Selection of Broilers with Improved Innate Immune Responsiveness to Reduce On-Farm Infection by Foodborne Pathogens

Christina L. Swaggerty,1 Igal Y. Pevzner,2 Haiqi He,1 Kenneth J. Genovese,1 David J. Nisbet,1 Pete Kaiser,3 and Michael H. Kogut1

Abstract
Economic pressure on the modern poultry industry has directed the selection process towards fast-growing broilers that have a reduced feed conversion ratio. Selection based heavily on growth characteristics could adversely affect immune competence leaving chickens more susceptible to disease. Since the innate immune response directs the acquired immune response, efforts to select poultry with an efficient innate immune response would be beneficial. Our laboratories have been evaluating the innate immune system of two parental broiler lines to assess their capacity to protect against multiple infections. We have shown increased in vitro heterophil function corresponds with increased in vivo resistance to Gram-positive and Gram-negative bacterial infections. Additionally, there are increased mRNA expression levels of pro-inflammatory cytokines/chemokines in heterophils isolated from resistant lines compared to susceptible lines. Collectively, all data indicate there are measurable differences in innate responsiveness under genetic control. Recently, a small-scale selection trial was begun. We identified sires within a broiler population with higher and/or lower-than-average pro-inflammatory cytokine/chemokine mRNA expression levels and subsequently utilized small numbers of high-expressing and low-expressing sires to produce progeny with increased or decreased, respectively, pro-inflammatory cytokine/chemokine profiles. This novel approach should allow us to improve breeding stock by improving the overall immunological responsiveness. This will produce a line of chickens with an effective pro-inflammatory innate immune response that should improve resistance against diverse pathogens, improve responses to vaccines, and increase livability. Ongoing work from this project is providing fundamental information for the development of poultry lines that will be inherently resistant to colonization by pathogenic and food-poisoning microorganisms. Utilization of pathogen-resistant birds by the poultry production industry would significantly enhance the microbiological safety of poultry products reaching the consumer.

Introduction
In the past 50 years, there have been three key initiatives that primary poultry breeders have employed to meet the ever-changing demands of a global food market: 1) eradication of vertically transmitted diseases; 2) selection within and between genetic lines for increased livability and disease resistance; 3) improved dissemination of husbandry practices and biosecurity (Flock et al., 2005). Even though significant progress has been made in improving overall poultry health and well-being, breeder companies continue to strive to produce products that are safer for consumers, with the primary focus shifting towards selection for a general increase in livability. There are numerous laboratories evaluating various aspects of the innate immune response in poultry. This review will focus on the work being conducted in our laboratory. One of the fundamental principles of our laboratory has been to develop a novel selection program for the poultry industry that is based on an effective innate immune response instead of the acquired response. The hypothesis driving these studies is that selection of poultry based on an effective/efficient innate immune response would produce a population of birds

1Agricultural Research Service, United States Department of Agriculture, College Station, Texas.
3Institute for Animal Health, Compton, Berkshire, United Kingdom.
that have multivalent protection against diverse pathogens and increased livability in the field. The purpose of this review is to connect 1) our initial studies evaluating two parental broiler lines and their F1 reciprocal crosses using in vitro heterophil function as an innate immune indicator of the overall immunological responsiveness of the line, to 2) our recent selection trials based on genes of the innate immune response.

Neonatal Poultry Immunity

Traditional management of infectious diseases in poultry depends on the use of vaccines, husbandry practices, biosecurity, and when necessary, use of broad-spectrum antibiotics. Public outcry for removal of growth-promoting antibiotics in animal feed will ultimately limit this husbandry practice. In fact, the European Union (EU) withdrew approval for the use of antibiotics as growth promoters in 2006 (Castanon, 2007). Further, the use of other prophylactic drugs, such as anticoccidiois and anti-helmintics, is also being curtailed for residue or resistance concerns.

