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IMMUNOLOGY

Differential Resistance to Staphylococcus aureus Challenge in Two Related Lines of Chickens

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ABSTRACT

Trials were conducted to determine whether differential resistance to challenge with Staphylococcus aureus was characteristic of two related lines of New Hampshire chickens differing genetically in size of the bursa of Fabricius. Neonatal small bursa line (SBL) chicks were superior to the unselected Lester J. Dreesen (LJD) line in five of six trials employing intracardiac challenge ($\chi^2 = 6.3, 0.05 > P > 0.01$). Older (7 to 12 wk) SBL chicks, challenged intravenously, had superior resistance than Line LID chicks in three of four trials where a direct comparison was possible. The mortality rate in Line SBL was 34% in all trials compared with a 54% mortality rate ($\chi^2 = 11.7, P<0.001$) in Line LID. Moreover, the development of morbidity was more rapid in Line LID. It is suggested that these lines can be of use in investigations of the nature of resistance to staphylococcal disease.

(Key words: Staphylococcus aureus, bursa of Fabricius, challenge, mortality rate, resistance)

INTRODUCTION

Losses due to staphylococcal infections have long been of concern to poultry producers (Smith, 1954). Vaccines have not proved beneficial, and the wide distribution of these bacteria rules out eradication as a means for its control (Gross, 1984). Antibiotics may sometimes be useful, but inclusion of low levels of penicillin in diets resulted in the emergence of resistant strains (Smith and Crabb, 1960). Perhaps an alternative approach using genetic selection may be worthwhile, as this was successful with Marek's disease (Cole, 1968) and coccidiosis (Johnson and Edgar, 1982).

Selection experiments should be facilitated if differential resistance can be demonstrated in related lines. These lines could be crossed to provide the offspring for further genetic studies needed to establish the feasibility of this approach. Thus, it was the objective of the present experiments to determine whether differential resistance to staphylococcal disease exists in related lines of New Hampshire chickens previously separated by selection for a difference in the size of their bursa of Fabricius (Glick and Dreesen, 1967).

MATERIALS AND METHODS

Chickens

The Lester J. Dreesen (LJD) line of New Hampshire chickens and a small bursa line (SBL) derived from the LJD line were used throughout these experiments. The SBL line was selected for smaller bursa size at early ages as described by Glick and Dreesen (1967). Three-day-old chicks reared in Horsfall isolation units were fed commercial diets and water for ad libitum intake. For both diets, crude protein = 16.4% and metabolizable energy = 1,429 kcal/kg.

Bacteria

The two Staphylococcus aureus isolates used in these studies were chosen on the basis of a previous demonstration of pathology in day-old chicks. In that pilot study, Isolate P4L, which was originally derived from a field case of chicken osteomyelitis, produced 46% mortality, and Isolate 3727, originally obtained from a cloacal swab of a healthy hen, showed a 67%
TABLE 1. Body weights in grams (± SD) of 20 placebo-challenged and 20 unchallenged control LJD and SBL chicks

<table>
<thead>
<tr>
<th>Day of age</th>
<th>Challenge</th>
<th>Control</th>
<th>Challenge</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>40 ± 4.1</td>
<td>42 ± 2.6</td>
<td>43 ± 3.3</td>
<td>43 ± 4.0</td>
</tr>
<tr>
<td>10</td>
<td>82 ± 13.2</td>
<td>82 ± 10.6</td>
<td>84 ± 10.9</td>
<td>88 ± 9.2</td>
</tr>
<tr>
<td>13</td>
<td>98 ± 14.1</td>
<td>99 ± 9.3</td>
<td>104 ± 12.6</td>
<td>107 ± 9.2</td>
</tr>
</tbody>
</table>

1LJD = Lester J. Dreesen line of New Hampshires; SBL = subline of LJD line selected for decreased bursa of Fabricius weight.

2Challenge was administered at 3 days of age.

3Total df = 119.

