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Functional comparison of heterophils isolated from commercial broiler chickens

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Heterophils from two pure lines (A and B) of commercial broiler chickens were isolated on days 1, 4, and 7 post-hatch to evaluate their ability to (1) phagocytose *Salmonella enteritidis* (SE) (2) degranulate when exposed to immune-IgG opsonized SE, and (3) produce an oxidative burst. On days 1 and 4, heterophils from line A were functionally more efficient compared to heterophils from line B ($p < 0.05$). By 7 days post hatch, heterophil functions for both lines were comparable. To further study the inheritance of heterophil functional efficiency, F1 reciprocal crosses (line C = male B × female A; line D = male A × female B) were evaluated for functional activity and compared with the immunologically efficient (A) and non-efficient (B) parent lines. Heterophils from line D had a more efficient heterophil function ($p < 0.05$) when compared to heterophils from C. These results suggest that heterophil function and efficiency can be genetically transferred to progeny. Moreover they indicate that heterophil function is sex-associated and genetically controlled by the rooster since progeny of line A males maintained immunologically efficient characteristics whereas heterophils from the progeny of line B roosters remained immunologically inefficient. To our knowledge, this is the first report to describe a functional relationship between pure and F1 reciprocal crosses of broiler chickens with regard to heterophils and the innate immune response.

Introduction

There are two functional components of the immune system: innate (natural) and acquired (adaptive) immunity. Historically, the innate immune response was thought of as the first-line of defence and functioned to limit infection until the acquired response was initiated (Kuby, 1992). However, recent literature suggests the innate immune response has additional functions and provides instruction for the acquired immune response (Fearon & Locksley, 1996; Bendelac & Fearon, 1997; Medzhitov & Janeway, Jr., 1997a; Parish & O'Neill, 1997). The instructional phase of the innate immune response begins with the recognition of self from (infectious) non-self by detecting molecules unique to invading organisms referred to as pathogen-associated molecular patterns (PAMPs) (Romagnani, 1992; Kogut *et al*., 1995; Fearon & Locksley, 1996; Anderson, 2000; Akira, 2001; Janeway, Jr. & Medzhitov, 2002). Recognition of PAMPs by pattern recognition receptors (PRRs) on the host immune cell directs acquired immunity to elicit the appropriate host response to the microbe by inducing co-stimulatory molecules and/or cytokines; therefore initiating a Th1 or Th2 immune response (Romagnani, 1992; Medzhitov & Janeway, Jr., 1997a, b; Imler & Hoffmann, 2001).

Studies show that selection of birds based on rapid growth characteristics can adversely affect

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disease resistance and/or the immune response (Han & Smyth, 1972; Qureshi & Havenstein, 1994; Bayyari *et al*., 1997). Selecting birds that are resistant to a single pathogen has proven to be complicated and ineffective. For example, a study by Janss and Bolder showed that broilers with a reduced mortality rate to *Salmonella* infections shed bacteria for longer periods of time (Janss & Bolder, 2000). Other studies showed that leghorn chickens resistant to *Salmonella typhimurium* were also resistant to *S. gallinarum*, *S. pullorum*, and *S. enteritidis* whereas lines susceptible to *S. typhimurium* were also susceptible to the other *Salmonella* species, suggesting a common mechanism of resistance/susceptibility between different *Salmonella* serotypes (Bumstead & Barrow, 1993) as a heritable trait (Beaumont *et al*., 1999). Instead of identifying birds resistant to a single pathogen, our laboratory is interested in identifying biomarker(s) for birds that will indicate which lines have the potential to mount an overall effective immune response against multiple microorganisms. In this regard, evaluation of commercial flocks for heterophil functional efficiency could be of superior benefit compared with further genetic selection for resistance to one specific pathogen.

Heterophils, the avian equivalent to the mammalian neutrophil, ingest and kill a variety of microbial pathogens. These professional phagocytic cells exhibit an assortment of cytoskeletal and biochemical activities that can be easily assayed *in vitro* to evaluate the efficiency of immune competence in relation to disease susceptibility and/or resistance of different lines of poultry. Functionally, heterophils rapidly kill bacterial pathogens by phagocytosis, degranulation, and generation of an oxidative burst (Desmidt *et al*., 1996; Maxwell & Robertson, 1998; Genovese *et al*., 2000; Kogut *et al*., 2001a,b,c). Because of their early response and ability to kill pathogens, heterophils are a useful functional biomarker for evaluating the innate immune competence in poultry.