The initial host immune response to pathogens is a critical determinant of disease resistance and susceptibility. Unfortunately, neonatal poultry exhibit a transient susceptibility to infectious diseases during the first week of life. This susceptibility can, and does, lead to significant economic losses and stems from an impairment of the avian innate and acquired host defense mechanisms. One of the most important features of this immune impairment is the functional inefficiency of the primary poultry polymorphonuclear cell (PMN), the heterophil, during the first 1–3 weeks post-hatch. Heterophils, the avian equivalent to the mammalian neutrophil, ingest and kill a variety of microbial pathogens. Specifically, heterophils are critical components of the host’s innate defenses, especially for neonatal poultry that lack efficient humoral and cell-mediated responses. Functionally, heterophils exhibit an assortment of cytoskeletal and biochemical activities which include adhesion, chemotaxis, phagocytosis, production of cytokines/chemokines, and the microbicidal activities of degranulation and production of a respiratory burst. Observations of functional variations between lines of birds appears to be due to heterogeneity in phagocytic and bactericidal activities of heterophils (Swaggerty et al., 2003a, 2003b). Chicken lines with functionally less active heterophils are more susceptible to infections than those with highly functional heterophils (Ferro et al., 2004; Swaggerty et al., 2003a, 2005b; Li et al., 2008).

Genetics and Poultry Immunity

Economic efficiencies demanded by the poultry industry has directed the selection process towards fast-growing broiler chickens (Gallus gallus) with improved feed conversion ratios and high yield. Over the past 50 years, selective breeding for increased body weight in broilers has brought about a dramatic reduction in the time required for a bird to reach processing weight. In 1953, 10.5 weeks were required for a broiler to reach a final live weight of 1.45 kg; however, by 2001 birds were reaching 2.67 kg in only 6 weeks (Havenstein et al., 2003; Flock et al., 2005). However, selection based heavily on growth characteristics and other phenotypic traits can adversely affect immune competence, thereby leaving chickens and turkeys more susceptible to disease (Han and Smyth, 1972; Nestor et al., 1996; Bayyari et al., 1997; Beaumont et al., 1997; Janss and Bolder, 2000). In the past, experimental genetic selection of poultry was based on immunity or inherent resistance to a specific infectious agent, such as Marek’s disease virus or avian leukosis virus, or to a vaccine (Zekarias et al., 2002). It is believed that this approach has resulted in inbred stocks of chickens resistant to specific pathogens with either poor growth characteristics or a poor response to other pathogens or vaccines. Since the poultry industry can suffer major economic losses from infectious diseases or contamination of food products due to transfer of microorganisms, there clearly would be great value in generating a line of birds with increased resistance to disease and improved livability in the field. Thus far, and to our knowledge, there is no commercially available line of poultry genetically selected and bred for polyvalent resistance to multiple infectious agents.