4ANOVA: Day of age, df = 2, F = 1,109, P<0.001; all other main effects and interactions were nonsignificant.

mortality rate after intracardiac challenges. Both isolates grew well on mannitol salt agar (MSA) plates routinely used for the identification of pathogenic *S. aureus*. They were coagulase-positive by the tube test using rabbit plasma; and both exhibited the typical microscopic characteristics as described by Baird-Parker (1974). The isolates were stored on brain-heart infusion (BHI) agar slants, grown out on MSA plates, and tested for coagulase production prior to each challenge study. Dosages were formulated by the dilution of overnight broth cultures, using the turbidity of a Number 1 MacFarland standard as a guide.

**Challenge**

Intracardiac challenges (3-day-old chicks only) were made using 1-mL tuberculin syringes fitted with small gauge (26-gauge) needles. Each dose was delivered in a .1-mL volume; care was taken to check for the presence of blood in the syringe barrel at delivery. Older chickens were injected in the wing vein using a 1-mL volume. The death of a neonate (3-day-old chick) occurring within the first 4 h postchallenge, was assumed to result from injection trauma. These deaths were not included in the data, and depending upon availability, such chicks were replaced with freshly challenged ones.

**Trauma Assessment**

Intracardiac challenge of neonates by itself could be expected to result in a certain amount of morbidity or mortality. Thus, a separate measurement of the potential for untoward effects was made by comparing morbidity, mortality, and short-term body weights in a group of 3-day-old chicks challenged with BHI broth alone, to unchallenged control groups.

**Statistical Analyses**

Total mortality was analyzed by the chi-square test in those trials in which both lines were represented and contained at least one survivor. Overall line or isolate effects were analyzed using the chi-square test after pooling the appropriate data within the neonatal or older chick age categories. Body weight data were analyzed by a three-factor ANOVA, with line, age in days, and placebo challenge as main effects.

**RESULTS**

Peracute (4 h) morbidity or mortality occurred in some trials (two or three chicks) but not in others. Neither morbidity nor mortality occurred in 20 3-day-old chicks challenged intracardially with a BHI broth placebo or in an equal number of unchallenged controls. Body weight data, given in Table 1, were not statistically different across lines, or placebo injected versus controls, but were highly significant for days of age (P<.001) as would be expected for growing chicks. None of the two- or three-way interactions between main effects were significant.

In contrast to chicks injected with placebo, there was a rapid onset of morbidity, and mortality was observed as early as 24 h, in neonates challenged with the bacteria. Necropsy examinations of chicks from each trial

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2Difco Lab., Detroit, MI 48232.
3BBL, Bethesda, MD 20814.
TABLE 2. Mortality from intracardiac challenge of neonatal (3 day) Line LJD and Line SBL chicks

<table>
<thead>
<tr>
<th>Trial</th>
<th>Isolate</th>
<th>LID</th>
<th>SBL</th>
<th>χ²</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. dead/</td>
<td>No. dead/</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>no. challenged</td>
<td>no. challenged</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percentage</td>
<td>Percentage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>P4L</td>
<td>15/36</td>
<td>9/36</td>
<td>2.25</td>
<td>.1&gt;P&gt;.05</td>
</tr>
<tr>
<td>2</td>
<td>27/40</td>
<td>27/40</td>
<td>12/40</td>
<td>11.25</td>
<td>P&lt;.01</td>
</tr>
<tr>
<td>3</td>
<td>3727</td>
<td>3727</td>
<td>6/24</td>
<td>.05</td>
<td>.75&gt;P&gt;.9</td>
</tr>
<tr>
<td>4</td>
<td>20/38</td>
<td>12/33</td>
<td>36</td>
<td>1.88</td>
<td>.25&gt;P&gt;.1</td>
</tr>
<tr>
<td>5</td>
<td>11/11</td>
<td>15/21</td>
<td>71</td>
<td>3</td>
<td>.64</td>
</tr>
<tr>
<td>6</td>
<td>22/40</td>
<td>16/35</td>
<td>46</td>
<td>.64</td>
<td>.5&gt;P&gt;.25</td>
</tr>
</tbody>
</table>

1LID = Lester J. Dreesen line of New Hampshires; SBL = subline of LID line selected for decreased bursa of Fabricius weight.
2Dose = 3 x 10⁷ bacteria per chick.
3Chi-square not calculable due to 100% LID mortality.

suggested acute septicemia. Bacteriological examinations of swab specimens of livers, spleens, or cardiac blood obtained from moribund chicks yielded MSA and coagulase-positive S. aureus.