The initial objective of this project was characterization and comparison of the heterophil function between two pure broiler chicken lines (A and B) within the first 7 days post hatch. Second, based on differences in heterophil function observed in the pure lines, characterization of the heterophil function of the F1 reciprocal crosses generated from the mating of the pure lines (C = B male × A female, and D = A male × B female) was performed. For both studies, heterophils isolated on days 1, 4, and 7 post hatch were assayed for the ability to (1) phagocytose *Salmonella enteritidis* (SE), (2) degranulate when stimulated with immune-IgG opsonized SE (OpSE), and (3) generate an oxidative burst upon stimulation with an inflammatory agonist (phorbol A-myristate 13-acetate [PMA]).

Materials and methods

Experimental birds

Broiler chickens used in this study were obtained from a commercial breeder. To maintain confidentiality, the lines were designated lines A, B, C, and D, where lines A and B are pure lines and lines C and D are F1 reciprocal crosses of the two parent lines (C = A female × B male, and D = A male × B female). Fertilized eggs were set in incubators (G.Q.F. Manufacturing Company, Savannah, GA or Jamesway Incubator Company, Inc., Ontario, Canada) and maintained at wet and dry bulb temperatures of 32.2°C and 37.8°C, respectively. After 10 days of incubation, the eggs were candled and viable eggs were returned to the incubator until day 18, when they were transferred to hatchers (Humidaire Incubator Company, New Madison, OH) and maintained under the same temperature and humidity conditions until day of hatch. Upon hatching, 200–250 birds per line were placed in their respective floor pens (10 feet × 10 feet) containing wood shavings, provided supplemental heat, water, and a balanced un-medicated corn and soybean meal based diet *ad libitum*. The feed was calculated to contain 23% protein and 3200 kcal metabolizable energy (ME)/kg of diet, and all other nutrient rations met or exceeded the standards established by the National Research Council (National Research Council, 1994).

Experimental design

Ninety, 45, and 30 birds per line were used on days 1, 4, and 7, respectively, as blood donors. The blood from each day was pooled for the line to provide sufficient numbers of heterophils to run all three assays on a given day to reduce variability. Each assay was conducted at least three times with pooled heterophils from different flocks of birds. At least 4 replicates were conducted for each assay with the heterophils from each pool of chickens. The data from the repeated experiments were pooled for presentation and statistical analyses.

Isolation of peripheral blood heterophils

Heterophils were isolated from the peripheral blood of chicks on days 1, 4, and 7 post hatch as previously described (Kogut *et al*., 2001a). Briefly, blood from chicks was collected in tubes containing disodium ethylenediaminetetraacetic acid (EDTA) and mixed thoroughly. The blood and EDTA was pooled and diluted 1:1 with RPMI 1640 media containing 1% methylcellulose and centrifuged at 25 *g* for 15 min. The supernatant was transferred to a new conical tube and washed with Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution (1:1) and layered onto discontinuous Histopaque® gradients (specific gravity, 1.077 over 1.119). The gradients were centrifuged at 250 *g* for 1 h. The histopaque layers were collected, washed with RPMI 1640 (1:1), then the cells were re-suspended in fresh RPMI 1640, counted on a hemacytometer, and diluted to the desired concentration in RPMI. All tissue culture reagents and chemicals including RPMI 1640, Hanks balanced salt solution, methylcellulose, Histopaque® 1119, and Histopaque® 1077 were obtained from Sigma Chemical Company, St Louis, MO.

Salmonella enteritidis *(SE) preparations*

A poultry isolate of SE #97-11771 was obtained from the National Veterinary Services Laboratory (Ames, IA) and approved by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service for use in our facilities. SE was cultured in tryptic soy broth (TSB) overnight at 41° C. The bacteria were pelleted (7700 *g* for 10 minutes) and washed with cold phosphate buffered saline (PBS), centrifuged at 7700 *g* for 10 minutes, the supernatant was discarded, and the pellet re-suspended in 1 ml cold PBS and diluted to 1×10^9 colony forming units (cfu)/ml in PBS using a Spectronic 20D spectrophotometer (Milton Roy Co., Golden, CO) with a 625 nm reference wavelength. SE was prepared fresh for each experiment and kept on ice until used.

Opsonization of SE

The SE bacteria were opsonized as previously described (Kogut *et al*., 2001a). Briefly, SE (10^9 cfu/ml) was suspended in serum from chickens

immunized against SE (v:v [4:1]) and incubated for 30 min at 39°C on a rotary shaker. The immune-IgG opsonized SE (OpSE) was stored at 4°C until used.

