Antibody Responses and Body Weights of Chicken Lines Selected for High and Low Humoral Responsiveness to Sheep Red Blood Cells.

1. Effect of Escherichia coli Lipopolysaccharide

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ABSTRACT

Antibody (Ab) responses to i.m. administered SRBC and BSA, and i.p. administered Escherichia coli lipopolysaccharide (LPS), and BW at various times after treatment, were measured in chicken lines divergently selected for high (H) and low (L) Ab responses to SRBC, and in a randombred control line (C).

The Ab responses to SRBC and BSA, but not LPS, were significantly affected by line by treatment interactions. Levels of antibodies to SRBC and BSA were higher in the H line than in either the C or L line (P < 0.05). Administration of LPS did not affect Ab responses to SRBC, but Ab responses to BSA were decreased in birds that received BSA and LPS simultaneously.

Body weights of C and L lines were significantly higher than BW of H line birds at all times. Lipopolysaccharide injection induced an acute, but transient reduction of BW gain, which was not affected by line. Antibody responses to SRBC and BSA were negatively correlated with BW. During the experimental period, however, percentage BW gain and humoral responsiveness were positively correlated. A higher percentage BW gain growth was seen in H line birds at the end of the experimental period.

The present results confirm the hypothesized acute cachectin nature of LPS, but the relationship between live BW (gain) and immune responsiveness in chickens remains to be further clarified.

(Key words: sheep red blood cells, bovine serum albumin, Escherichia coli lipopolysaccharide, antibody, body weight)


INTRODUCTION

Chickens have been divergently selected for either high (H line) or low (L line) antibody (Ab) responses to SRBC at 5 d after i.m. immunization at 37 d of age (Van der Zijpp and Nieuwland, 1986). These lines differ with respect to Ab responses to various T cell-dependent antigens (Parmentier et al., 1994; Kreukniet et al., 1996) and vaccines (Parmentier et al., 1996). Apart from the H and L lines, a randombred control (C line), which resembles the genetic pool of the original parental stock of layers (Pinard et al., 1993), has been maintained.

Hens and cocks of the current H line have lower BW than those of the C and L lines (Parmentier et al., 1996). The relation between divergent BW and immune responsiveness of the current lines might be based on the cachectin activities of acute phase proteins such as interleukin (IL)-1 and IL-6, or tumor necrosis factor (TNF)-α-like substances. Cachectins are produced by extravascular effector cells, such as macrophages, in response to invasive stimuli. Among various multiple effects, cachectins induce a state of anorexia, as occurs during neoplastic and infectious diseases (Beutler and Cerami, 1988; Grimble, 1994). Immune stimulation (Klasing et al., 1987), and injection of chickens with IL-1-containing supernatant from bacterial endotoxin-stimulated chicken HD11 macrophages (Klasing, 1987) induced a reduction of growth and feed utilization in chickens, and accelerated muscle protein degradation. The degree of sanitation may affect growth rate and feed efficiency (Klasing and Johnstone, 1991). Continuous stimulation with ubiquitous microorganisms may thus account for enhanced cachectin release in, and lower BW of the present H line.

Release of IL-1, IL-6, or TNF by macrophages in vivo can be induced with bacterial endotoxins such as

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Abbreviation Key: Ab = antibody; C = randombred control line; H = line selected for high antibody responses to SRBC; IL = interleukin; L = line selected for low antibody responses to SRBC; LPS = lipopolysaccharide; TNF = tumor necrosis factor.
Escherichia coli endotoxin (lipopolysaccharide; LPS) (Klasing and Peng, 1987). In the present study, acute and long term effects of i.p. administered E. coli LPS on BW and humoral response of the selection lines to the particulate antigen SRBC and the soluble antigen BSA lines were studied. We hypothesized that higher immune responsiveness of the H line is related with higher acute phase protein activity. Therefore, effects of injection with LPS on Ab responses or BW were expected to be affected by line.