Avian Innate Immunity

The host immune response to pathogens in the earliest stages of infection is a critical determinant of disease resistance and susceptibility. The relatively recent discovery that the innate immune response directs the acquired response (Parish and O’Neill, 1997) supports efforts to select poultry with an efficient early innate immune response. Microbial recognition by cells of the innate response activates intracellular signaling pathways that result in the 1) activation of microbicidal killing mechanisms, 2) release of cytokines and chemokines, and 3) production of costimulatory molecules required for antigen presentation to the acquired immune system (Romagnani 1992; Medzhitov and Janeway, 1997a, 1997b; O’Connell et al., 2005; Takeuchi and Akira, 2007). Cytokines are essential effector molecules of innate and acquired immunity that initiate and coordinate responses aimed at eradicating pathogens. Chemokines are small, structurally related chemoattractant molecules that regulate movement of leukocytes (Zlotnick and Yoshie, 2000). Detecting avian cytokines and chemokines is limited by the lack of specific antibodies and reliable bioassays. However, cloning of chicken cytokines and chemokines has generated a more comprehensive array of reagents for investigating innate and acquired immune responses at cellular and molecular levels. Chicken orthologs of the T1 cytokines interferon-γ (IFN-γ) (Digby and Lowenthal, 1995), interleukin (IL)-18 (Schneider et al., 2000), and IL-12 (Balu and Kaiser, 2003; Degen et al., 2004); the pro-inflammatory cytokines IL-1β (Weining et al., 1998) and IL-6 (Schneider et al., 2001); the chemokines CXCL12 (formerly IL-8) (Bedard et al., 1987; Sugano et al., 1987) and CCL2; the T2-specific cytokines IL-4 and IL-13 (Avery et al., 2004); and the anti-inflammatory cytokines transforming growth factor-β4 (TGF-β4) (Jakowlew et al., 1988) and IL-10 (Rothwell et al., 2004) have all been cloned and sequenced. This makes it possible to design probes and primers to quantify signature cytokine and chemokine mRNA expression using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Although measuring cytokine mRNA expression levels by qRT-PCR does not necessarily equate to the production of bioactive protein, recent publications demonstrate qRT-PCR is the most highly sensitive method available to reliably quantify avian cytokines and chemokines (Kaiser et al., 2000, 2002, 2003; Beal et al., 2004; Withanage et al., 2004; Swaggerty et al., 2004, 2008; Smith et al., 2005).
Strong pro-inflammatory cytokine and chemokine responses are associated with increased resistance against disease (Heinrich et al., 2001; Sebastiani et al., 2002; Coussens et al., 2004; Ferro et al., 2004; Withanage et al., 2004). Heterophils migrate to the liver and intestinal villi of newly hatched chickens infected with Salmonella Typhimurium (ST), accompanied by elevated levels of pro-inflammatory cytokines in tissues (Withanage et al., 2004) and the ileum of Salmonella Enteritidis (SE)-infected chickens (Chappell et al., 2009), collectively indicating a potential role of the acute inflammatory response in neonatal chickens. In vitro stimulation of macrophages isolated from Salmonella-resistant chickens results in a significant increase in pro-inflammatory cytokine and chemokine mRNA expression levels, including IL-1β, IL-6, and CCL2 (Wigley et al., 2006). A strong pro-inflammatory cytokine response is likely to be very important in determining the response and subsequent outcome following Salmonella infections in poultry. Pro-inflammatory cytokines and chemokines have a key role in initiating an innate immune response and assist in generating a local inflammatory response (Ferro et al., 2004; Hughes et al., 2007). Namely, the pro-inflammatory cytokine IL-1β and chemokine IL-8 (CXCL2) are found in the gut of newly hatched chickens, and the mRNA expression levels continue to increase during the first week post-hatch (Bar-Shira and Friedman, 2006). IL-8 (CXCL2) mRNA expression is upregulated in Salmonella-resistant chickens (Sadeyen et al., 2004), and IL-1β and IL-6 mRNA expression levels increase following infection with SE (Cheeseman et al., 2007). An increase in IL-1β, IL-6, and IL-8 (CXCL2) mRNA expression is associated with increased resistance to extraintestinal SE infections in chickens (Ferro et al., 2004). In addition to an effective pro-inflammatory cytokine response, a strong chemotactic response is also influential in determining resistance to SE. In chickens, an IL-8-like chemokine is involved in heterophil recruitment to the site of infection following challenge with SE (Kogut, 2002).