Phagocytosis of SE

Heterophils were assayed for the ability to phagocytose live SE as previously described with the following modifications (Genovese *et al*., 2000; Kogut *et al*., 2001a). Heterophils (2 ml) were diluted to 4×10^6 cells/ml in RPMI and SE (1×10^9 cfu/ml) was added to the diluted heterophils (100 SE:1 heterophil) and incubated for 30 min at 39°C on a rocker to allow for phagocytosis. The samples were washed with an equal volume of RPMI and centrifuged at 190 *g* for 10 min and the supernatant was discarded. The heterophils were washed an additional three times with RPMI, and the pellet was re-suspended in the original volume (2 ml). Cytospin smears were prepared from each sample in a Shandon cytospin3 (Shandon Inc., Pittsburgh, PA), stained with Hematology 3-step stain (Biochemical Sciences, Inc., Swedesboro, NJ), and examined by light microscopy with the oil immersion objective (100 ×). Results are reported as the percent heterophils containing SE and the average number of SE/heterophil.

Degranulation assay

Degranulation was monitored by quantifying the levels of β-D-glucuronidase in the supernatant of heterophils as previously described (Kogut *et al*., 2001a). Heterophils (8×10^6 cells/ml) were treated with OpSE or RPMI for 1 h at 39°C on a rotary shaker. The samples were immersed in ice for 10 min to stop the reaction. Cells were removed by centrifugation (250 *g* for 15 min at 4°C), and the supernatants collected then assayed for β-D-glucuronidase activity. Briefly, samples and standards (25 µl) were added to a black flat-bottom ELISA plate and incubated with 50 µl freshly prepared substrate (10 mM 4-methylumbelliferyl-β-D-glucuronide dissolved in 0.1 M sodium acetate buffer containing 0.1% Triton X-100; pH 4.0 [Sigma]) for 4 h at 41°C. The reaction was stopped by the addition of 200 µl stop solution (0.05 M glycine, 5 mM EDTA; pH 10.4). Released 4-methylumbelliferone was measured on an *f*max fluorescence microplate reader (Molecular Devices, Sunnyvale, CA) (excitation = 355 nm, emission = 460 nm) and converted using a standard curve of known concentrations.

Oxidative burst assay

Oxidative burst was quantified by LDCL as previously described (Merrill *et al*., 1996). Briefly, heterophils (4×10^6 cells/ml) were incubated with either phorbol A-myristate 13-acetate (PMA [2.2 µg/ml], Sigma) or RPMI alone (comparable volume as was added for PMA treatment) for 30 minutes at room temperature. Samples were inverted every five minutes to ensure sufficient mixing. After the 30 min incubation, 400 µl of the samples were added to Beckman polypropylene scintillation vials containing 5-amino-2,3-dihydro-1,4-phthalazinedione sodium salt (luminol [500 µl of 0.01 M in RPMI 1640], Sigma). Samples were counted for 1 min/vial in a LKB A19 liquid scintillation counter (LKB Products, Turku, Finland) using the tritium channel and the incoincidence mode. Each sample was assayed in replicates of ten vials/experiment and expressed as an average counts per minute (cpm).

Statistical analyses

Statistical analyses were performed using the SigmaStat statistical software (SigmaStat statistical software, 1994). Results of pure line were compared using the Student's *t*-test. However, when crosses and pure lines were compared to one another, a one-way analysis of variance (ANOVA) was employed to determine statistical significance. Mean values were further analysed for significance with Tukey's Test. ($p < 0.05$).

Results

Pure Lines A and B

Phagocytosis of live SE. Heterophils were isolated from lines A and B and evaluated for the ability to phagocytize live SE on days 1, 4, and 7 post hatch (data not shown). On day 1, significantly ($p < 0.05$) more of the heterophils isolated from line A (78.8%) contained SE when compared to heterophils isolated from line B (64.1%). There were no significant differences in the average number of SE in heterophils between the two pure lines on day 1 post hatch. By days 4 and 7 post hatch, no significant differences were found in the percent heterophils with SE or the average number of SE that were phagocytized.

Degranulation. The release of bactericidal intracellular granules was compared in heterophils isolated from lines A and B (Figure 1). On day 1 post hatch, heterophils isolated from line A (■) released significantly ($p < 0.05$) more β-D-glucuronidase when compared with heterophils isolated from line B (●) (58.4 ± 5.6 and 38.3 ± 4.6 µM, respectively). The significant difference was maintained through day 4 as heterophils isolated from line A released 84.7 ± 17.2 µM β-D-glucuronidase compared to 49.7 ± 15.0 µM for heterophils isolated from line B. There were no significant differences observed in the ability of isolated heterophils to degranulate on day 7 between the two pure lines (Figure 1).

