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Heterophil cytokine mRNA profiles from genetically distinct lines of chickens with differential heterophil-mediated innate immune responses

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Previously we demonstrated that increased *in-vitro* heterophil function translates to increased *in-vivo* resistance to *Salmonella enteritidis* infections in broilers (line A > B). Heterophils produce cytokines and modulate acute protection against *Salmonella* in neonatal poultry. We hypothesized that heterophils from *S. enteritidis*-resistant chickens produce an up-regulated pro-inflammatory cytokine/chemokine response compared with *S. enteritidis*-susceptible chickens. In this study, heterophils were isolated 1, 14, and 28 days post-hatch, treated with RPMI or phagocytic agonists, and the cytokine/chemokine mRNA expression assessed using quantitative real-time reverse transcriptase-polymerase chain reaction. At all time-points, heterophils from *S. enteritidis*-resistant chickens (line A) had higher levels of pro-inflammatory cytokine/chemokine mRNA expression upon stimulation compared with heterophils from *S. enteritidis*-susceptible chickens (line B). Furthermore, heterophils from line A chickens had decreased mRNA expression of transforming growth factor-β4, an anti-inflammatory cytokine, compared with line B. These data indicate a relationship between cytokine/chemokine mRNA expression by heterophils and determining overall immune competence. Therefore, heterophil functional efficiency, accompanied by evaluating cytokine/chemokines produced by heterophils, may be useful biomarkers for breeders to consider when developing new immunocompetent lines.

Introduction

Studies over the past decade indicate that innate immunity, in addition to the first line of defence, provides instruction for acquired immunity (Medzhitov & Janeway, 2002; Hoebe *et al*., 2004). This cascade of events begins with specific receptors (pattern recognition receptors) on the host's innate immune response cells recognizing conserved molecular motifs unique to invading organisms (pathogen-associated molecular patterns) (Akira, 2001; Janeway & Medzhitov, 2002). The end product of microbial recognition by the innate system is activation of intracellular signalling pathways that initiate cellular processes, such as activation of microbicidal killing mechanisms, production of pro-inflammatory and/or anti-inflammatory cytokines/chemokines, and production of co-stimulatory molecules required for antigen presentation to the acquired immune system (Medzhitov & Janeway, 1997; Imler *et al*., 2001).

As the first cells to migrate to the infection site, polymorphonuclear leukocytes (PMN) are critical components of innate immunity and subsequent inflammatory response (Hachicha *et al*., 1998; Kobayashi *et al*., 2002). Heterophils, the primary PMN in chickens, are the avian counterpart to mammalian neutrophils (Burton & Harrison, 1969; Maxwell & Robertson, 1998). Functionally, heterophils modulate the acute innate host response through phagocytosis of invading microbes and foreign particles, production of oxygen intermediates, and releasing proteolytic enzymes (Desmidt *et al*., 1996; Kogut *et al*., 2001; Swaggerty *et al*., 2003b). Until recently, heterophils were thought to be terminally differentiated cells, deficient of transcriptional activity, and with little protein synthesis. However, using quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) measuring specific mRNA expression of cytokines and chemokines, we showed that heterophils isolated from 1-day-old chickens have a distinct pro-inflammatory or anti-inflammatory response dependent on the stimulant (Kogut *et al*., 2003b). We have demonstrated that increased *in-vitro* heterophil functional efficiency translates to increased *in-vivo* resistance to *Salmonella enteritidis* and vancomycin-resistant *Enterococcus gallinarum* infections utilizing a pair of parental broiler lines (line A is more resistant than line B) (Swaggerty *et al*., 2003b, 2005a,b; Ferro *et al*., 2004). Additionally, heterophils isolated from 1-day-old line A and B chickens have a differential constitutive and agonist-stimulated cytokine profile (Swaggerty *et al*., 2004).

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Cytokines/chemokines are essential effector molecules of innate and acquired immunity that initiate and coordinate responses aimed at eradicating pathogens. Detecting avian cytokines is limited by the lack of specific antibodies and reliable bioassays. However, recent cloning of chicken cytokines has enabled development of a more comprehensive array of reagents for investigating innate and acquired immune responses at cellular and molecular levels. Chicken orthologues of T1 cytokines interferon-γ (Digby & Lowenthal, 1995), interleukin (IL)-18 (Schneider *et al*., 2000), and IL-12 (Balu & Kaiser, 2003; Degen *et al*., 2004), pro-inflammatory cytokines IL-1β (Weining *et al*., 1998) and IL-6 (Schneider *et al*., 2001), chemokine CXCLi2 (formerly IL-8) (Bedard *et al*., 1987; Sugano *et al*., 1987), T2-specific cytokines, IL-4, IL-13, and IL-3 (Avery *et al*., 2004), and anti-inflammatory cytokines transforming growth factor-β4 (TGF-β4) (Jakowlew *et al*., 1988) and IL-10 (Rothwell *et al*., 2004) have been cloned and sequenced (Kaiser *et al*., 2005). This makes it possible to design probes and primers to quantify cytokine mRNA expression using qRT-PCR in a specific population of cells, such as heterophils. Measuring cytokine mRNA expression by qRT-PCR does not equate to production of bioactive protein. However, recent publications show qRT-PCR is the most highly sensitive method available to reliably quantify a broad spectrum of avian cytokines, particularly in the absence of effective bioassays (Kaiser *et al*., 2000, 2002, 2003; Beal *et al*., 2004; Swaggerty *et al*., 2004; Withanage *et al*., 2004; Smith *et al*., 2005).

Economic pressure on the poultry industry has directed genetic selection to develop birds that grow rapidly while consuming less feed. However, selection based on growth characteristics alone can adversely affect immune competence (Han & Smyth, 1972; Bayyari *et al*., 1997; Beaumont *et al*., 1999). Assessment of innate immune indices, specifically cytokine mRNA expression by heterophils, may be a useful selection tool that will indicate the overall immunological responsiveness of a particular line of commercial poultry. The objectives of the present study were to further characterize the cytokine/chemokine profile of heterophils isolated from chickens from lines A and B and to determine whether the differences observed 1-day post-hatch are maintained over time. Specifically, we used qRT-PCR to measure mRNA expression levels of IL-6, CXCLi2, IL-18, and TGF-β4 following treatment with phagocytic agonists 1, 14, and 28 days post-hatch.

