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Differential Resistance to Staphylococcus aureus Challenge in Major Histocompatibility (B) Complex Congenic Lines

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Differential Resistance to Staphylococcus aureus Challenge in Major Histocompatibility (B) Complex Congenic Lines

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ABSTRACT
Ten inbred B-congenic Leghorn lines were challenged with two isolates of Staphylococcus aureus at 3 days and 6 wk of age. Significant differences in mortality were observed among such lines when challenged at 3 days with either S. aureus Isolate P4L (moderately pathogenic) or S. aureus Isolate 3727 (highly pathogenic). Line 331 (B2/B2 genotype) had lower mortality than either Line 004 (B17/B17, \( \chi^2 = 4.13, P < .05 \)) or Line 253 (B18/B18, \( \chi^2 = 4.23, P < .05 \)) challenged with Isolate P4L. The use of a susceptibility index allowed for the detection of additional differences among the various lines challenged by Isolate 3727. Line 336 (BQ/BQ) was more resistant than either Line 335 (B19/B19, \( P < .01 \)) or Line 330 (B21/B21, \( P < .01 \)). No significant differences were found among the lines challenged at 6 wk by either isolate. The results provide additional evidence for the importance of the B complex in genetically determined disease resistance, and further demonstrate the usefulness of congeneric lines in such investigations. (Key words: B complex, congeneric chickens, Staphylococcus aureus, disease resistance, age)

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INTRODUCTION
The importance of the B complex (chicken MHC) to immune function and disease resistance is well known. Association between Rous sarcomas, coccidiosis, and other diseases has been reported (reviewed in Gavora, 1990). Resistance to fowl cholera caused by Pasteurella multocida infection was mapped to the B-G subregion of the chicken MHC (Lamont et al., 1987). On the other hand, little published information is available concerning specific B complex haplotypes and resistance to challenge with Staphylococcus aureus. Superior resistance to S. aureus was consistently found in Line SC chickens (genotype B2/B2) compared with another homozygous Leghorn line (genotype B14/B14), but because these lines differed at other genetic loci, the observed resistance could not be ascribed solely to an effect of the B complex (R. F. Smith, Department of Microbiology and Environmental Health, Colorado State University, Fort Collins, CO 80523, personal communication).
The recent development of a number of congenic lines having a common highly inbred genetic background (Line UCD 003) and differing in their MHC (Abplanalp et al., 1992) has afforded an opportunity to make comparisons between the relative degree of disease resistance provided by specific B complex haplotypes. Therefore, the purpose of the present study was to observe the resistance in such congenic lines after challenge by two isolates of *S. aureus*; each known to be pathogenic in chickens (Cotter and Taylor, 1991).

**MATERIALS AND METHODS**

**Chickens**

Inbred congenic lines were established by outcrossing single hens of Line UCD 003 (B17/B17) to males from source lines carrying different B complex haplotypes. This mating was followed by repeated backcrosses to Line UCD 003. Each resulting congenic line was estimated to contain 98.4% of the genetic background of Line UCD 003. The present study used a subline of Line 003 (004, B17/B17) that had been separated from Line 003 for the two preceding generations. Thus, all congenic lines used in the current study possess B complex haplotypes as the differential genes with the exception of Line UCD 317, which has the *dw* allele as the differential gene. A detailed description of the breeding program and the various sublines has been reported (Abplanalp et al., 1992).

The chicks were hatched at Davis, California and air-shipped to the University of New Hampshire where all challenge experiments were conducted. Three-day-old chicks were placed in isolation units. Older chickens (6 wk) were housed in rows of wire cages at a density of three to five per unit, and were observed daily for morbidity and mortality for 3 wk following the challenge. The numbers of chicks used in each trial are shown in Table 1. All chickens were fed commercial diets and water for *ad libitum* consumption until the termination of the trials at 21 days posthatch. The diets used for both age groups were similar and contained 20% crude protein and 2,860 kcal ME/kg.

**Bacteria and Challenge**

Two isolates of *S. aureus* known to be pathogenic in chickens were used. The origin and the properties of each isolate have been described elsewhere. Briefly, Isolate P4L is of moderate pathogenicity, whereas Isolate 3727 is usually more pathogenic in neonatal chicks or in older chicks challenged through 12 wk of age (Cotter and Taylor, 1991).

Dosages were formulated by further dilution of log phase brain-heart infusion (BHI) broth cultures of each isolate. The turbidity of a Number 1 MacFarland standard was used as a guide so that the resulting dosages were estimated to be $3 \times 10^7$ and $3 \times 10^8$ cfu per chick for the 3-day-old and 6-wk-old groups, respectively.