Oxidative burst. Luminol-dependent chemiluminescence (LDCL) was used to determine if there was a difference in the functional ability of stimulated heterophils isolated from the pure lines (A [■] and B [●]) to produce an oxidative burst (Figure 2). On days 1 and 4 post hatch, heterophils isolated from line A generated a significantly ($p < 0.05$) higher oxidative burst than heterophils isolated from line B (day 1, $34.6 \pm 4.8 \times 10^6$ and $19.6 \pm 2.9 \times 10^6$, respectively; day 4, $21.2 \pm 2.0 \times 10^6$ and $13.2 \pm 1.8 \times 10^6$, respectively). By day 7 post hatch, there were no statistical differences in the ability of heterophils isolated from line A or B to generate an oxidative burst (Figure 2).

F1 reciprocal Crosses (Lines C and D)

Phagocytosis of live SE. Heterophils isolated from lines C and D, the F1 reciprocal crosses of lines A and B, were evaluated for their ability to phagocytose SE on days 1, 4, and 7 post hatch (data not shown). For all experiments, birds from both parent lines were used as controls. On days 1 and 4 post hatch, there were no significant differences in the average number of SE phagocytosed per heterophil between either cross when compared to heterophils isolated from the parent lines. On day 7 post hatch, there were significantly ($p < 0.05$) fewer

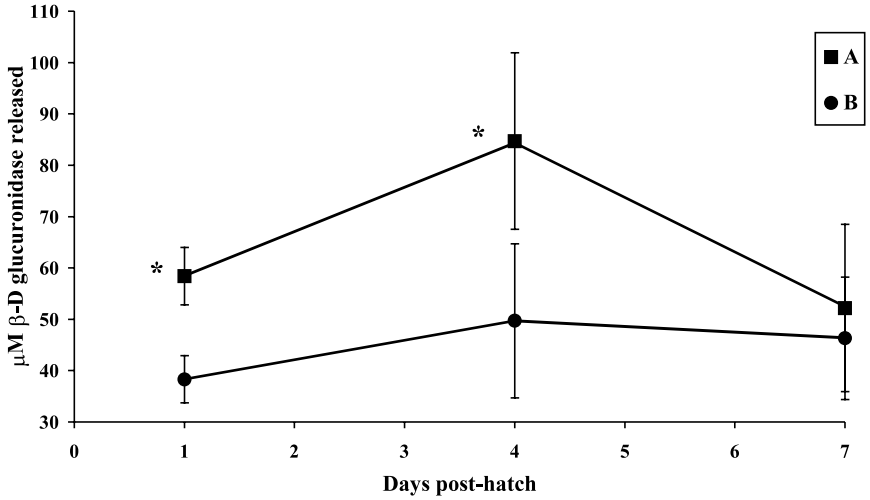

Figure 1. *Heterophils isolated from lines A (■) and B (●) degranulate when stimulated with opsonized SE. The x-axis is the day post hatch and the y-axis is μM β-D glucuronidase released. Data are presented as the mean ± standard error mean (SEM) of triplicate assays from at least three separate experiments. [* = (p < 0.05)].*

SE per heterophil in cells isolated from cross C when compared to heterophils isolated from the parental line B. On day 1 post hatch, heterophils isolated from both crosses had a significantly ($p < 0.05$) lower percentage of heterophils with SE when compared with heterophils isolated from the parental line A, but not when compared with heterophils from parent line B. By 7 days post hatch, the percentage of heterophils with SE isolated from cross D was significantly ($p < 0.05$) lower than those isolated from parental line B. There were no differences in the percent heterophils with SE nor the average number of SE/heterophil between cells isolated from cross C when compared with cross D.

Degranulation. The ability of heterophils isolated from the F1 reciprocal crosses (lines C and D) to degranulate was evaluated and compared to heterophils isolated from the parent lines A and B (Figure 3). On days 1 and 4 post hatch the amount of β-D-glucuronidase released by heterophils isolated from parental line A was significantly (* = $p < 0.05$) greater than the μM amount of β-D-glucuronidase released by heterophils isolated from line C (day $1 = 61.5 \pm 5.6$ and 36.9 ± 6.7, respectively; day 4, 75.7 ± 24.3 and 25.8 ± 12.5, respectively). No differences were observed on days 1 and 4 post hatch between heterophils isolated from parent line B when compared to heterophils isolated from line C (day $1 = 39.5 \pm 5.4$ and 36.9 ± 6.7, respectively; day $4 = 34.8 \pm 7.2$ and 25.8 ± 12.5, respectively). On days 1 and 4 post hatch, μM β-D Glucuronidase released by heterophils from line D was significantly higher than that released by heterophils from line B († = $p < 0.05$). There were no statistical differences between cells isolated from parental line A and the D crosses for their ability to degranulate. Heterophils isolated from cross D released significantly more β-D-