Materials and Methods

Chickens. Chickens were obtained from a commercial breeder and designated line A and line B. Fertilized eggs were incubated and hatched under standard conditions (Stromberg, 1975). At hatch, chickens were placed in floor pens containing wood shavings, provided supplemental heat, water, and unmedicated corn and soybean meal-based chick starter diet *ad libitum* until day 14, then transitioned to grower diet. Both feeds met or exceeded standards established by the National Research Council (1994).

Bacteria. A poultry isolate of *S. enteritidis* (#97-11771) was obtained from the National Veterinary Services Laboratory (Ames, IA, USA) and approved by the United States Department of Agriculture. *S. enteritidis* was cultured in tryptic soy broth (Difco Laboratories, Becton Dickinson Co., Sparks, Maryland, USA) overnight at 41°C. Stock *S. enteritidis* (1×10^9 colony forming units/ml) was prepared as previously described (Swaggerty *et al*., 2003b). *S. enteritidis* were opsonized with normal chicken serum (NCS-OpSE) or hyper-immune serum from adult chickens immunized against *S. enteritidis* (IgY-OpSE) as previously described (Kogut *et al*., 2001).

Heterophil isolation. Heterophils were isolated from peripheral blood of 50 chickens per line on day 1, 12 chickens per line on day 14 and eight chickens per line on day 28. Blood from 1-day-old chickens was collected in vacutainer tubes containing disodium ethylenediamine tetraacetic acid (EDTA) (BD vacutainer, Franklin Lakes, New Jersey, USA) and mixed thoroughly. Blood was collected into 50 ml tubes containing 5 ml K_3EDTA (0.15 M) on days 14 and 28. Following blood collection, heterophils were isolated as previously described (Swaggerty *et al*., 2003b). Tissue culture reagents obtained from Sigma Chemical Company (St Louis, Missouri, USA).

RNA isolation. Heterophils (1×10^7) were treated with 300 μl RPMI, SE, NCS-OpSE, or IgY-OpSE for 30 min at 39°C on a rotary shaker. Treated heterophils were pelleted and washed with RPMI ($485 \times g$ for 15 min at 4°C), the supernatant discarded, and the cells re-suspended in lysis buffer (Qiagen RNeasy mini RNA extraction kit; Qiagen Inc., Valencia, California, USA), and frozen. Total RNA was extracted from homogenized (QIAshredder columns) lysates according to the manufacturer's instructions.

Quantitative real-time reverse transcriptase-polymerase chain reaction. Cytokine/chemokine mRNA expression in control and agonist-treated heterophils were quantitated using a method described by Kaiser *et al*. (2000) and Moody *et al*. (2000). Primers and probes have been described (Kaiser *et al*., 2000; Kogut *et al*., 2003b; Swaggerty *et al*., 2004). qRT-PCR was performed using the Reverse Transcriptase qPCR Master Mix RT-PCR kit (Eurogentec, San Diego, California, USA). Amplification and detection of products were performed using the ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). Results are expressed as the fold change from controls.

Statistical analyses. Anti-coagulated blood from 50, 12, and eight chickens per line (1, 14, and 28 days post-hatch, respectively) were pooled and heterophils were isolated. Each blood collection and heterophil isolation was conducted on three separate days (heterophils pooled from 50, 12, and eight chickens for each experiment; i.e. 150, 36, and 24 total chickens were used per line as cell donors on days 1, 14, and 28, respectively). The mean and standard error of the mean were calculated from pooled data. Statistical analyses (Student's t test) were performed using Microsoft® Excel 2000 ($P \leq 0.05$).

Results and Discussion

We recently showed that heterophils express increased levels of cytokine mRNA upon receptor-mediated phagocytosis (Kogut *et al*., 2003b), indicating that heterophils are capable of transcriptional activity and protein synthesis. Previously, we quantified basal and agonist-stimulated cytokine mRNA expression in heterophils from 1-day-old line A and B chickens (Swaggerty *et al*., 2004). The ability to initiate effective inflammatory and T1 responses results in efficient stimulation and direction of acquired immunity (Fearon & Locksley, 1996; Medzhitov & Janeway, 1997). *In-vivo* challenges using line A and B chickens showed line A chickens are more resistant to *S. enteritidis*-induced mortality (Swaggerty *et al*., 2005a) and extra-intestinal organ invasion (Ferro *et al*., 2004) and vancomycin-resistant *E. gallinarum* organ invasion (Swaggerty *et al*., 2005b).

Herein, heterophils were isolated from line A and B chickens and the mRNA expression of cytokines and chemokines quantified through 28 days post-hatch. Data indicate heterophils from genetically distinct chickens have a differential cytokine/chemokine profile and responsiveness to phagocytic agonists. The differences

in mRNA expression are maintained to 28-days post-hatch and therefore may be potential selection parameters to identify poultry resistant to diverse pathogens.

There were differences ($P \leq 0.05$) between IL-6 mRNA expression of heterophils isolated from the two lines regardless of the phagocytic agonist (SE, NCS-OpSE or IgY-OpSE) (Figure 1). Heterophils isolated from line A chickens expressed significantly more IL-6 mRNA (Figure 1a) compared with heterophils from line B chickens (Figure 1b). The increase in IL-6 expression was maintained from 1, 14, and 28 days post-hatch. The fold change levels ranged from 7.88 to 10.39 in heterophils from line A chickens compared with 1.4 to 4.67 in heterophils from line B chickens.

