillehoj, 1988). Furthermore, levels of disease resistance to *E. acervulina* infection in 15I5-B congenic chickens correlate with the T cell but not with the antibody response to a recombinant merozoite antigen, p150 (Lillehoj et al., 1988). Definitive proof that the enhanced disease susceptibility to *E. tenella* infection is due to a defective cell-mediated immunity (CMI) needs further investigation.

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