The mortality rates seen in the neonates were highly variable, ranging from 17 to 100% depending on the individual trial, the line, or the isolate used for the challenge (Table 2). Although statistical significance of a line difference was achieved in one neonatal trial only, the mortality rate of Line LJD was higher than that of Line SBL in five of the six trials. The overall LTD mortality rate across isolates and trials was 50% (102 of 205) as compared with 37% (70 of 189) for the SBL line (χ² = 6.3, .05 > P > .01). The mortality rate for Isolate 3727 across lines and trials was 54% (96 of 178) compared with 35% (76 of 216) for Isolate P4L (χ² = 13.4, P<.001).

The onset of morbidity and mortality was more gradual in the older chicks challenged intravenously. Morbidity, characterized by lameness, recumbency, and the production of watery feces discolored with bile, began by Day 2 or 3 postchallenge. Mortality began by Day 4 or 5 and increased more rapidly in Line LJD than in Line SBL (Figure 1). Older Line LJD chicks had a higher mortality rate over all trials (54%, 52 of 97) compared with Line SBL (34%, 44 of 128, χ² = 7.7, P<.001).

Isolate 3727 was more pathogenic for older

TABLE 3. Mortality in Line LJD and Line SBL chicks 7 to 12 wk of age challenged intravenously

<table>
<thead>
<tr>
<th>Trial</th>
<th>Isolate</th>
<th>LID line</th>
<th>SBL line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. dead/</td>
<td>No. dead/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no. challenged</td>
<td>no. challenged</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percentage</td>
<td>Percentage</td>
</tr>
<tr>
<td>1</td>
<td>P4L</td>
<td>7</td>
<td>25/27</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0/16</td>
<td>0/21</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>9/20</td>
<td>1/20</td>
</tr>
<tr>
<td>4</td>
<td>3727</td>
<td>7</td>
<td>7/28</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>14/20</td>
<td>6/20</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>11/20</td>
<td>5/12</td>
</tr>
</tbody>
</table>

1LID = Lester J. Dreesen line of New Hampshires; SBL = subline of LID line selected for decreased bursa of Fabricius weight.
2Dose = 3 x 10⁸ bacteria per chick.
3NA = not available.
4Chi-square not calculable.
5Morbidity for LID was 5/16 (31%) and for SBL was 2/21 (9%).
6Morbidity for LID was 16/20 (80%), and for SBL was 9/12 (75%).
chicks than was Isolate P4L (Table 3). A total of 61 of 121 (50%) of the older chicks succumbed to the former isolate as compared with 35 of 104 (34%) to the latter ($\chi^2 = 5.75, .05 > P > .25$). As was the case in the neonates, the mortality rates in individual trials were highly variable, ranging from a low of 0% for both lines, (Trial 2) to a high of 93% for the usually resistant Line SBL (Trial 1).

DISCUSSION

Development of staphylococcal disease probably occurs as a result of relocation of the bacteria from their normal residence in mucosal areas to sensitive deeper tissues. Resistance may depend in part on the rapid removal of these invasive bacteria by phagocytosis. Support for the first supposition comes from a report of Cotter et al. (1987a), who isolated MSA-positive bacteria from cloacal swabs obtained from 248 of 294 (84%) healthy hens in lay. Of the 100 of these isolates tested for coagulase production, 30% were found to be positive. Six of these isolates, and an additional coagulase-negative isolate obtained during the same study, were tested for pathogenicity by intracardiac challenge of day-old chicks. The six coagulase-positive isolates were pathogenic, but the coagulase-negative isolate was not.

Some lines of chickens are better able than others to phagocytose SRBC, which show immunological crossreactivity with S. aureus (Schoof and Tempelis, 1982; Dietert, R. R., Poultry and Avian Sciences, Cornell University, Ithaca, NY, 14853, personal correspondence, 1989). Line SBL has been shown to be an efficient producer of anti-SRBC antibody (Yamamoto and Glick, 1982). Perhaps resistant lines like SBL can remove the invasive bacteria more efficiently than susceptible lines.