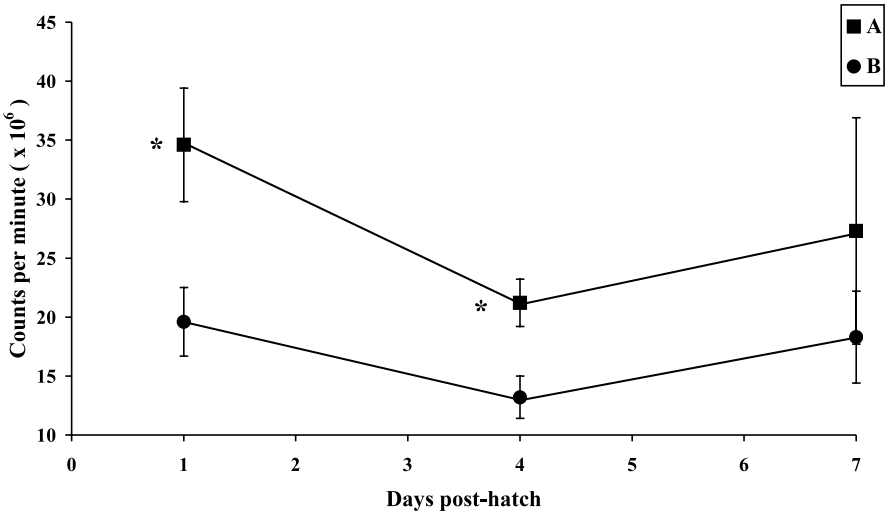

Figure 2. *Heterophils isolated from lines A (■) and B (●) generate an oxidative burst response when stimulated with the inflammatory agonist PMA. The x-axis is the day post hatch and the y-axis is the counts per minute ($\times 10^6$). Data are presented as the mean ± SEM of triplicate assays from at least three experiments. [* = (p < 0.05)].*

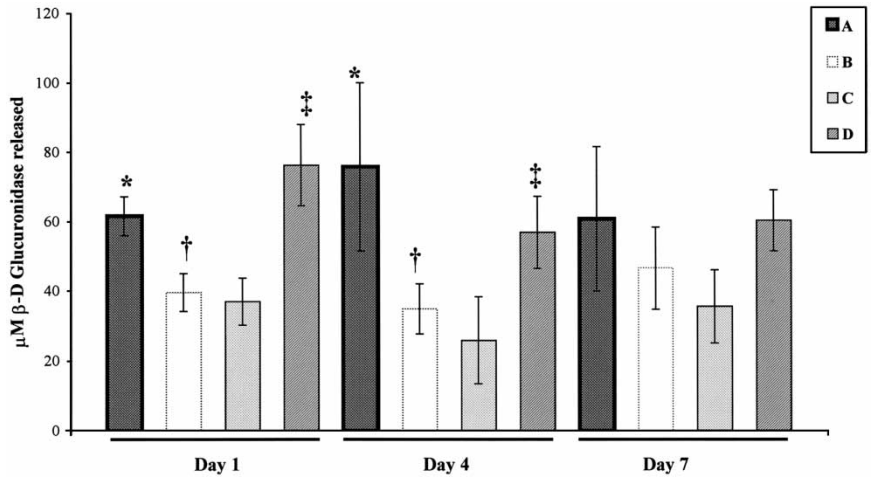

Figure 3. *Heterophils isolated from F1 crosses (lines C and D) compared to heterophils isolated from parental lines (A and B) regarding their ability to degranulate when stimulated with opsonized SE. The x-axis is the day post hatch and is grouped by day (—). The y-axis is μM β-D Glucuronidase released. Data are presented as the mean ± SEM of triplicate assays from at least three separate experiments. All statistical differences are for line (A, B, C, and D) comparisons are on an individual day and not between different days post-hatch. On days 1 and 4 post hatch, μM β-D Glucuronidase released by heterophils from line A was significantly greater than that released by heterophils from line C [* = (p < 0.05)]. On days 1 and 4 post hatch, μM β-D Glucuronidase released by heterophils from line D was significantly higher than that released by heterophils from line B [† = (p < 0.05)]. Heterophils from line D released significantly more μM β-D Glucuronidase than heterophils from line C on days 1 and 4 post hatch [‡ = (p < 0.05)].*

glucuronidase on days 1 and 4 post hatch when compared with heterophils from cross line C (‡ = $p < 0.05$). There were no differences observed at 7 days post hatch between any of the lines examined.