Pro-inflammatory and Th1 cytokines are important in immunological responses. In mammals, IL-6 stimulates neutrophils to degranulate (Borish *et al*., 1989). It is possible that the increased degranulation observed between lines A and B (Swaggerty *et al*., 2003b) is attributed to increased IL-6 expression, thus creating an environment where heterophils respond more efficiently and eliminate pathogens. However, it is also likely that IL-6 alone is not sufficient to confer resistance against *Salmonella*, as recombinant chicken IL-6 does not affect *in-vitro* heterophil function (Ferro *et al*., 2005). In mammals, IL-6 accompanied with other cytokines may act collaboratively (Keller *et al*., 1996). It is likely that additional cytokines and/or other factors influence resistance of chickens to *S. enteritidis*. Heterophils migrate to the liver and intestinal villi of newly hatched chickens infected with *Salmonella typhimurium*, accompanied by elevated levels of pro-inflammatory cytokine in the tissues (Withanage *et al*., 2004), further indicating a potential role of the heterophil and the acute inflammatory response in neonatal chickens. Taken together, these data indicate previously observed increased resistance against bacterial infections by line A chickens compared with line B chickens is influenced, in part, by the presence of a strong pro-inflammatory cytokine response, including IL-6, elicited by heterophils.

Expression of the pro-inflammatory chemokine CXCLi2 (formerly IL-8) was up-regulated in heterophils from both lines of chickens following treatment and was maintained throughout the experiment (Figure 2). Recent analysis of the chicken genome sequence revealed that the previous nomenclature for chicken inflammatory chemokines was incorrect; therefore, a new nomenclature has been proposed (Kaiser *et al*., 2005). As with IL-6, expression of CXCLi2 mRNA was higher ($P \leq 0.05$) in heterophils from line A chickens (Figure 2a) compared with line B (Figure 2b). The fold changes for heterophils from line A chickens ranged from 9.83 to 11.51 over the course of the experiment compared with 2.72 to 4.3 for heterophils from line B chickens.

In addition to an effective pro-inflammatory cytokine response, a strong chemotactic response may also influence susceptibility of chickens to *S. enteritidis*. Chemokines are small, structurally related chemoattractant molecules that regulate movement of various leukocytes (Zlotnick & Yoshie, 2000). In mammals, IL-8 is a pro-inflammatory chemokine involved in recruitment of PMN to the site of infection (Rot, 1991), and an IL-8-like (CXCLi2) chemokine is involved in heterophil recruitment to the site of infection in *S. enteritidis*-infected neonatal chickens (Kogut, 2002). One-day-old chickens infected with *S. typhimurium* had elevated mRNA expression of pro-inflammatory cytokines/chemokines in the liver and small intestine (Withanage *et al*., 2004). Additionally, there is increased infiltration of heterophils into tissues of *S. typhimurium*-infected chickens. Based on studies from our laboratory, increased numbers of heterophils in tissues may contribute to up-regulation of pro-inflammatory cytokine mRNA. We have shown a differential IL-8 (CXCLi2) response in heterophils from *S. enteritidis*-challenged chickens and observed a significant up-regulation in line A chickens compared with line B (Ferro *et al*., 2004). Similarly, IL-8 (CXCLi2) mRNA expression was up-regulated in other *Salmonella*-resistant chickens (Sadeyen *et al*., 2004). Since heterophils from line A chickens are functionally more efficient *in vitro* (Swaggerty *et al*., 2003a,b) it is likely that the recruited heterophils are more efficient at eliminating an infection. In fact, we have shown that chickens from line A are more resistant to bacterial

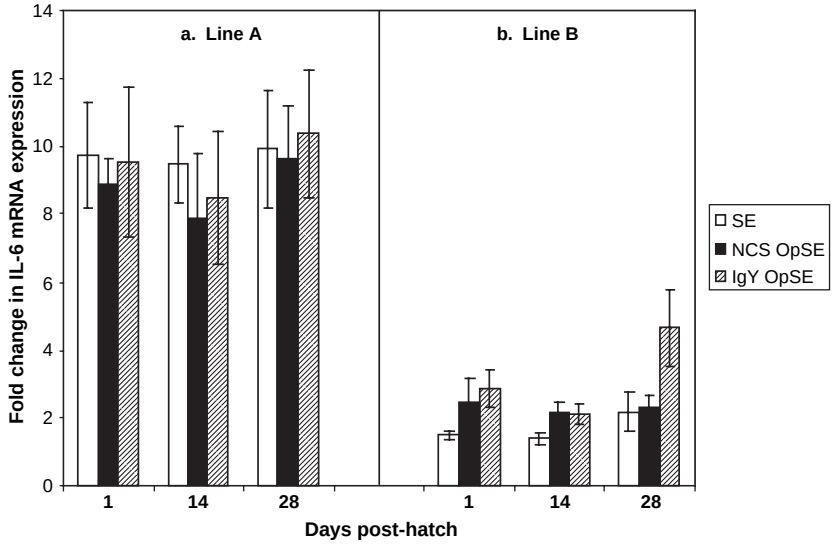

Figure 1. *Quantitation of IL-6 mRNA expression in heterophils isolated from (1a) line A chickens and (1b) line B chickens on days 1, 14, and 28 post-hatch. Data are expressed as the fold change in IL-6 mRNA levels when treated samples (*S. enteritidis *[SE], NCS-OpSE, and IgY-OpSE) were compared with control heterophils treated with RPMI. Error bars show the standard error of the mean from triplicate experiments.*