MATERIALS AND METHODS

Chickens

The experiment was conducted with 120 female chicks originating from an ISA Warren cross (medium heavy layers), which had been selected in the past for H and L primary antibody responses at Day 5 after primary i.m. immunization with SRBC at 37 d of age, and the randombred C line (Van der Zijpp and Nieuwland, 1986). From the 15th generation, 40 5-wk-old chicks of each line from one hatch were used. Chicks were housed in battery cages (50 × 100 cm) with a maximum bird density of 12 chicks per cage and free access to feed (152 g/kg CP, 2,817 kcal ME/kg) and water. The birds were vaccinated against Marek’s disease, infectious bronchitis, and infectious bursal disease at 0, 2, and 15 d of age, respectively, and Newcastle disease at hatch.

General Reagents

Bovine serum albumin (Factor V),2 and Escherichia coli derived LPS (LPS, 055:B5) were used.3

Experimental Design

Effects of i.m. immunization with SRBC and BSA, or i.p. injection with LPS on Ab responses to SRBC, BSA, and LPS, and on BW, were studied using a 3 × 4 factorial arrangement of treatments: three lines (H, C, and L lines), and four treatments. Treatment 1 consisted of 10 birds of each line, that at 5 wk of age were sensitized i.m. in the left breast muscle with 1 mL PBS containing a mixture of 1 mg BSA and 25% packed SRBC. Treatment 2 consisted of birds that received 1 mL PBS i.m. (negative control). Birds of Treatment 3 were i.m. sensitized similarly as Treatment 1, but simultaneously received 1 mg/kg BW LPS i.p. Birds of Treatment 4 received 1 mg/kg BW LPS i.p., and 1 mL PBS i.m. BW were measured 1 d before the day of sensitization, 24 h after sensitization, and at 9, 11, and 13 wk of age.

Humoral Immune Response to SRBC, BSA, and LPS

Total Ab titers to SRBC were determined by agglutination according to Van der Zijpp and Leenstra (1980) in serum from all 120 birds 1 d before, and at 2, 5, 7, 9, 14, 21, and 28 d after sensitization. Antibody titers measured against SRBC were expressed as the logs of the reciprocal of the highest serum dilution giving complete agglutination. Titrations were assessed the same day in 96-well microtiter plates, using SRBC from the same stock as used for the immunizations.

Total Ab titers to BSA, and E. coli LPS were determined by ELISA in serum from all 120 birds 1 d before, and at 2, 5, 7, 9, 14, 21, and 28 d after sensitization. Briefly, 96-well plates were coated with either 4 μg/mL BSA, or 4 μg/mL LPS, respectively. After subsequent washing with PBS and 0.05% Tween, the plates were incubated with serial dilutions of serum. Binding of antibodies to BSA, and LPS antigen were detected using 1:20,000 diluted rabbit anti-chicken/IgG1 coupled to peroxidase. After washing, tetramethylbenzidine and 0.05% H2O2 were added and incubated for 10 min at room temperature (21°C). The reaction was stopped with 2.5 N H2SO4. Extinctions were measured with a Multiskan4 at a wavelength of 450 nm. Titers were expressed as the log2 values of the highest dilution giving a positive reaction. Positivity was derived from the extinction values of a standard positive serum present on every microtiter plate.

Statistical Analysis

Serum Ab titers to SRBC, BSA, and LPS, were analyzed by a three-way ANOVA for the effect of line, type of sensitization (PBS, or antigen simultaneously with or without LPS), time, and their interactions using the repeated measurement procedure (SAS Institute, 1985). Body weights at 5, 9, 11 and 13 wk of age were analyzed by a two-way ANOVA for the effect of line, type of sensitization, and their interactions (SAS Institute, 1985). Differences in BW between the four types of sensitization and between lines were tested with Bonferroni’s test.

Correlations between Ab titers to SRBC, BSA, and LPS at various times after immunization were analyzed by Pearson’s partial correlation, adjusted for line and treatment, for birds that were sensitized with SRBC/BSA either simultaneously with or without i.p. injection with LPS. Similarly, partial correlations adjusted for line, and treatment between antibody responses to all antigens at all times, and BW at 5, 9, 11 and 13 wk of age, and growth, were calculated.

RESULTS

Humoral Immune Response to SRBC, BSA, and E. coli LPS

Least square means of mean serum Ab titers to SRBC, BSA, and E. coli LPS of all lines during 4 wk after sensitization at 5 wk of age are shown in Table 1.