Oxidative burst. Oxidative burst was measured in the F1 reciprocal crosses (lines C and D) and compared with the parent lines (lines A and B) (Figure 4). On days 1 and 4 post hatch, the oxidative burst generated by heterophils from line A was significantly (* = $p < 0.05$) higher than generated by heterophils from line C (day 1 = $34.8 \pm 1.5 \times 10^6$ and $13.5 \pm 3.3 \times 10^6$, respectively; day 4 = $24.0 \pm 1.1 \times 10^6$ and $10.0 \pm 2.4 \times 10^6$, respectively). There were no statistical differences between heterophils from parent line B and cross line C to produce an oxidative burst response on day 1 or 4 post-hatch (day 1, 15.8 ± 5.4 and $13.5 \pm 3.3 \times 10^6$, respectively; day 4 = $15.0 \pm 2.7 \times 10^6$ and $10.0 \pm 2.4 \times 10^6$, respectively). Heterophils isolated from cross line D produced a significantly († = $p < 0.05$) higher oxidative burst than heterophils isolated from parental line B on day 1 ($35.3 \pm 2.4 \times 10^6$ and 15.8 ± 5.4, respectively). On day 1, hetero-

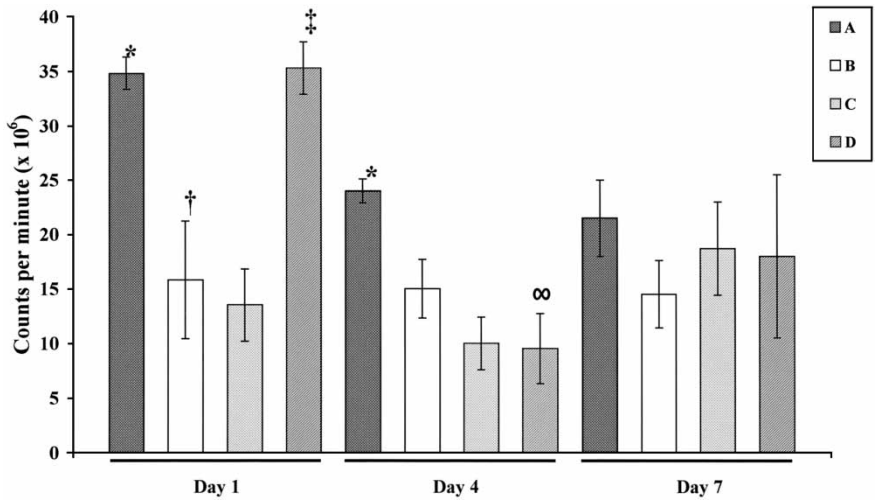

Figure 4. *Heterophils isolated from F1 crosses (lines C and D) compared with heterophils isolated from parental lines (A and B) regarding their ability to generate an oxidative burst response when stimulated with the inflammatory agonist PMA. The x axis is the day post hatch and is grouped by day, and the y axis is the counts per minute ($\times 10^6$). Data are presented as the mean ± SEM of triplicate assays from at least three separate experiments. On days 1 and 4 post-hatch, the oxidative burst generated by heterophils from line A was significantly higher than generated by heterophils from line C (*$p < 0.05$). On day 1 post-hatch, heterophils from line D produced a significantly greater response than heterophils from line B († $p < 0.05$). Heterophils from line D produced a significantly higher response than heterophils from line C on day 1 post-hatch (‡ $p < 0.05$). On day 4 post-hatch, the oxidative burst response by heterophils isolated from line D was significantly lower than the response of heterophils from line A (∞ $p < 0.05$).*

phils isolated from cross line D generated a significantly ($\ddagger = p < 0.05$) higher oxidative response than heterophils from cross line C ($35.3 \pm 2.4 \times 10^6$ and $13.5 \pm 3.3 \times 10^6$, respectively). By day 4, heterophils isolated from cross D generated a significantly ($\infty = p < 0.05$) lower oxidative burst than heterophils isolated from parent line A ($9.5 \pm 3.2 \times 10^6$ and $24.0 \pm 1.1 \times 10^6$, respectively). No differences were observed after 7 days post hatch by any of the lines to generate an oxidative burst in response to stimulation with an inflammatory agonist.

Discussion

Genetic engineering and/or selecting for chickens that are resistant to various pathogens has been shown to be difficult (Bumstead & Barrow, 1988, 1993; Gavora, 1993; Beaumont *et al*., 1999; Lamont *et al*., 2002). Furthermore, selecting birds with a strong general immunological response can reduce the growth characteristics and qualities required by the poultry industry (Han & Smyth, 1972; Qureshi & Havenstein, 1994; Bayyari *et al*., 1997). The emerging evidence indicating that the innate immune response directs the acquired response (Bendelac & Fearon, 1997; Parish & O'Neill, 1997) supports efforts to direct the selection process toward the early innate immune response. The evaluation of heterophil functional efficiency as a biomarker in neonatal chickens seems a practical approach to select for an efficient innate immune response in poultry. The general immunological responsiveness of four lines of commercial broilers was assessed using heterophil functional efficacy as a biomarker of the innate immune response.