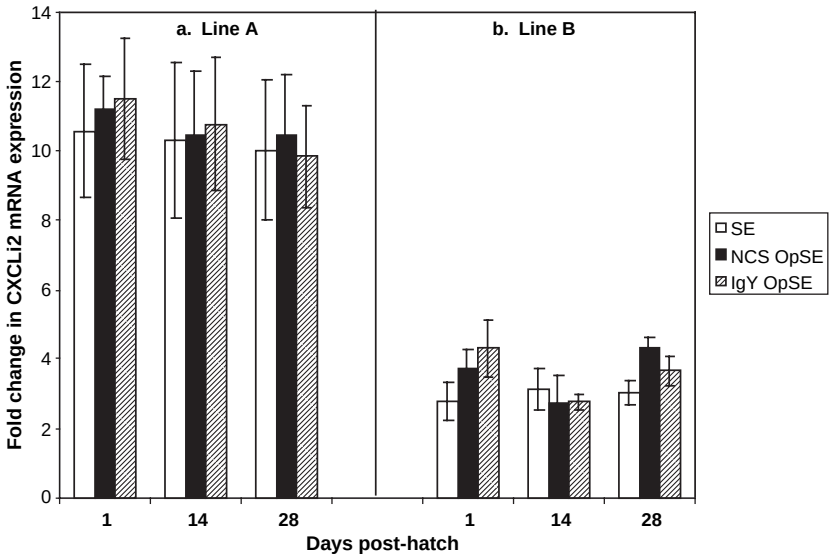

Figure 2. *Quantitation of CXCLi2 mRNA expression in heterophils isolated from (2a) line A chickens and (2b) line B chickens on days 1, 14, and 28 post-hatch. Data are expressed as the fold change in CXCLi2 mRNA levels when treated samples (*S. enteritidis *[SE], NCS-OpSE, and IgY-OpSE) were compared with control heterophils treated with RPMI. Error bars show the standard error of the mean from triplicate experiments.*

infections than chickens from line B (Ferro *et al*., 2004; Swaggerty *et al*., 2005a,b).

IL-18 mRNA expression was evaluated in heterophils from line A and B chickens at 1, 14, and 28-days post-hatch (Figure 3). Again, heterophils from line A chickens had higher ($P \leq 0.05$) IL-18 mRNA expression than heterophils from line B chickens. Line A fold changes ranged between 2.85 and 5.12 compared with -0.94 to 1.72 for line B.

IL-18 is critical in initiating an inflammatory response, is associated with a T1 cytokine response, and dramatically enhances innate and acquired immunity, thereby affecting host resistance to bacterial infections (Okamura *et al*., 1998; Garcia *et al*., 1999; Barreiros *et al*., 2000). In humans, increased IL-18 plays a protective role against systemic *Salmonella* infections (Mizuno *et al*., 2003). In the present study, heterophils from chickens more resistant to *S. enteritidis* produced higher mRNA expression levels of IL-18. In mammals, IL-18 activates neutrophils by promoting cell migration, cytokine production, generation of a respiratory burst, and degranulation (Leung *et al*., 2001) mediated by p38 mitogen-associated protein kinase (Wyman *et al*., 2002). Avian heterophils also undergo degranulation mediated by p38 mitogen-associated protein kinase (Kogut *et al*., 2003a). Since PMN are activated by IL-18, increased mRNA expression of IL-18 may result in a population of cells primed and subsequently more able to respond.

As would be expected, mRNA expression of the anti-inflammatory cytokine TGF-β4 is different from the pro-inflammatory profile. At 1, 14, and 28 days post-hatch, TGF-β4 mRNA levels in line A heterophils were

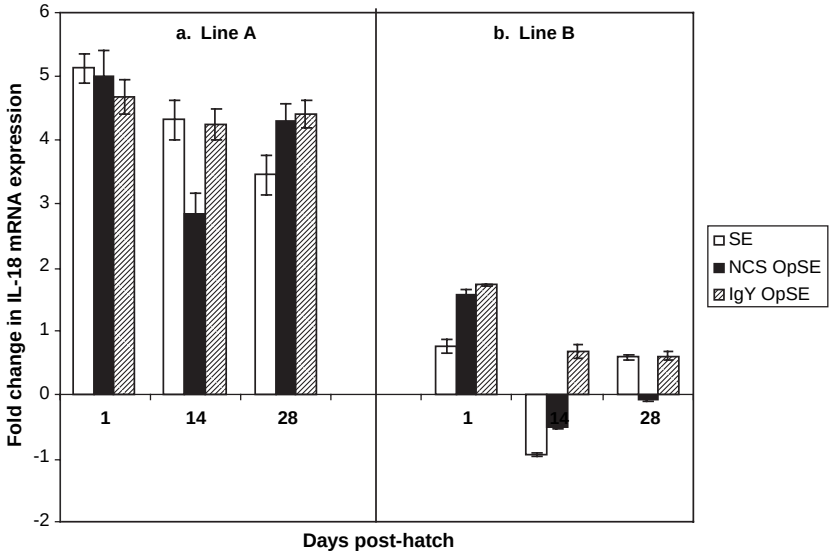

Figure 3. *Quantitation of IL-18 mRNA expression in heterophils isolated from (3a) line A chickens and (3b) line B chickens on days 1, 14, and 28 post-hatch. Data are expressed as the fold change in IL-18 mRNA levels when treated samples (*S. enteritidis *[SE], NCS-OpSE, and IgY-OpSE) were compared with control heterophils treated with RPMI. Error bars show the standard error of the mean from triplicate experiments.*