2Sigma Chemical Co., St. Louis, MO 63178-9916.
3Nordic, Tilburg, The Netherlands.
4Labsystems, Helsinki, Finland.
FIGURE 1. Antibody titers in serum from High line (A), Control line (B), and Low line (C) chickens to SRBC after i.m. sensitization at 5 wk of age with SRBC and BSA, simultaneously with i.p. sensitization with Escherichia coli lipopolysaccharide (LPS) (Treatment 3, ●), or without LPS (Treatment 1, ◊). Treatment 4 (○) consisted of sensitization with PBS, whereas birds of Treatment 2 (□) only received LPS i.p. Data represent the mean ± SEM of 10 hens per group.

**SRBC.** Anti-SRBC serum antibody titers in H, C, and L lines during 4 wk after sensitization to SRBC and BSA are shown in Figure 1. Antibody responses to SRBC were significantly affected by a line by treatment by time interaction. During the entire experimental period, levels of Ab binding SRBC were higher in the H line (Figure 1A) than in the C line (Figure 1B) and the L line (Figure 1C) (P < 0.05). No Ab responses to SRBC were found in the L line. Treatment 1 (SRBC i.m.) and Treatment 3 (SRBC i.m. and simultaneously LPS i.p.) significantly enhanced titers to SRBC in the H and C lines as compared to the negative control (Treatment 2). Simultaneous i.p. administration of LPS (Treatment 3) did not significantly reduce Ab responses to SRBC in the H line at Days 5, 7, and 9 after immunization (P > 0.05). Also, line differences were not affected by LPS treatment.

**BSA.** Anti-BSA serum antibody titers in H, C, and L lines during 4 weeks after sensitization to SRBC and BSA are shown in Figure 2. As was true for SRBC, the humoral response to BSA was significantly affected by a line by treatment by time interaction. During the entire period, significantly higher titers (P < 0.05) were found in the H line (Figure 2A) than in the C line (Figure 2B) and L line (Figure 2C). Also, the C line differed significantly (P < 0.05) from the L line. Treatment 1 (BSA i.m.) and Treatment 3 (BSA i.m. and simultaneously LPS i.p.) significantly enhanced titers as compared to the negative control (Treatment 2). Significantly lower titers to BSA were found in birds that received BSA, and simultaneously LPS i.p. (Treatment 3) as compared to birds that received BSA alone (Treatment 1). Especially in the C line, titers to BSA were negatively affected by simultaneous LPS injection (Figure 2B). Peak titers in all lines were 2 d earlier in birds that received BSA simultaneously with LPS. Although almost similar at the first moments after sensitization, levels of Ab titers to BSA decreased more rapidly in the C line compared to the H line.

**E. coli LPS.** Anti-LPS serum antibody titers in H, C, and L lines during 4 wk after sensitization to SRBC and BSA are shown in Figure 3. From Day 5 after sensitization titers were enhanced and remained at a plateau for 4 wk. Neither line nor treatment nor time effects were found, but titers to LPS were affected by a line by treatment interaction (Table 1). During the entire period, titers to LPS were similar in all three lines, and were not enhanced by i.p. sensitization with LPS.

**Body Weights and Antibody Titers**

At 5 wk of age, 1 d before treatment, BW of C and L line pullets were both significantly higher than BW of H line pullets (Table 2). The same was true for BW 48 h later, i.e., 24 h after i.m. sensitization with SRBC/BSA simultaneously with or without i.p. sensitization with LPS. Forty-eight-hour BW gain was affected by treatment. Growth of birds that received LPS i.p. (Treatments 3 and 4) was significantly lower than birds that received SRBC/BSA or PBS without simultaneous LPS injection (Treatments 1 and 2). Also percentage growth of these birds was retarded. There was no line by treatment interaction, birds of all three lines were negatively affected by LPS treatment; however, significant differences were found within the C line with respect to immunization treatments.
FIGURE 2. Antibody titers in serum from High line (A), Control line (B), and Low line (C) chickens to BSA after i.m. sensitization at 5 wk of age with SRBC and BSA, simultaneously with i.p. sensitization with *Escherichia coli* lipopolysaccharide (LPS) (Treatment 3, ●), or without LPS (Treatment 1, □). Treatment 4 (▴) consisted of sensitization with PBS, whereas birds of Treatment 2 (○) only received LPS i.p. Data represent the mean ± SEM of 10 hens per group.