Hypothesis and Objectives

The purpose of this review is to summarize our studies evaluating a parental pair of commercial broiler lines (lines A and B) and their F1 reciprocal crosses (C = B sire × A dam; D = A sire × B dam). Our initial hypothesis was based on the premise that selecting poultry based on an efficient innate immune response would likely produce birds that are more resistant to diverse foodborne pathogens. For the past 6 years, we have extensively characterized the innate immune response of these parental broilers and their F1 reciprocal crosses and show that the sire is more influential in determining in vitro heterophil functional efficiency (Swaggerty et al., 2003a, 2003b), which translates to increased in vivo resistance to organ invasion and/or colonization by Enterococcus gallinarum (Swaggerty et al., 2005b), SE (Ferro et al., 2004; Swaggerty et al., 2005a), and Campylobacter jejuni (Li et al., 2008). Additionally, we know there are increased mRNA expression levels of pro-inflammatory cytokines in heterophils isolated from the more resistant lines compared to the susceptible lines (Swaggerty et al., 2004). Collectively, all data indicate there are clear and measurable differences in heterophil function and innate responsiveness that are under genetic control. We recently initiated a small-scale selection trial to move our research from basic science to applying it in the poultry industry (Swaggerty et al., 2008). These studies were conducted to determine if there were differences in pro-inflammatory cytokine and chemokine mRNA expression levels within sires from a broiler population. Subsequently, a simple “progeny test,” as described by Lush (1945) and reviewed by Hunton (2006), was conducted to determine if the desired profile obtained in the sires was passed onto progeny. This novel approach will allow for improvement of broiler breeding stock by enhancing the immunological responsiveness and overall livability. Instead of identifying a line that is resistant to a single pathogen, we have identified biological markers that will predict a general innate immunological efficiency that can be used to identify highly productive poultry that are less susceptible to a broad range of pathogens and that have increased livability in the field.

In Vitro Screening Model

Early in the development of the in vitro screening model, neonatal chickens less than 4 days of age were utilized as peripheral blood donors. Large numbers of chickens (usually 100) from each line were bled, heterophils isolated from the pooled blood sample, and the in vitro functional assays performed. This allowed us to screen across genetic lines, but did not allow us to screen individuals within a particular line. The initial experiments evaluated in vitro heterophil function, including phagocytosis and killing of SE, and the biochemical killing mechanisms of degranulation and oxidative burst (Swaggerty et al., 2003b). The data are summarized in Table 1 and show heterophils isolated from line A chickens are significantly (p ≤ 0.05) more efficient at phagocytizing and killing SE compared to heterophils from line B chickens.

<table>
<thead>
<tr>
<th>Line</th>
<th>Average no. of SE per heterophil</th>
<th>Phagocytic index</th>
<th>SE killed (%)</th>
<th>Degranulation (µM)</th>
<th>Oxidative burst (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.3 ± 0.3*</td>
<td>601 ± 24*</td>
<td>68.7 ± 2.6*</td>
<td>61.5 ± 5.6*</td>
<td>34.8 ± 1.5*</td>
</tr>
<tr>
<td>B</td>
<td>5.9 ± 0.5</td>
<td>410.7 ± 30.8</td>
<td>46.7 ± 2.4</td>
<td>39.5 ± 5.4</td>
<td>15.8 ± 5.4</td>
</tr>
<tr>
<td>C</td>
<td>5.9 ± 0.9</td>
<td>371.5</td>
<td>51.2</td>
<td>36.9 ± 6.7</td>
<td>13.5 ± 3.3</td>
</tr>
<tr>
<td>D</td>
<td>8.0 ± 1.1*</td>
<td>497.2*</td>
<td>62.9*</td>
<td>76.2 ± 13.1*</td>
<td>35.3 ± 2.4*</td>
</tr>
</tbody>
</table>

SE, Salmonella Enteritidis.

*aHeterophils were isolated from the peripheral blood of day-old chickens and in vitro functional assays to quantify phagocytosis, killing efficiency, degranulation, and oxidative burst were measured.

*bC, B sire × A dam; D, A sire × B dam.

*p ≤ 0.05 Student t test between parent lines (A and B) and then a separate analysis between the crosses (C and D). No comparisons were made between parents and crosses.
Likewise, heterophils from cross D are more efficient compared to heterophils from cross C chickens (C = B sire x A dam; D = A sire x B dam), therefore indicating the sire is more influential in determining heterophil functional efficiency. Development of the in vitro screening model allows us to predict the projected immunological responsiveness of a line of chickens and how the line will respond to challenge with foodborne pathogens.