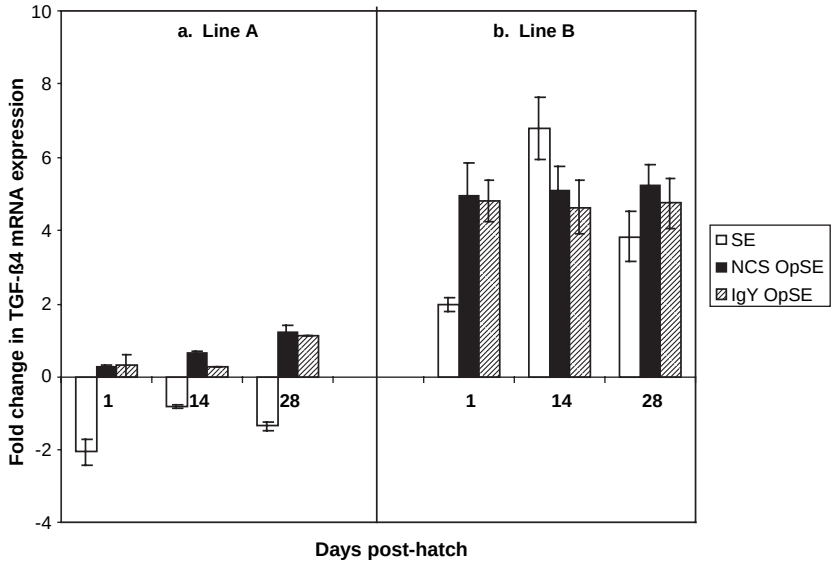

Figure 4. *Quantitation of TGF-β4 mRNA expression in heterophils isolated from (4a) line A chickens and (4b) line B chickens on days 1, 14, and 28 post-hatch. Data are expressed as the fold change in TGF-β4 mRNA levels when treated samples (*S. enteritidis *[SE], NCS-OpSE, and IgY-OpSE) were compared with control heterophils treated with RPMI. Error bars show the standard error of the mean from triplicate experiments.*

slightly down-regulated or changed very little from control values following treatment (-2.05 to 1.35) (Figure 4a). In contrast, expression levels in line B heterophils were higher ($P \leq 0.05$) regardless of the time post-hatch or the agonist (1.97 to 6.78) (Figure 4b).

In the current study, heterophils from resistant line A chickens had up-regulated mRNA expression of pro-inflammatory (IL-6), T1 (IL-18), and chemotactic (CXCLi2) cytokines and chemokines, while mRNA expression of the anti-inflammatory cytokine, TGF-β4, was down-regulated compared with the response observed in heterophils from line B chickens. These data provide evidence that down-regulation of TGF-β4 in heterophils from line A chickens enables these chickens to quickly and efficiently initiate an acute pro-inflammatory cytokine/chemokine response, resulting in increased resistance to bacterial infections. In mammals, high levels of TGF-β are associated with increased mortality in *Plasmodium* infections (Omer *et al*., 2003). A separate study showed TGF-β4 mRNA expression was up-regulated in the spleen from *S. typhimurium*-infected 6-week-old Leghorn chickens (Beal *et al*., 2004), which is similar to those observed in our *Salmonella*-susceptible chickens (line B). It is likely that *S. typhimurium* and *S. enteritidis* produce a differential cytokine response or that the differences observed are attributed to the type of chickens evaluated. Age is probably not the key factor in the differences, as the current study evaluated 4-week-old chickens compared with 6-week-old chickens (Beal *et al*., 2004). We postulate that there is a direct relationship between the up-regulation of TGF-β4, decreased heterophil functional efficiency, and increased susceptibility to extra-intestinal *S. enteritidis* infections in poultry.

Based on data from the present study accompanied by our previous findings utilizing these parental lines of chickens (Swaggerty *et al*., 2003a,b, 2005a,b; Ferro *et al*., 2004), we propose isolating heterophils and evaluating cytokine mRNA expression as a novel method to identify poultry with an efficient immune response while maintaining the desired growth parameters required by industry.

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References

Akira, S. (2001). Toll-like receptors and innate immunity. *Advances in Immunology*, *78*, 1–56.

Avery, S., Rothwell, L., Degen, W.D.J., Schijns, V.E.J.C., Young, J. & Kaiser, P. (2004). Characterization of the first nonmammalian T2 cytokine gene cluster: the cluster contains functional single-copy genes for IL-3, IL-4, IL-13, and GM-CSF, a gene for IL-5 that appears to be a pseudogene, and a gene encoding another cytokine-like transcript, KK34. *Journal of Interferon & Cytokine Research*, *24*, 600–610.

Balu, S. & Kaiser, P. (2003). Avian interleukin-12b (p40): cloning and characterization of the cDNA and gene. *Journal of Interferon & Cytokine Research*, *23*, 699–707.

Barreiros, A.P., Schirmacher, P., Laufenberg-Feldmann, R., Meyer Zum Buschenfelde, K.-H. & Schlaak, J.F. (2000). The early immune response in the liver of BALB/c mice infected with S. typhimurium. *Scandinavian Journal of Immunology*, *51*, 472–478.

Bayyari, G.R., Huff, W.E., Rath, N.C., Balog, J.M., Newberry, L.A., Villines, J.D., Skeeles, J.K., Anthony, N.B. & Nestor, K.E. (1997). Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. *Poultry Science*, *76*, 289–296.

Beal, R.K., Powers, C., Wigley, P., Barrow, P.A. & Smith, A.L. (2004). Temporal dynamics of the cellular, humoral and cytokine responses in chickens during primary and secondary infection with *Salmonella enterica* serovar Typhimurium. *Avian Pathology*, *33*, 25–33.

Beaumont, C., Protais, J., Guillot, J.F., Colin, P., Proux, K., Millet, N. & Pardon, P. (1999). Genetic resistance to mortality of day-old chicks

and carrier-state of hens after inoculation with *Salmonella enteritidis*. *Avian Pathology*, *28*, 131–135.

Bedard, P.-A., Alcorta, D., Simmons, D.L., Luk, K.-C. & Erikson, R.L. (1987). Constitutive expression of a gene encoding a polypeptide homologous to biologically active human platelet protein in Rous sarcoma virus-transformed fibroblast. *Proceedings of the National Academy of Sciences of the United States of America*, *84*, 6715–6719.

Borish, L., Rosenbaum, R., Albury, L. & Clark, S. (1989). Activation of neutrophils by recombinant interleukin 6. *Cellular Immunology*, *121*, 280–289.

Burton, R.R. & Harrison, J.S. (1969). The relative differential leukocyte count of the newly hatched chick. *Poultry Science*, *48*, 451–453.

Degen, W.D.J., van Daal, N., van Zuilekom, H.I., Burnside, J. & Schijns, V.E.C.J. (2004). Identification and molecular cloning of functional chicken IL-12. *Journal of Immunology*, *172*, 4371–4380.

Desmidt, M., Nerom, A.V., Haesebrouck, F., Ducatelle, R. & Ysebaert, M.T. (1996). Oxygenation activity of chicken blood phagocytes as measured by luminol- and lucigenin-dependent chemiluminescence. *Veterinary Immunology and Immunopathology*, *53*, 303–311.