FIGURE 3. Antibody titers in serum from High line (A), Control line (B), and Low line (C) chickens to LPS after i.m. sensitization at 5 wk of age with SRBC and BSA, simultaneously with i.p. sensitization with *Escherichia coli* lipopolysaccharide (LPS) (Treatment 3, ●), or without LPS (Treatment 1, □). Treatment 4 (▴) consisted of sensitization with PBS, whereas birds of Treatment 2 (○) only received LPS i.p. Data represent the mean ± SEM of 10 hens per group.

The C line birds that received LPS i.p. simultaneously with or without antigenic sensitization had significantly lower BW gain and percentage growth than birds that did not receive LPS.

At 28 d after sensitization, i.e., 9 wk of age, BW of H line birds were significantly lower (*P* < 0.05) than BW of the C and L lines, but during the period from 5 to 9 wk of age relatively higher growth was found in the H line (Table 3). Birds of Treatments 3 (antigen i.m., simultaneously with LPS i.p.) and 4 (LPS i.p.) showed significantly higher actual and percentage body growth than birds that did not
TABLE 1. Serum antibody titers for different antigens from High (H), Control (C), and Low (L) line chickens after sensitization to SRBC, BSA, and *Escherichia coli* lipopolysaccharide (LPS) at 5 wk of age.

<table>
<thead>
<tr>
<th>Line</th>
<th>Treatment</th>
<th>Antigen</th>
<th>SRBC</th>
<th>BSA</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1: SRBC/BSA</td>
<td>4.16</td>
<td>4.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2: PBS</td>
<td>0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3: SRBC/BSA + LPS</td>
<td>3.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4: PBS + LPS</td>
<td>0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.14</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1: SRBC/BSA</td>
<td>2.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2: PBS</td>
<td>0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3: SRBC/BSA + LPS</td>
<td>2.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4: PBS + LPS</td>
<td>0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.09</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>1: SRBC/BSA</td>
<td>0.54</td>
<td>1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2: PBS</td>
<td>0.16</td>
<td>0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3: SRBC/BSA + LPS</td>
<td>0.59</td>
<td>1.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4: PBS + LPS</td>
<td>0.03</td>
<td>0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.94</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.34</td>
<td>0.19</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

**Main effects**
- **Line**: ***NS***
- **Treatment**: ***NS***
- **Line × Treatment**: ***NS***
- **Time**: ***NS***
- **Line × Time**: ***NS***
- **Treatment × Time**: ***NS***
- **Line × Treatment × Time**: ***NS***

<sup>a,b</sup>Means per antigen within line (column) with no common superscript differ significantly (<i>P</i> ≤ 0.05).

<sup>1</sup>Least squares means of the entire observation period.

Receives LPS; however, this result was not so obvious for the actual growth in the C line. The enhanced growth in birds that initially grew slower due to the LPS treatment, might represent a recovery of initial weight loss. This result was true for all lines, but significantly enhanced absolute and percentage growth was found in birds of the L line.

At 11 and 13 wk of age, birds of the C (1,368 g at 11 wk, and 1,528 g at 13 wk) and L line (1,434 g at 11 wk, and 1,615 g at 13 wk) were significantly heavier than H line birds (1,294 g at 11 wk, and 1,475 g at 13 wk) (<i>P</i> < 0.05), but a higher percentage growth of the H line (774 g from 5 to 11, and 955 g from 5 to 13 wk) was found, as compared to the C line (762 from 5 to 11 wk, and 922 from 5 to 13 wk) and the L line (830 from 5 to 11 and 1,011 from 5 to 13 wk). Body weight, growth from 5 to 11 and 5 to 13 wk of age, and percentage BW; were not affected by any treatment H line: 151 g and 186 g; C line: 126 g and 153 g; and L line 139 and 169 g, from 5 to 11 and from 5 to 13 wk of age, respectively.