The comparison of heterophils isolated from lines A and B revealed that heterophils from line A were more immunologically competent than heterophils from line B, as shown by our established *in vitro* assays (Figures 1 and 2). The most significant differences in functional efficacy were apparent within the first four days post hatch. Based on the initial results with the pure lines (Figures 1 and 2), studies were performed to assess heterophils isolated from F1 reciprocal crosses of the pure lines (Figures 3 and 4). We identified line D as an immunologically efficient cross demonstrated by *in vitro* heterophil functional efficiency. This observation suggests that heterophil functional efficiency observed in the immunologically dominant line A chicks is a heritable trait. Birds from lines A and D are therefore more likely to survive and do well during infection with SE in the first week of life compared with birds from lines B and C as their heterophils are more proficient at phagocytosing bacteria, undergoing degranulation upon stimulation and generating an oxidative burst response. In fact, the mortality data through the first 6 weeks of life for lines A (3.6%) and B (5.7%) support these findings (data not shown). The resistance and/or susceptibility of the lines to SE or other pathogens is not known at this time. Therefore, *in vivo* infection studies are required to fully understand the complex relationship between *in vitro* heterophil function and the overall immunological competence of the birds.

As the receptors for recognition of microbial pathogens by the innate immune response are encoded in the host genome (Medzhitov & Janeway, Jr., 1997b), we wanted to determine if the heterophil functional efficiency observed in line A could be genetically transferred to F1 progeny. Indeed, heterophils from cross line D (A male × B female) chicks are immunologically efficient compared to heterophils from cross C (B male × A female) chicks. These data suggest the genetic contribution from a line A female is not sufficient to confer heterophil efficiency. The data clearly demonstrate efficient heterophil function elicited by line A was not an autosomal dominant trait since there was a significant difference in the ability of heterophils to function efficiently between lines C and D, both of which had an "A" line parent. Genetically, the only difference between the two reciprocal crosses is that for line C the hen was from line A while for line D the rooster was from line A. These results suggest an immunologically efficient heterophil function is a heritable trait that may be contributed, in part, by the rooster. However, additional studies are required to fully elucidate the heritability and sex-linkage of heterophil function(s) in broiler chickens.

A study by Klingensmith and colleagues showed that broilers homozygous for the sex-linked dwarfism (*dw*) gene may have a more competent cellular immune response, specifically more efficient T cell subpopulations, when compared to normal birds (Klingensmith *et al*., 1983). This study indicates there may be a relationship between immunity and sex-linked traits. To our knowledge, there are no reports to indicate that heterophil function or the innate immune response of chickens is under the influence of being a sex-associated trait. Further studies examining other lines of birds and their genetic crosses are required to elucidate the innate immune response and/or heterophil functional efficiency.

The information gained from this study indicates a role for utilizing immunologically competent birds with an effective heterophil function. Evaluation of genetically diverse lines and F1 reciprocal crosses allowed us to show that lines respond differently and that an effective heterophil response is heritable and passed on to progeny flocks. Selection for lines that have an immunologically efficient heterophil function may also select for birds with a strong innate immune response. An effective heterophil and/or innate immune response

will allow birds to (1) eliminate or at least limit an infection within the first week post hatch and (2) to rapidly mount reactive immune responses. Evaluation of commercial flocks for an innate immune response biomarker such as heterophil functional efficiency within the first week post hatch would be advantageous compared to further genetic selection for resistance against a single pathogen.

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RÉSUMÉ

Comparaison fonctionnelle des hétérophiles isolés chez des poulets de chair commerciaux

Les hétérophiles de deux lignées pures (A et B) de poulets de chair commerciaux ont été prélevés à 1, 4 et 7 jours après l'éclosion pour évaluer leur capacité à (1) la phagocytose de *Salmonella enteritidis* (SE), (2) la dégranulation quand ils sont exposés à SE opsonisée par des IgG et (3) la production d'une oxydation. Les hérérophiles de la lignée A, prélevés à un et quatre jours ont eu une activité plus efficace comparés à ceux de la lignée B ($p < 0.05$). Sept jours après l'éclosion, les fonctions des hétérophiles des deux lignées ont été comparables. Une étude complémentaire d'hérédité de l'efficacité des fonctions des hétérophiles chez les différents croisements de la génération F1 (lignée C: mâle B × femelle A; lignée D = mâle A × femelle B) a été réalisée; ces croisements ont été comparés aux lignées parentales, A immunologiquement efficace et B non efficace. Les hétérophiles de la lignée D ont présenté une activité significativement supérieure ($p < 0.05$) comparés à ceux de la lignée C. Ces résultats suggèrent que la fonction des hétérophiles et leur activité peuvent être transmises génétiquement à la descendance. De plus, ils montrent que la fonction des hétérophiles est liée au sexe et est contrôlée génétiquement par le coq puisque la descendance de la lignée mâle A a gardé les caractéristiques d'efficacité immunologiques