Digby, M.R. & Lowenthal, J.W. (1995). Cloning and expression of the chicken IFN-g gene. *Journal of Interferon & Cytokine Research*, *15*, 939–945.

Fearon, D.T. & Locksley, R.M. (1996). The instructive role of innate immunity in the acquired immune response. *Science*, *272*, 50–54.

Ferro, P.J., Swaggerty, C.L., Kaiser, P., Pevzner, I.Y. & Kogut, M.H. (2004). Heterophils isolated from chickens resistant to extraintestinal *Salmonella enteritidis* infection express higher levels of pro-inflammatory cytokine mRNA following infection than heterophils from susceptible chickens. *Epidemiology and Infection*, *132*, 1029–1037.

Ferro, P.J., Swaggerty, C.L., He, H., Rothwell, L., Kaiser, P. & Kogut, M.H. (2005). Recombinant IL-6 does not activate heterophils isolated from day-old chickens *in vitro*. *Developmental & Comparative Immunology*, *29*, 375–383.

Garcia, V.E., Uyemura, K., Sieling, P.A., Ochoa, M.T., Morita, C.T., Okamura, H., Kurimoto, M., Rea, T.H. & Modlin, R.L. (1999). IL-18 promotes type 1 cytokine production from NK cells and T cells in human intracellular infection. *The Journal of Immunology*, *162*, 6114–6121.

Hachicha, M., Rathanaswami, P., Nacche, P.H. & McColl, S.R. (1998). Regulation of chemokine gene expression in human peripheral blood neutrophils phagocytosing microbial pathogens. *The Journal of Immunology*, *160*, 449–454.

Han, P.F.S. & Smyth, J.R. (1972). The influence of growth rate on the development of Marek's disease in chickens. *Poultry Science*, *51*, 975–985.

Hoebe, K., Janssen, E. & Beutler, B. (2004). The interface between innate and adaptive immunity. *Nature Immunology*, *5*, 971–974.

Imler, J.-L. & Hoffmann, J.A. (2001). Toll receptors in innate immunity. *Trends in Cell Biology*, *11*, 304–311.

Jakowlew, S.B., Dillard, P.J., Sporn, M.B. & Roberts, A.B. (1988). Complementary deoxyribonucleic acid cloning of a messenger ribonucleic acid encoding transforming growth factor-b 4 from chicken embryo chondrocytes. *Molecular Endocrinology*, *2*, 1186–1195.

Janeway, C.A., Jr. & Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology*, *20*, 197–216.

Kaiser, P., Rothwell, L., Galyov, E.E., Barrow, P.A., Burnside, J. & Wigley, P. (2000). Differential cytokine expression in avian cells in response to invasion by *Salmonella typhimurium*, *Salmonella enteritidis*, and *Salmonella gallinarum*. *Microbiology*, *146*, 3217–3226.

Kaiser, P., Rothwell, L., Vasicek, D. & Hala, K. (2002). A role for IL-15 in driving the onset of spontaneous autoimmune thyroiditis? *The Journal of Immunology*, *168*, 4216–4220.

Kaiser, P., Underwood, G. & Davison, F. (2003). Differential cytokine responses following Marek's disease virus infection of chickens differing in resistance to Marek's disease. *The Journal of Virology*, *77*, 762–768.

Kaiser, P., Yeow-Poh, T., Rothwell, L., Avery, S., Balu, S., Pathania, U.S., Hughes, S., Goodchild, M., Morrell, S., Watson, M., Bumstead, N., Kaufman, J. & Young, J.R. (2005). A genomic analysis of chicken cytokines and chemokines. *Journal of Interferon & Cytokine Research*, *25*, 467–484.

Keller, E.T., Wanagat, J. & Ershler, W.B. (1996). Molecular and cellular biology of interleukin-6 and its receptor. *Frontiers in Bioscience*, *1*, 340–357.

Kobayashi, S.D., Voyich, J.M., Buhl, C.L., Stahl, R.M. & DeLeo, F.R. (2002). Global changes in gene expression by human polymorphonuclear leukocytes during receptor-mediated phagocytosis: cell fate is regulated at the level of gene expression. *Proceedings of the National Academy of Sciences of the USA*, *99*, 6901–6906.

Kogut, M.H. (2002). Dynamics of a protective avian inflammatory response: the role of an IL-8-like cytokine in the recruitment of heterophils to the site of organ invasion by *Salmonella enteritidis*. *Comparative Immunology, Microbiology & Infectious Diseases*, *25*, 159–172.

Kogut, M.H., Genovese, K.J. & Lowry, V.K. (2001). Differential activation of signal transduction pathways mediating phagocytosis, oxidative burst, and degranulation by chicken heterophils in response to stimulation with opsonized *Salmonella enteritidis*. *Inflammation*, *25*, 7–15.

Kogut, M.H., Lowry, V.K. & Farnell, M.B. (2003a). Selective pharmacological inhibitors reveal the role of Syk tyrosine kinase, phospholipase C, phosphatidylinositol-3′-kinase, and p38 mitogen-activated protein kinase in Fc receptor-mediated signaling of chicken heterophil degranulation. *International Immunopharmacology*, *2*, 963–973.

Kogut, M.H., Rothwell, L. & Kaiser, P. (2003b). Differential regulation of cytokine gene expression by avian heterophils during receptor-mediated phagocytosis of opsonized and non-opsonized *Salmonella enteritidis*. *Journal of Interferon and Cytokine Research*, *23*, 319–327.

Leung, B.P., Culshaw, S., Gracie, J.A., Hunter, D., Canetti, C.A., Campbell, C., Cunha, F., Liew, F.Y. & McInnes, I.B. (2001). A role for IL-18 in neutrophil activation. *The Journal of Immunology*, *167*, 2879–2886.

Maxwell, M.H. & Robertson, G.W. (1998). The avian heterophil leucocyte: a review. *World's Poultry Science Journal*, *54*, 155–178.