From Day 5 after sensitization, Ab responses to SRBC and BSA were positively correlated, 0.53 < <i>r</i> < 0.75 (<i>P</i> < 0.05). No correlations between titers to SRBC and BSA on the one hand and titers to LPS on the other hand were found. Antibody responses to SRBC and BSA in birds from Treatments 1 and 3 were negatively correlated (<i>P</i> < 0.05) with BW at 24 h before (<i>r</i> = −0.3) and at 24 h after sensitization with SRBC and BSA (i.e., 5 wk of age), respectively. Only at 5 d after sensitization, a significant negative correlation between antibody response to BSA and acute 48 h BW gain was found (<i>r</i> = −0.311). Body weights at 9, 11, and 13 wk of age were significantly correlated with titers to SRBC and BSA (<i>r</i> = −0.2). Percentage growth until 9 wk of age, i.e., during the experimental period was, however, significantly positively correlated with antibody titers to BSA and SRBC (0.26 < <i>r</i> < 0.42) (<i>P</i> < 0.05). Antibody responses to LPS at 5 wk of age were significantly correlated with BW at 5 wk of age (<i>P</i> < 0.05), and at 9, 11, and 13 wk of age, but negatively correlated with percentage BW gain at 9, 11 and 13 wk of age (<i>r</i> = −0.22).

**DISCUSSION**

Pullets of the current H line of the 15th generation had significantly lower BW than C and L line pullets. Lower Ab responses to SRBC were reported for chicken lines selected for higher BW (Miller *et al.*, 1992), whereas
TABLE 2. Acute effects of sensitization with SRBC/BSA i.m., and *Escherichia coli* lipopolysaccharide (LPS) i.p. on body weights and 48-h growth at 5 wk of age of High (H), Control (C), and Low (L) line hens of the 15th generation

<table>
<thead>
<tr>
<th>Line</th>
<th>Treatment1</th>
<th>Day -12</th>
<th>Day +12</th>
<th>Net BW gain3</th>
<th>Percentage growth4</th>
</tr>
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<tbody>
<tr>
<td>H</td>
<td>1: SRBC/BSA</td>
<td>549</td>
<td>585</td>
<td>37</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>2: PBS</td>
<td>519</td>
<td>563</td>
<td>44</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>3: SRBC/BSA + LPS</td>
<td>514</td>
<td>637</td>
<td>23</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>4: PBS + LPS</td>
<td>521</td>
<td>544</td>
<td>23</td>
<td>4.6</td>
</tr>
<tr>
<td>C</td>
<td>1: SRBC/BSA</td>
<td>639ab</td>
<td>690a</td>
<td>51ab</td>
<td>7.9ab</td>
</tr>
<tr>
<td></td>
<td>2: PBS</td>
<td>612ab</td>
<td>670b</td>
<td>58a</td>
<td>9.4b</td>
</tr>
<tr>
<td></td>
<td>3: SRBC/BSA + LPS</td>
<td>575b</td>
<td>607a</td>
<td>32a</td>
<td>5.6b</td>
</tr>
<tr>
<td></td>
<td>4: PBS + LPS</td>
<td>601ab</td>
<td>631ab</td>
<td>30b</td>
<td>5.2b</td>
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<tr>
<td>L</td>
<td>1: SRBC/BSA</td>
<td>589</td>
<td>641</td>
<td>52</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>2: PBS</td>
<td>587</td>
<td>640</td>
<td>53</td>
<td>9.1</td>
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<tr>
<td></td>
<td>3: SRBC/BSA + LPS</td>
<td>645</td>
<td>674</td>
<td>29</td>
<td>4.6</td>
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<tr>
<td></td>
<td>4: PBS + LPS</td>
<td>596</td>
<td>633</td>
<td>37</td>
<td>6.9</td>
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<tr>
<td>SEM</td>
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<td>19.0</td>
<td>19.5</td>
<td>6.5</td>
<td>1.3</td>
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Main effects

<table>
<thead>
<tr>
<th>Line</th>
<th>Treatment</th>
<th>***</th>
<th>***</th>
<th>NS</th>
<th>NS</th>
</tr>
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<tbody>
<tr>
<td>H</td>
<td>&lt; C, L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>&lt; C, L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>L</td>
<td>≤ C, L</td>
<td></td>
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<table>
<thead>
<tr>
<th>Treatment</th>
<th>NS</th>
<th>NS</th>
<th>***</th>
<th>***</th>
</tr>
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<tbody>
<tr>
<td>1, 2 &gt; 3, 4</td>
<td>2 ≥ 1 ≥ 4 ≥ 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a,b*Means per antigen within line (column) with no common superscript differ significantly (*P* < 0.05).