Although line differences influenced resistance, the present data show that bacterial virulence factors are important as well. Isolate 3727, derived from a healthy hen, produced 54% mortality (96 of 178) in the neonates, compared with 35% (76 of 216) for Isolate P4L, obtained from an osteomyelitic lesion ($\chi^2 = 4.4, .05 > P > .01$). Similar results were obtained in older chicks in which challenge with Isolate 3727 resulted in a 50% (61 of 121) mortality rate compared with 35% (35 of 104) for Isolate P4L ($\chi^2 = 5.0, .05 > P > .01$). The similarity of mortality rates in the two age categories suggests that virulence is a stable property of these two isolates and that isolate origin is not necessarily a good indicator of pathogenicity. Three interacting factors, the number and virulence of the pathogens, the existence of innate (inherited) resistance, and the capacity to develop resistance as the animal ages (acquired immunity), are generally assumed to determine the probability for infection.

The S. aureus virulence factors, including coagulases, fibrinolysins, and hemolysins, are thought to play a role in establishing the infection (Baird-Parker, 1974). Inherited immunity to other bacterial diseases of chickens has been reported (Gavora, 1990). Acquired immunity, presumably dependent on the development of protective antibody or cell-mediated immunity (T-cells) may be problematic in chickens, as it seems to be in other species. The failure to acquire protective immunity would explain why staphylococcal vaccines, usually dependent on the development of these defense mechanisms, have not been successful.

Apart from the delayed onset of symptoms observed in older chicks, little evidence was obtained to suggest an important role for age-dependent (acquired) immunity as a determinant of staphylococcal resistance. The latter observation, coupled with the lack of trauma associated with intracardiac challenges (Table 1), suggests a practical application. Because there is little evidence for the development of age-dependent immunity, commercially important lines could be screened for staphylococcal susceptibility at early ages using a direct intracardiac challenge.
Inherited resistance to staphylococci has been proposed to be associated with the major histocompatibility complex in mice and humans (Marrack and Kappler, 1990). Accordingly, resistance is mediated by a genomic restriction in the capacity of T-lymphocytes and macrophages to react with bacterial toxins. The resistance to P4L associated with the B complex (chicken major histocompatibility complex) was reported by Smith (R. F., Department of Microbiology and Environmental Health, Colorado State University, Fort Collins, CO 80523, personal correspondence, 1989), and Cotter et al. (1987b) found B complex associated differences in hypersensitivity (wattle) reactions to an antigen derived from Isolate P4L. Perhaps resistance results from the accumulation of favorable B-haplotypes in some lines and their scarcity in others.

The degree of resistance demonstrated by the lines used in the present experiment was quite variable, suggesting that the severity of staphylococcal disease was influenced by other factors. One factor may be concurrent infection with another disease. Concurrent infection with infectious bursal disease virus can make chickens highly susceptible to staphylococci (Santivatr et al., 1981). This may account for the 93% mortality observed with Isolate P4L in 7-wk-old SBL chicks (Table 3, Trial 1).

The results of these experiments indicate that a higher degree of staphylococcal resistance accompanied selection for small bursal size. Perhaps such selection resulted in a reduction of the chicken homolog of IgE, an antibody that has been suggested to enhance colonization by staphylococci (Sheagren, 1984). Susceptible lines could be producing higher levels of such antibody, perhaps resulting in the impairment of bacterial clearance by phagocytosis. This explanation is plausible, as resistance is present in neonates and bacterial clearance by phagocytosis has been demonstrated in chick embryos (Powell, 1987).

In conclusion, the explanation of the superior resistance shown by the SBL line is not yet known, but it may be associated with the B complex. If this is the case, then such resistance favoring B complex alleles could serve as convenient selection markers for the establishment of resistant breeding stock. Moreover, Lines LJQ and SBL should be useful in further investigation of staphylococcal pathogenesis.

## Acknowledgment

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## References