**In Vivo Challenge Models**

Upon establishing differences in the in vitro heterophil responses between the lines, it was imperative to show that increased in vitro heterophil function correlates with increased resistance against diverse foodborne pathogens. To this end, chickens from lines A and B and their F1 reciprocal crosses were challenged, in separate experiments, with SE (Ferro et al., 2004; Swaggerty et al., 2005a), C. jejuni (Li et al., 2008), and a vancomycin-resistant E. gallinarum (Swaggerty et al., 2005b). In each challenge trial, the appropriate parameters were evaluated to determine if there were differences in resistance between the lines. Regardless of the bacteria used for the challenge, line A chickens were always more resistant than line B chickens. Additionally, cross D chickens were more resistant than cross C chickens. Increased resistance was measured by fewer infected chickens, reduced bacterial load, or less mortality. The data are summarized in Table 2. All challenge trials followed the trends established by the in vitro model, leading credence to employing the model as a quick, reliable, and cost-effective means for the poultry industry to evaluate the immunological responsiveness of existing and experimental lines of poultry.

**Gene Expression**

Cytokines and chemokines are essential effector molecules produced by cells during innate and acquired immune responses and are involved in initiating and coordinating immune responses aimed at eradicating pathogens. Pro-inflammatory cytokines and chemokines have key roles in initiating an innate immune response and assist in generating local inflammatory responses. Both in vitro and in vivo SE challenge trials showed that line A and cross D chickens have higher pro-inflammatory cytokine and chemokine mRNA expression levels compared to line B and cross C chickens, respectively. The higher levels are indicative that a stronger/more efficient pro-inflammatory profile reduces invasion early after infection and therefore contributes, in part, to increased resistance against these foodborne pathogens.

The consistent differences in cytokine and chemokine mRNA expression levels led us to consider upstream receptors as contributors to the differential response. Toll-like receptors (TLR) are an integral component in the host’s response against microbial invasion. TLR-4 recognizes lipopolysaccharide, TLR-5 responds to flagellin, and TLR-15 is a recently described chicken-specific TLR (Higgs et al., 2006). Evaluation of heterophils from line A and B chickens show there are no differences in mRNA expression levels of either TLR-4 or -5; however, there is a significantly higher level of TLR-15 in line A heterophils prior to and following stimulation with SE (Nerren et al., 2009). These data indicate TLR-15 contributes, in part, to the differential responses observed between lines A and B.

**Preliminary Selection Based on Innate Immune Genes**

Based on previous genetic analysis with parent lines A and B and their F1 reciprocal crosses, we hypothesized it would be possible to 1) identify and select sires within a broiler population with inherently high or low pro-inflammatory cytokine and chemokine profiles and 2) evaluate the progeny of these sires to determine if they have a high or low profile that is similar to the sire. The goal of the selection trial is to increase the pro-inflammatory cytokine and chemokine responses of a population of broilers. In this case, line B was selected as the original population of sires. We understand that selection based on specific parameters will alter the line and therefore, the progeny will no longer be line B, but hereafter referred to as a broiler population. The long-term goal is to produce a stable broiler population with increased livability in the field accompanied by increased resistance against diverse pathogens.