Medzhitov, R. & Janeway, C.A., Jr. (1997). Innate immunity: impact on the adaptive immune response. *Current Opinion in Immunology*, *9*, 4–9.

Medzhitov, R. & Janeway, C.A., Jr. (2002). Decoding the patterns of self and nonself by the innate immune response. *Science*, *296*, 298–300.

Mizuno, Y., Takada, H., Nomura, A., Jin, C.H., Hattori, H., Ihara, K., Aoki, T., Eguchi, K. & Hara, T. (2003). Th1 and Th1-inducing cytokines in *Salmonella* infection. *Clinical and Experimental Immunology*, *131*, 111–117.

Moody, A., Sellers, S. & Bumstead, N. (2000). Measuring infectious bursal disease virus RNA in blood by multiplex real-time quantitative RT-PCR. *Journal of Virological Methods*, *85*, 55–64.

National Research Council. (1994). *Nutrient Requirements of Poultry* 9th edn (pp. 19–34). Washington, DC: National Academy Press.

Okamura, H., Tsutsui, H., Kashiwamura, S.-I., Yoshimoto, T. & Nakanishi, K. (1998). Interleukin-18: a novel cytokine that augments both innate and acquired immunity. *Advances in Immunology*, *70*, 281–312.

Omer, F.M., de Souza, J.B. & Riley, E.M. (2003). Differential induction of TGF-b regulates proinflammatory cytokine production and determines the outcome of lethal and nonlethal *Plasmodium yoelii* infections. *The Journal of Immunology*, *171*, 5430–5436.

Rot, A. (1991). Chemotactic potency of recombinant human neutrophil attractant/activation protein-1 (interleukin-8) for polymorphonuclear leukocytes of different species. *Cytokine*, *3*, 21–27.

Rothwell, L., Young, J.R., Zoorob, R., Whittaker, C.A., Hesketh, P., Archer, A., Smith, A.L. & Kaiser, P. (2004). Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*. *The Journal of Immunology*, *173*, 2675–2682.

Sadeyen, J.R., Trotereau, J., Velge, P., Marly, J., Beaumont, C., Barrow, P.A., Bumstead, N. & Lalmanach, A.C. (2004). Salmonella carrier state in chicken: comparison of expression of immune response genes between susceptible and resistant animals. *Microbes and Infection*, *6*, 1278–1286.

Schneider, K., Puehler, F., Baeuerle, D., Elvers, S., Staeheli, P., Kaspers, B. & Weining, K.C. (2000). cDNA cloning of biologically active chicken interleukin-18. *Journal of Interferon & Cytokine Research*, *20*, 879–883.

Schneider, K., Klaas, R., Kaspers, B. & Staeheli, P. (2001). Chicken interleukin-6. cDNA structure and biological properties. *European Journal of Biochemistry*, *268*, 4200–4206.

Smith, C.K., Kaiser, P., Rothwell, L., Humphrey, T., Barrow, P.A. & Jones, M.A. (2005). *Campylobacter jejuni*-induced cytokine responses in avian cells. *Infection and Immunity*, *73*, 2094–2100.

Stromberg, J. (1975). A guide to better hatching. In L. Stromberg (Ed.), *A Guide to Better Hatching*. Fort Dodge, IA: Stromberg Publishing Company.

Sugano, S., Stoeckle, M.Y. & Hanafusa, H. (1987). Transformation by Rous sarcoma virus induces a novel gene with homology to a mitogenic platelet protein. *Cell*, *49*, 321–328.

Swaggerty, C.L., Pevzner, I.Y., Ferro, P.J., Crippen, T.L. & Kogut, M.H. (2003a). Association between *in vitro* heterophil function and the feathering gene in commercial broiler chickens. *Avian Pathology*, *32*, 483–488.

Swaggerty, C.L., Pevzner, I.Y., Lowry, V.K., Farnell, M.B. & Kogut, M.H. (2003b). Functional comparison of heterophils isolated from commercial broiler chickens. *Avian Pathology*, *32*, 95–102.

Swaggerty, C.L., Kogut, M.H., Ferro, P.J., Rothwell, L., Pevzner, I.Y. & Kaiser, P. (2004). Differential cytokine mRNA expression in heterophils isolated from *Salmonella*-resistant and -susceptible chickens. *Immunology*, *113*, 139–148.

Swaggerty, C.L., Ferro, P.J., Pevzner, I.Y. & Kogut, M.H. (2005a). Heterophils are associated with resistance to systemic *Salmonella enteritidis* infection in genetically distinct lines of chickens. *FEMS Immunology and Medical Microbiology*, *43*, 149–154.

Swaggerty, C.L., Lowry, V.K., Ferro, P.J., Pevzner, I.Y. & Kogut, M.H. (2005b). Disparity in susceptibility to vancomycin-resistant *Enterococcus* organ invasion in commercial broiler chickens that differ in innate immune responsiveness. *Food and Agricultural Immunology*, *16*, 1–15.

Weining, K.C., Sick, C., Kaspers, B. & Staeheli, P. (1998). A chicken homolog of mammalian interleukin-1: cDNA cloning and purification of active recombinant protein. *European Journal of Biochemistry*, *258*, 994–1000.

Withanage, G.S.K., Kaiser, P., Wigley, P., Powers, C., Mastroeni, P., Brooks, H., Barrow, P., Smith, A., Maskell, D. & McConnell, I. (2004). Rapid expression of chemokines and proinflammatory cytokines in newly hatched chickens infected with *Salmonella enterica* Serovar typhimurium. *Infection and Immunity*, *72*, 2152–2159.

Wyman, T.H., Dinarello, C.A., Banerjee, A., Gamboni-Robertson, F., Hiester, A.A., England, K.M., Kelher, M. & Silliman, C.C. (2002). Physiological levels of interleukin-18 stimulate multiple neutrophil functions through p38 MAP kinase activation. *Journal of Leukocyte Biology*, *72*, 401–409.