Intracardiac challenge of the 3-day-old chicks was accomplished by using a volume of .1 mL of the diluted broth cultures delivered by a tuberculin-type syringe fitted with .5 in (1.27-cm)-26 gauge needle.

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2BBL, Bethesda, MD 20814.
3Difco Laboratories, Detroit, MI 48232.
TABLE 2. Analysis of variance for Staphylococcus aureus susceptibility index for all experimental chickens

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td>9.8</td>
<td>4.9</td>
<td>.027</td>
</tr>
<tr>
<td>Line</td>
<td>9</td>
<td>6.3</td>
<td>3.1</td>
<td>.001</td>
</tr>
<tr>
<td>Bacterial isolate</td>
<td>1</td>
<td>13.0</td>
<td>6.5</td>
<td>.011</td>
</tr>
<tr>
<td>Error</td>
<td>594</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The presence of blood in the syringe barrel was confirmed immediately prior to each injection, thus assuring successful penetration of the heart chamber. Intravenous challenges of 6-wk-old chicks were made by using the wing vein as access, and a 1 mL volume of log phase cultures appropriately diluted with BHI broth. All chickens were checked daily for the development of characteristic symptoms, including acute prostration, recumbency, and lameness as described (Smith, 1954; Cotter and Taylor, 1991).

Statistical Analyses

Neonatal mortality was analyzed by pairwise chi-square tests between congenic lines having a common genetic background (UCD Line 003). Line 317 contains the dwarf (dw) allele, therefore it was compared with Line 004 only, having the same $B^{17}$ haplotype.

An index value for the severity of symptoms seen in each chick was constructed as follows: 1 = asymptomatic; 2 = morbidity for 1 or 2 days; 3 = morbidity greater than 2 days; 4 = mortality 8 to 14 days postchallenge; 5 = mortality 1 to 7 days postchallenge. The susceptibility index values obtained for each line were subjected to ANOVA followed by pairwise contrasts between lines as stated above.

RESULTS

There was a rapid onset of morbidity in the neonates beginning at 24 h postchallenge, with mortality appearing shortly thereafter. Mortality usually peaked within the 1st wk, but extended into the 2nd wk postchallenge in some cases. The susceptibility index value, which incorporates both morbidity and mortality, was assigned to each chick using the scale described above. The ANOVA for the susceptibility index for all lines, both ages, and both bacterial isolates is given in Table 2.

Death of the neonates was caused by a patent septicemia as evidenced by their grossly enlarged livers and spleens containing necrotic foci. Cardiac blood specimens from moribund chicks consistently yielded coagulase-positive $S$. aureus. Septicemia occurred in older chicks as well, but more often these demonstrated lameness alone, or lameness followed by recumbency. These chicks were killed by cervical dislocation and were considered as specific mortality.

The total mortality for all sublines inoculated with Isolate P4L was 15% whereas those chicks inoculated with Isolate 3727 at 3 days had 23% mortality (Table 1). Six-week-old chicks inoculated with Isolate P4L had 4% mortality. There was 10% mortality in chicks of the same age inoculated with Isolate 3727.

The results of P4L challenge for 3-day-old chicks is shown in Table 3. Data for Trials 1 and 2 were pooled after a contingency chi-square test for a trial effect ($\chi^2 = 2.94, .10 > P > .05$). This allowed a better opportunity to observe haplotype-associated mortality differences. In Table 3, the column headed $\chi^2$ refers to the results of a test for a difference in mortality between that particular line (genotype) and each of the other genotypes. This analysis showed that genotype $B^2/B^2$ had significantly lower mortality than genotypes $B^{17}/B^{17}$ or $B^{18}/B^{18}$; $\chi^2 = 4.13$ and 4.23, respectively ($P < .05$). No other statistically significant mortality differences were detected among the remaining genotypes. The susceptibility indices showed that $B^24/B^24$ had significantly lower values than either $B^{17}/B^{17}$ or $B^{21}/B^{21}$. In addition, $B^2/B^2$ had a
TABLE 3. Mortality and susceptibility indices for chicks challenged at 3 days of age with *Staphylococcus aureus* Isolate P4L, Trials 1 and 2 combined