One of the limiting factors associated with the early in vitro model was that we were characterizing lines of birds but not individuals. Using more advanced genetic and immunological studies allows us to identify and select individuals within a population with the desired characteristics, which is obviously critical to the broiler breeder industry. Profiling individuals allows for the freedom to sample at ages relevant and important to the industry; for example, sires for our trial were evaluated at 21 weeks of age and progeny were sampled at 6 weeks of age. To this end, we conducted a preliminary

<table>
<thead>
<tr>
<th>Lineb</th>
<th>SE (% positive)</th>
<th>SE (% mortality)</th>
<th>EG (%+ hearts [livers])</th>
<th>Cj (log10 CFU recovered)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55.8*</td>
<td>1*</td>
<td>5.1* [8.5]*</td>
<td>1.39*</td>
</tr>
<tr>
<td>B</td>
<td>70</td>
<td>33.7</td>
<td>48.3 [66.7]</td>
<td>3.5</td>
</tr>
<tr>
<td>C</td>
<td>45.8</td>
<td>10.8</td>
<td>31 [65.6]</td>
<td>1.91</td>
</tr>
<tr>
<td>D</td>
<td>40.8</td>
<td>1*</td>
<td>6.8* [22]*</td>
<td>0.31*</td>
</tr>
</tbody>
</table>

SE, Salmonella Enteritidis; EG, Enterococcus gallinarum; CJ, Campylobacter jejuni; CFU, colony-forming units.

*Day-old chickens were challenged orally with SE (5 x 10⁶ CFU/chicken) or EG (5 x 10⁶ CFU/chicken). Twenty-four hours post-challenge, chickens were humanely euthanized, and livers/spleens or hearts/livers collected for bacterial enumeration for SE and EG, respectively. CJ were administered orally to day-old chickens (3 x 10⁶ CFU/chicken). Seven days post-challenge the chickens were euthanized and cecal contents collected and log10 CFU CJ recovered was determined.

*C, B sire x A dam; D, A sire x B dam.

*p ≤ 0.05 Student t test between parent lines (A and B) and then a separate analysis between the crosses (C and D). No comparisons were made between parents and crosses.
TABLE 3. RANGE OF PRO-INFLAMMATORY CYTOKINE AND CHEMOKINE mRNA EXPRESSION LEVELS (CORRECTED 40-Ct) OBSERVED IN INITIAL SIRE SCREENING (N=119 SIRESN)A

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Range (corrected 40-Ct)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0–18.75</td>
</tr>
<tr>
<td>IL-6</td>
<td>0–16.34</td>
</tr>
<tr>
<td>CXCL12</td>
<td>0.42–18.28</td>
</tr>
<tr>
<td>CCL2</td>
<td>0–17.85</td>
</tr>
</tbody>
</table>

*Peripheral blood leukocytes (PBL), including heterophils and monocytes, were isolated from blood samples (3–5 mL) collected from 21-week-old broilers from the big vein in the wing. Total RNA was extracted using QiShredder homogenizer columns and RNeasy mini RNA extraction kit (Qiagen Inc., Valencia, CA). Cytokine and chemokine mRNA expression levels determined using quantitative reverse transcription polymerase chain reaction (qRT-PCR) performed on an Applied Biosystems 7500 Fast Real-Time PCR System using TaqMan fast universal PCR master mix and one-step RT-PCR master mix reagents (Applied Biosystems, Cheshire, UK). To correct for differences in RNA levels between samples within the experiment, the correction factor for each sample was calculated by dividing the mean Ct value for 28S rRNA-specific product for each sample, by the overall mean Ct value for the 28S rRNA-specific product from all samples. The corrected cytokine mean is calculated as follows: (Average of each replicate×cytokine slope)/28s slope×28s correction factor. n=5 mRNA expression levels of pro-inflammatory cytokines and chemokines (Swaggerty et al., 2008). Sires in the top/bottom of each parameter were used in the selection process and showed a strong/weaks pro-inflammatory cytokine and chemokine response were transferred to progeny and therefore potentially increase/decrease the efficiency of the innate immune response. A total of 120 and 94 progeny from high and low sires, respectively, were analyzed and their pro-inflammatory cytokine and chemokine profile determined by qRT-PCR. In all instances, progeny from the high sires had significantly (p ≤ 0.02) higher levels of mRNA expression levels compared to the progeny from low sires (summarized in Table 4).