1Treatment 1 = SRBC/BSA i.m.; Treatment 2 = PBS i.m.; Treatment 3 = SRBC/BSA i.m., and LPS i.p.; Treatment 4 = PBS i.m., LPS i.p.

2Means estimates of BW.

348-h increase in BW (grams).

4Percentage increase in BW after 48 h.

*P* < 0.05.

****P* < 0.001.

A negative relationship between BW and Ab titers was found in commercial broilers (Siegel et al., 1984), and chicken lines divergently selected for Ab responses to SRBC (Martin et al., 1990). Intraperitoneal stimulation of chickens with IL-1, SRBC, LPS or Sephadex resulted in reduced growth and feed intake, probably due to the cachectin activities of IL-1, IL-6, or TNF-α (Klasing et al., 1987). These cytokines are the earliest mediators secreted by the host in response to LPS and other injurious stimuli (Van Miert, 1995). Acute phase proteins act in concert to decrease food intake (partially as a result from loss of appetite (Grimble, 1994), increase resting expenditure, gluconeogenesis, glucose oxidation, and synthesis of fatty acids and acute phase proteins by the liver. Also, metabolism of circulating insulin, glucagon, and corticosterone are affected (Klasing, 1988). Amino acids and glutamine released from muscle, and glucose derived from increased hepatic gluconeogenesis may provide endogenous sources of nutrition of the immune system. Diversion of nutrient pools of the host as a consequence of cachectin release may also lead to retarded growth (Grimble, 1994).

In the present experiment, acute growth (live weight gain) of birds was significantly retarded after i.p. sensitization with *E. coli* LPS, but no line differences were found. These results correspond with the earlier reported absence of differences in macrophage (phago-cytic) activity between the lines (Van der Zijpp et al., 1988, 1989; Kreukniet et al., 1994). The effect of LPS treatment appeared to be temporary; birds treated with LPS showed higher weight gain during the rest of the experimental period.

Antibody titers to LPS were found in all birds, regardless of immunization with LPS. These titers may reflect environmental stimulation by ubiquitous bacteria. Assuming that anti-LPS Ab titers reflect IL-1-dependent immune responses, the similarity of anti-LPS Ab titers in all lines indicated equal activation by exogenous LPS that did not affect BW in this experiment. Intramuscular sensitization with SRBC/BSA did not result in acute BW loss, and also during the experimental period, the humoral immune response did not induce retarded growth, although a negative relation between BW and titers was found.

Negative correlations between BW and antibody response might be based on pleiotropic effects for genes associated with immunoresponsiveness (Martin et al., 1990). On the other hand, arguments for resource allocation or prioritization of resource use for various demands by chickens artificially selected for body growth have been provided (Dunnington and Siegel, 1996). In the present lines, a negative co-selection due to linkage cannot be excluded. The absence of line effects, and the consistent negative (phenotypic) relation be-
TABLE 3. Body weights and growth of High (H), Control (C), and Low (L) line hens of the 15th generation at 9 wk of age