<table>
<thead>
<tr>
<th>Line</th>
<th>Mortality</th>
<th>Percentage</th>
<th>$\chi^2$</th>
<th>Index</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>331</td>
<td>$B^2/B^2$</td>
<td>1/37</td>
<td>3</td>
<td>17*, 18*</td>
<td>1.4</td>
</tr>
<tr>
<td>312</td>
<td>$B^{24}/B^{24}$</td>
<td>3/37</td>
<td>8</td>
<td>NS</td>
<td>1.6</td>
</tr>
<tr>
<td>313</td>
<td>$B^3/B^3$</td>
<td>3/29</td>
<td>10</td>
<td>NS</td>
<td>1.8</td>
</tr>
<tr>
<td>335</td>
<td>$B^{19}/B^{19}$</td>
<td>5/38</td>
<td>13</td>
<td>NS</td>
<td>1.9</td>
</tr>
<tr>
<td>254</td>
<td>$B^{15}/B^{15}$</td>
<td>3/20</td>
<td>15</td>
<td>NS</td>
<td>1.9</td>
</tr>
<tr>
<td>336</td>
<td>$BQ/BQ$</td>
<td>3/14</td>
<td>21</td>
<td>NS</td>
<td>1.9</td>
</tr>
<tr>
<td>330</td>
<td>$B^{21}/B^{21}$</td>
<td>9/29</td>
<td>21</td>
<td>NS</td>
<td>2.2</td>
</tr>
<tr>
<td>264</td>
<td>$B^{17}/B^{17}$</td>
<td>8/32</td>
<td>25</td>
<td>NS</td>
<td>2.3</td>
</tr>
<tr>
<td>253</td>
<td>$B^{18}/B^{18}$</td>
<td>4/11</td>
<td>36</td>
<td>NS</td>
<td>2.3</td>
</tr>
<tr>
<td>317</td>
<td>$B^{17}/B^{17}dw$</td>
<td>6/22</td>
<td>27</td>
<td>NS</td>
<td>2.4</td>
</tr>
</tbody>
</table>

1 B haplotype

*P < .05.

**P < .01.

significantly lower susceptibility index than $B^{21}/B^{21}$.

The mortality and the susceptibility indices for 3-day-old chicks challenged by the highly pathogenic Isolate 3727 are given in Table 4. Total mortality in genotype $B^{18}/B^{18}$ was significantly lower than that for genotype $B^{21}/B^{21}$, ($\chi^2 = 4.52$, $P < .05$). The mortality obtained in genotype $BQ/BQ$ was also significantly lower than that for either genotypes $B^{21}/B^{21}$, or $B^{19}/B^{19}$ ($\chi^2 = 5.34$ and 3.88, respectively; $P < .05$). Additional susceptibility differences between the genotypes were detected through the use of the susceptibility indices. Both $BQ/BQ$ and $B^3/B^3$ were significantly lower than $B^{15}/B^{15}$, $B^{19}/B^{19}$, $B^{21}/B^{21}$, and $B^{24}/B^{24}$. The $B^{17}/B^{17}$ chickens had significantly lower susceptibility indices than $B^{17}/B^{17}dw$, $B^{19}/B^{19}$, and $B^{21}/B^{21}$ chickens. Finally, $B^{18}/B^{18}$ was lower than $B^{19}/B^{19}$.

Few deaths and low morbidity were observed in 6-wk-old chicks challenged by either isolate; therefore the susceptibility index alone was used for making comparisons among the different genotypes. These data are given in Table 5, which shows the

TABLE 4. Mortality and susceptibility indices for chicks challenged at 3 days of age with *Staphylococcus aureus* Isolate 3727, Trial 1