Conclusions

The host immune response to pathogens in the early stages of infection is a critical determinant of disease resistance and susceptibility. For the past 6 years we have extensively characterized the innate immune response of parental broilers and have shown that, as in vitro heterophil function increases (Swaggerty et al., 2003b), there is a corresponding increase in resistance against organ invasion by SE (Ferro et al., 2004; Swaggerty et al., 2005a). More importantly the increased resistance against SE is accompanied by increased mRNA expression levels of pro-inflammatory cytokines and chemokines (Ferro et al., 2004). Collectively, these data suggest mRNA expression levels of the innate immune pro-inflammatory cytokines and chemokines are influenced in part by the levels observed in the sires. Our initial study evaluating a parental pair of broilers and their F1 reciprocal crosses show the sire is more influential in determining in vitro heterophil functional efficiency, which translates to increased resistance against SE (Ferro et al., 2004; Swaggerty et al., 2005a), E. gallinarum (Swaggerty et al., 2005b), and C. jejuni (Li et al., 2008).

TABLE 4. CYTOKINE AND CHEMOKINE mRNA EXPRESSION LEVELS (CORRECTED 40-Ct) OF PROGENY FROM DEFINED HIGH AND LOW SIRESN

<table>
<thead>
<tr>
<th>Progeny from</th>
<th>Progeny from</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>high sires (n=120)</td>
<td>low sires (n=119)</td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>9.9±0.2c</td>
<td>9.4±0.2</td>
</tr>
<tr>
<td>IL-6</td>
<td>17.7±0.3</td>
<td>16.7±0.3</td>
</tr>
<tr>
<td>CXCL12</td>
<td>10.2±0.3</td>
<td>8.7±0.4</td>
</tr>
<tr>
<td>CCL2</td>
<td>17.1±0.3</td>
<td>15.8±0.3</td>
</tr>
</tbody>
</table>

*Peripheral blood leukocytes (PBL), including heterophils and monocytes, were isolated from blood samples (3–5 mL) collected from 6-week-old broilers from the big vein in the wing. Total RNA was extracted using QiShredder homogenizer columns and RNeasy mini RNA extraction kit. Cytokine and chemokine mRNA expression levels determined using quantitative reverse transcription polymerase chain reaction (qRT-PCR) performed on an Applied Biosystems 7500 Fast Real-Time PCR System using TaqMan fast universal PCR master mix and one-step RT-PCR master mix reagents. To correct for differences in RNA levels between samples within the experiment, the correction factor for each sample was calculated by dividing the mean Ct value for 28S rRNA-specific product for each sample, by the overall mean Ct value for the 28S rRNA-specific product from all samples. The corrected cytokine mean is calculated as follows: (Average of each replicate×cytokine slope)/28s slope×28s correction factor.

Our recent selection trial advances the research from the laboratory and moves us one step closer to affecting the animals on the farm (Swaggerty et al., 2008). Additional rounds of selection based on increased pro-inflammatory cytokine and chemokine profiles will need to be carried out to confirm that the line is stable. In the future, production parameters such as growth, feed conversion, livability, and leg stability must be determined. Additionally, the high and low populations should be subjected to diverse stress challenge trials to determine if there are differences in resistance/susceptibility. If we are correct, this novel selection approach will allow us to improve broiler breeding stock by improving the overall immunological responsiveness including resistance against diverse pathogens, improved responses to vaccines, and increased livability.

Ongoing work from this project is providing fundamental information for the development of poultry lines that will be inherently resistant to colonization and/or invasion by pathogenic and food-poisoning microorganisms. Utilization of a more pathogen-resistant bird by the poultry production industry would significantly enhance the microbiological safety of poultry meat products reaching the consumer.

Acknowledgments

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Christina L. Swaggerty, B.S., Ph.D.
United States Department of Agriculture
Agricultural Research Service
2881 F&B Road
College Station, TX 77845

E-mail: christi.swaggerty@ars.usda.gov