Zlotnick, A. & Yoshie, O. (2000). Chemokines: a new classification system and their role in immunity. *Immunity*, *12*, 121–127.

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Non-English Abstracts

Heterophil cytokine mRNA profiles from genetically distinct lines of chickens with differential heterophil-mediated innate immune responses

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Profils des ARNm des cytokines d'hétérophiles de lignées de poulet génétiquement différentes, présentant des réponses immunitaires à médiation hétérophile différentes dès l'éclosion

Lors d'études préalables nous avons montré qu'une fonction hétérophile accrue *in vitro* entraîne une résistance accrue *in vivo* aux infections par *Salmonella enteritidis* (SE) chez les poulets de chair (lignée A > B). Les hétérophiles produisent des cytokines et modulent la protection contre les salmonelles chez le poussin de 1 jour. Nous avons émis l'hypothèse que les hétérophiles des poulets résistants à SE présentent une sur-expression des cytokines/chemokines pro-inflammatoires comparée à celle des poulets sensibles à SE. Dans cette étude, les hétérophiles ont été isolés à 1, 14, et 28 jours après éclosion, traités avec du RPMI ou des agonistes phagocytaires, et l'expression de l'ARNm des cytokines/chemokines a été évaluée en utilisant la RT-PCR quantitative en temps réel. A toutes les dates d'analyse les hétérophiles des poulets résistants à SE (A) avaient des niveaux plus élevés d'expression de l'ARNm des cytokines/chemokines pro-inflammatoires lors de stimulations, comparés à ceux des hétérophiles des poulets sensibles à SE (B). De plus les hétérophiles des poulets de la lignée A présentaient une diminution de l'expression de l'ARNm du TGF-4, une cytokine anti-inflammatoire, comparée à la lignée B. Ces données indiquent une relation entre l'expression des ARNm des cytokines/chemokines par les hétérophiles et la détermination de la compétence immunitaire globale. Par conséquent, l'efficacité de la fonction hétérophile ainsi que l'évaluation des cytokines/chemokines produites par les hétérophiles, peuvent être des biomarqueurs utiles à prendre en considération par les sélectionneurs lors du développement de lignées immunocompétentes.

mRNS-Profile von heterophilen Zytokinen aus genetisch unterschiedlichen Hühnerlinien mit verschiedenen angeborenen von Heterophilen vermittelten Immunantworten

Vor Kurzem konnten wir nachweisen, dass eine gesteigerte *in vitro*-Funktion der Heterophilen in eine erhöhte Resistenz gegenüber *Salmonella enteritidis* (SE)-Infektionen bei Broilern (Linie A > B) übertragen wurde. Heterophile produzieren Zytokine und modulieren akute Schutzmaßnahmen gegen Salmonellen in frisch geschlüpften Geflügelküken. Wir stellten die Hypothese auf, dass Heterophile von SE-resistenten Hühnern im Vergleich zu SE-empfänglichen Hühnern die pro-inflammatorische Zytokin/Chemokin-Antwort hochregulieren. In dieser Studie wurden Heterophile 1, 14 und 28 Tage nach dem Schlupf isoliert, mit RPMI oder phagozystischen Antagonisten behandelt und die Exprimierung der Zytokin/Chemokin-mRNS wurde mittels Real Time RT-PCR gemessen. Zu allen Zeitpunkten zeigten die Heterophilen aus den SE-resistenten Hühnern (A) nach der Stimulation eine höhere Exprimierung der pro-inflammatorischen Zytokin/Chemokin-mRNS als die Heterophilen aus den SE-empfänglichen Hühnerküken (B). Außerdem wiesen die Heterophilen aus der Hühnerlinie A im Vergleich zu denen aus der Linie B eine erniedrigte Exprimierung des transformierenden Wachstumsfaktors-4, eines anti-inflammatorischen Zytokins, auf. Diese Ergebnisse weisen auf eine Beziehung zwischen der Zytokin/Chemokin-mRNS-Exprimierung und der bestimmenden Gesamtimmunkometenz hin. Aus diesem Grund kann die Bestimmung der Effizienz der Heterophilenfunktion zusammen mit der Zytokin/Chemokin-Produktion der Heterophilen ein nützlicher Biomarker für Züchter sein bei der Entwicklung neuer immunkompetenter Linien.

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Perfiles de mRNA de citoquinas de heterófilos procedentes de distintas líneas genéticas de pollos con respuestas inmunes innatas diferenciadas mediadas por heterófilos

Anteriormente demostramos que funciones heterofílicas incrementadas *in vitro* se traducen en un incremento *in vivo* de la resistencia a infecciones por *Salmonella enteritidis* (SE) en pollos de engorde (línea A > B). Los heterófilos producen citoquinas y modulan la protección aguda frente a *Salmonella* en aves neonatales. Hipotetizamos que los heterófilos procedentes de pollos SE-resistentes producían una respuesta pro-inflamatoria de citoquinas/quimioquinas sobreregulada en comparación con pollos SE-susceptibles. En este estudio, se aislaron heterófilos a 1-, 14-, y 28-días post eclosión, se trataron con RPMI o agonistas fagocíticos, y se evaluó la expresión de mRNA de citoquinas/quimioquinas mediante RT-PCR a tiempo real. En todos los tiempos, los heterófilos procedentes de pollos SE-resistentes (A) tenían niveles más altos de expresión de mRNA de citoquinas/quimioquinas pro-inflamatorias bajo estimulación en comparación con los heterófilos de pollos SE-susceptibles (B). Además, los heterófilos de la línea de pollos A tenían la expresión de mRNA del factor de crecimiento de transformación 4, una citoquina anti-inflamatoria, disminuída en comparación con la línea B. Estos resultados indican una relación entre la expresión de mRNA de citoquina/quimioquina por heterófilos y una competencia inmune media determinante. Por lo tanto, la eficiencia funcional de los heterófilos, podría ser un biomarcador útil en el desarrollo de nuevas líneas inmunocompetentes de aves reproductoras.