<table>
<thead>
<tr>
<th>Line</th>
<th>Mortality</th>
<th>Percentage</th>
<th>$\chi^2$</th>
<th>Index</th>
<th>Probability1</th>
</tr>
</thead>
<tbody>
<tr>
<td>336</td>
<td>$BQ/BQ$</td>
<td>0/13</td>
<td>0</td>
<td>19**, 21*</td>
<td>1.3</td>
</tr>
<tr>
<td>313</td>
<td>$B^3/B^3$</td>
<td>1/12</td>
<td>6</td>
<td>NS</td>
<td>1.3</td>
</tr>
<tr>
<td>204</td>
<td>$B^{17}/B^{17}$</td>
<td>2/18</td>
<td>11</td>
<td>NS</td>
<td>1.5</td>
</tr>
<tr>
<td>253</td>
<td>$B^{18}/B^{18}$</td>
<td>1/19</td>
<td>5</td>
<td>21*</td>
<td>1.8</td>
</tr>
<tr>
<td>331</td>
<td>$B^2/B^2$</td>
<td>6/20</td>
<td>30</td>
<td>NS</td>
<td>2.2</td>
</tr>
<tr>
<td>312</td>
<td>$B^{24}/B^{24}$</td>
<td>4/20</td>
<td>20</td>
<td>NS</td>
<td>2.3</td>
</tr>
<tr>
<td>254</td>
<td>$B^{15}/B^{15}$</td>
<td>2/12</td>
<td>17</td>
<td>NS</td>
<td>2.4</td>
</tr>
<tr>
<td>317</td>
<td>$B^{17}/B^{17}dw$</td>
<td>8/20</td>
<td>40</td>
<td>NS</td>
<td>2.5</td>
</tr>
<tr>
<td>330</td>
<td>$B^{21}/B^{21}$</td>
<td>9/20</td>
<td>45</td>
<td>NS</td>
<td>2.6</td>
</tr>
<tr>
<td>335</td>
<td>$B^{19}/B^{19}$</td>
<td>7/20</td>
<td>35</td>
<td>NS</td>
<td>2.9</td>
</tr>
</tbody>
</table>

1 Probability of susceptibility index difference by ANOVA.

2 B haplotype.

*P < .05.

**P < .01.
relative rank for each line or genotype and for the two bacterial isolates as well. No statistically significant differences were detected among the genotypes challenged at 6 wk, presumably due to the small numbers of chicks available for that part of the study.

**DISCUSSION**

The results of the present study suggest that resistance to *S. aureus* challenge is influenced by different B complex haplotypes. They further show that certain alleles provided superior protection when compared with that provided by other alleles. Some alleles had lower mortality whereas others provided a degree of protection that could be detected only through the use of the susceptibility index. Line 336, containing haplotype B_Q, which was derived from the Red Jungle Fowl, showed superior resistance to both isolates, and at either age tested. This line was among the most resistant at 6 wk and at 3 days. High resistance of B_2 and B_Q congenics was also found to three strains of Marek's disease (Abplanalp et al., 1984). Conversely, Line 330, containing haplotype B_21, was highly susceptible to Isolate 3727 and moderately susceptible to Isolate P4L. Haplotype B_21 consistently provides superior resistance to Marek's disease (Bacon, 1987).

Lines 253 and 254, containing haplotypes B_18 and B_15, respectively, were moderately susceptible at either age and to both isolates. Some lines showed inconsistent behavior, however; Line 004 was moderately resistant to the more pathogenic isolate (3727) at 3 days, but was highly susceptible to the usually less pathogenic Isolate P4L. Line 313 was usually seen as resistant, but ranked near the bottom when challenged at 6 wk by Isolate 3727. These observations may not be unlike the genotype by challenge dose interactions described by Lamont et al. (1987) in the *Pasteurella multocida* system.

Line 004, containing haplotype B_17, could be considered only moderately susceptible. When the same B_17 haplotype is placed in a congenic background with another gene, a different picture emerges. Line 317, containing the dw allele, was highly susceptible. This observation suggests the existence of other resistance or susceptibility genes that might map to non-MHC loci, and is reminiscent of the results in the Rous sarcoma system reported by Gebriel and Nordskog (1983). Those authors found that cellular resistance (tv) genes mapped separately from the tumor regression (rs) genes, which were linked to the B complex.

A complete explanation for the observed B complex-associated resistance to *S. aureus* must await a better understanding of the immunological principles involved in staphylococcal disease. Marrack and Kappler (1990) have found that inherited resistance to staphylococci is as-

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1. Least symptomatic = lowest rank.
2. 5 indicates a tie in ranking between two sublines.
associated with the MHC in mice and humans; the present results show that this is also the case in chickens. Moreover, Miller et al. (1990) have found that antigens similar to those expressed by the B-G subregion of the chicken MHC are expressed in intestinal epithelial tissue. These authors suggested that such intestinal expression of B-G genes may serve some immune recognition function, and perhaps protection. A protective function may explain why a high intestinal carriage of pathogenic staphylococci was found in commercial layers (Cotter and Taylor, 1987) without causing any symptoms.

The results using congenic Leghorn lines extends and explains earlier observations of the existence of genetic resistance to staphylococci in New Hampshire chickens, which was probably also related to the MHC (Cotter and Taylor, 1991). The association of varying degrees of resistance with particular haplotypes, as shown in the current study, provides additional evidence for the importance of the B complex in staphylococcal immunity, and further demonstrates the utility of congenic chickens in investigations of infectious disease.

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REFERENCES