<table>
<thead>
<tr>
<th>Line</th>
<th>Treatment¹</th>
<th>BW² (g)</th>
<th>Net body weight gain³</th>
<th>Percentage growth⁴ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1: SRBC/BSA</td>
<td>1,081</td>
<td>533</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>2: PBS</td>
<td>1,039</td>
<td>521</td>
<td>102.8</td>
</tr>
<tr>
<td></td>
<td>3: SRBC/BSA + LPS</td>
<td>1,047</td>
<td>533</td>
<td>103.8</td>
</tr>
<tr>
<td></td>
<td>4: PBS + LPS</td>
<td>1,087</td>
<td>565</td>
<td>108.4</td>
</tr>
<tr>
<td>C</td>
<td>1: SRBC/BSA</td>
<td>1,182</td>
<td>543</td>
<td>85.4</td>
</tr>
<tr>
<td></td>
<td>2: PBS</td>
<td>1,171</td>
<td>559</td>
<td>91.2</td>
</tr>
<tr>
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<td>3: SRBC/BSA + LPS</td>
<td>1,106</td>
<td>533</td>
<td>93.1</td>
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<td></td>
<td>4: PBS + LPS</td>
<td>1,161</td>
<td>560</td>
<td>93.2</td>
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<td>L</td>
<td>1: SRBC/BSA</td>
<td>1,103</td>
<td>515¹</td>
<td>88.3¹</td>
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<tr>
<td></td>
<td>2: PBS</td>
<td>1,124</td>
<td>538¹</td>
<td>91.9¹</td>
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<td>3: SRBC/BSA + LPS</td>
<td>1,232</td>
<td>588¹</td>
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<td></td>
<td>4: PBS + LPS</td>
<td>1,212</td>
<td>616¹</td>
<td>105.0¹</td>
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<tr>
<td>SEM</td>
<td></td>
<td>34.2</td>
<td>21.5</td>
<td>4.0</td>
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</table>

Main effects
Line *** NS ***
H < C, L H > C, L
Treatment NS **
1 ≤ 2, 3 ≤ 4 1 ≤ 2, 3 ≤ 4

Line × Treatment NS NS NS

²-³ Means per antigen within line (column) with no common superscript differ significantly (P < 0.05).
³Treatment 1 = SRBC/BSA i.m.; Treatment 2 = PBS i.m.; Treatment 3 = SRBC/BSA i.m., LPS i.p.; Treatment 4 = PBS i.m., LPS i.p.
³Mean estimates of BW.
³Increase in BW from 5 to 9 wk of age.
³Percentage increase in BW from 5 to 9 wk of age.
*P < 0.05.
**P < 0.01.
***P < 0.001.

Takahashi et al. (1995) failed to demonstrate the cachectin effects of LPS sensitization, but presented evidence that high dietary protein concentration and multiple injections of LPS (negatively) affected the acute phase responses by LPS. Also the involvement of IL-1 and other known cytokines in the protein breakdown after infection has been questioned (Goldberg et al., 1988). In the current experiment, LPS administration was limited to one i.p. sensitization of a limited number of birds per group. Simultaneous administration of LPS did not significantly affect Ab responses to SRBC; however, antibody titers to BSA were significantly lower, especially in the C line, and the peak titer was found earlier. Antibody responses to SRBC were measured by agglutination, but ELISA was applied to measure Ab titer to BSA. Quantifications of Ab titers to BSA via ELISA is usually more sensitive than agglutination assays to SRBC. Furthermore, different types of Ab may have been measured. The primary response to SRBC in the current lines consists predominantly of IgM (Parmentier et al., 1997), whereas IgM, IgG, and low amounts of IgA are mounted to BSA (Parmentier et al., 1994). At present the effect of simultaneous LPS administration on different isotypes could not be elucidated.

Interleukin-1 release by macrophages may enhance release of glucocortisteroids via the pituitary-adrenal axis (Klasing, 1988; Grimble, 1994), which may negatively affect T cell-dependent Ab responses. Lipopolysaccharide-induced IL-1 in addition to IL-1 induced by antigen administration may have initiated suppression of immune responses to BSA next to decreased growth.

In summary, a transient reduction of BW gain in all three lines was found after LPS injection, but most pronounced in the C line. Correlations between BW and Ab responses were present; however, the small numbers of birds in each group, especially when line is taken into account, implicate a preliminary nature of the current experiment. Also, line by treatment interactions with respect to BW and treatments were not found. Continuous selection during 15 generations for Ab responses to SRBC may have resulted in decreased genetic variability of the H and L lines. Experiments are in progress to determine the level of IL-1/TNF release by LPS-stimulated macrophages of both H and L lines in vitro.
and in vivo, and the effects of immunization on feed uptake and metabolism.

ACKNOWLEDGMENTS

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REFERENCES