alors que les hétérophiles de la descendance de la lignée mâle B sont immunologiquement inefficaces. A notre connaissance, il s'agit du premier rapport décrivant les relations fonctionnelles entre deux lignées pures et les produits de la génération F1 chez le poulet de chair relatives aux hétérophiles et à la réponse immunologique innée.

ZUSAMMENFASSUNG

Funktionsvergleich von aus kommerziellen Broilern isolierten heterophilen Granulozyten

Aus kommerziellen Broilerküken von zwei reinen Zuchtlinien (A und B) wurden am Tag 1, 4 und 7 nach dem Schlupf heterophile Granulozyten isoliert, um deren Fähigkeit (1) *Salmonella enteritidis* (SE) zu phagozytieren, (2) nach Exposition mit Immunglobulin G opsonierten SE zu degranuleren und (3) einen oxidativen Ausbruch zu produzieren. Am 1. und 4. Tag waren die Heterophilen aus Linie A funktionell effizienter im Vergleich zur Linie B ($p < 0.05$). Am 7. Tag nach dem Schlupf waren die Funktionen in beiden Linien ähnlich. Zur weiteren Untersuchung der Vererbung der Effizienz heterophiler Funktionen wurden die F1 reziproken Kreuzungen (Linie C = männliche B × weibliche A; Linie D = männlicheA × weibliche B) auf ihre funktionelle Aktivität überprüft und mit den immunologisch effizienten (A) und nicht effizienten (B) Elternlinien verglichen. Die Heterophilen von D wiesen im Vergleich zu Heterophilen von C eine effizientere Funktion auf ($p < 0.05$). Diese Ergebnisse suggerieren, dass die Funktion und Effizienz der Heterophilen genetisch auf die Nachkommen übertragen weden kann. Überdies zeigen sie, dass die Funktion der Heterophilen geschlechtsgebunden ist und genetisch von den Hähnen bestimmt wird, da die Nachkommen der männlichen Tiere von Linie A die immunologisch effizienten Charakteristika behielten, während die Heterophilen der Nachkommen der Hähne von Linie B immunologisch ineffizient blieben. Unseres Wissens nach ist dies der erste Bericht, der die funktionalen Beziehungen hinsichtlich der heterophilen Granulozyten und deren angeborener Immunantwort zwischen reinen Linien und den F1 reziproken Kreuzungen von Broilern beschreibt.

RESUMEN

Comparación funcional de heterófilos aislados de pollos de engorde comerciales

Se asilaron los heterófilos de dos líneas puras (A y B) de pollos de engorde comerciales a los días 1, 4 y 7 tras el nacimiento con el objetivo de valorar la habilidad de estas células para (1) fagocitar *Salmonella enteritidis* (SE) (2) degranular al ser expuestos a SE opsonizada con IgG, y (3) producir un estallido respiratorio. A los días 1 y 4, los heterófilos de la línea A eran funcionalmente más eficientes en comparación con los de la línea B ($P < 0.05$). Al séptimo día tras el nacimiento, la funcionalidad de los heterófilos de ambas líneas era similar. Con el objetivo de estudiar en más profundidad la herencia de la eficiencia de la función heterofílica, se evaluó la actividad funcional de los F1 de dos cruces recíprocos (línea C = macho B × hembra A; línea D = macho A × hembra B) y se compararon con las líneas paternas inmunológicamente eficientes (A) y no eficientes (B). Los heterófilos de la línea D presentaron una función heterofílica mucho más eficiente ($P < 0.05$) que los de la línea C. Estos resultados sugieren que la función y la eficiencia de los heterófilos puede ser genéticamente transmitida a la progenie. Además, indica que la función de los heterófilos está asociada al sexo y está genéticamente controlada por los gallos ya que la progenie de la línea de machos A mantuvo las características de eficiencia inmunológica, mientras que los heterófilos de la progenie de la línea de gallos B permanecieron inmunológicamente ineficaces. Según nuestro conocimiento, esta es la primera descripción de la relación funcional entre líneas puras y los F1 de cruces recíprocos en relación a los heterófilos y la respuesta inmune innata.