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Heterophils isolated from chickens resistant to extra-intestinal *Salmonella enteritidis* infection express higher levels of pro-inflammatory cytokine mRNA following infection than heterophils from susceptible chickens

PAMELA J. FERRO[1], CHRISTINA L. SWAGGERTY[2], PETE KAISER[3], IGAL Y. PEVZNER[4] AND MICHAEL H. KOGUT[2]*

[1] *Texas A&M University, Poultry Science, College Station, TX 77843, USA*
[2] *USDA-ARS, SPARC, 2881 F&B Road, College Station, TX 77845, USA*
[3] *Institute for Animal Health, Compton, Berkshire RG20 7NN, UK*
[4] *Cobb-Vantress Inc., Siloam Springs, AR 72761-1030, USA*

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SUMMARY

Previous studies showed differences in *in vitro* heterophil function between parental (A > B) broilers and F1 reciprocal crosses (D > C). Our objectives were to (1) determine if *in vitro* variations translate to differences in resistance to *Salmonella enteritidis* (SE) and (2) quantitate cytokine mRNA in heterophils from SE-infected chicks. One-day-old chicks were challenged and organs were cultured for SE. Chicks with efficient heterophils (A and D) were less susceptible to SE compared to chicks with inefficient heterophils (B and C). Heterophils were isolated from SE-infected chicks and cytokine mRNA expression was evaluated using quantitative real-time RT–PCR. Pro-inflammatory cytokine mRNA was up-regulated in heterophils from SE-resistant chicks compared to susceptible chicks. This is the first report to quantitate cytokine mRNA in heterophils from SE-infected chicks. These data show a relationship between *in vitro* heterophil function, increased pro-inflammatory cytokine mRNA expression, and increased resistance to SE in 1-day-old chicks.

INTRODUCTION

The vertebrate immune system consists of two functional divisions, the innate and acquired immune responses. Historically, the innate immune response has been considered as the first line of defence which functioned to limit infection until the acquired response was initiated [1]. However, more recent findings indicate the innate immune response also provides instruction for the acquired immune response [2–5]. The innate immune system uses germ-line-encoded receptors, known as pattern recognition receptors (PRRs), to recognize self from (infectious) non-self by detecting molecules unique to invading organisms referred to as pathogen-associated molecular patterns (PAMPs) [2, 6–10]. Recognition of PAMPs by PRRs on the host immune cell directs the acquired immune response to that appropriate to the microbe by inducing pro-inflammatory cytokines and co-stimulatory molecules [4, 6, 11, 12].

Cytokines are soluble gene products that are produced by different cells during the activation phase of the immune response. They are pivotal for communication between cells and are potent effector molecules [13]. Proper communication between cells via PRRs and PAMPs initiates signal transduction pathways which in turn induce the expression of cytokines and co-stimulatory molecules that control immune function and direct the appropriate immune response

* Author for correspondence.
(Email: kogut@ffsru.tamu.edu)

[10]. Pro-inflammatory cytokines are important in the recruitment of immune cells to the site of infection. The pro-inflammatory cytokines IL-1β and IL-6, as well as IL-8, a chemokine, have been cloned and sequenced in the chicken [14–17].

As the first cells to migrate to the site of infection, polymorphonuclear leukocytes (PMNs) are vital cellular components of inflammatory and innate immune responses [18–20]. The primary PMN in poultry is the heterophil, the avian equivalent of the mammalian neutrophil. Like the neutrophil, avian heterophils are involved in the phagocytosis of invading microbes and foreign particles, the production of oxygen intermediates, and the release of proteolytic enzymes [21–23]. In mammals, neutrophil activation, following receptor-mediated phagocytosis, induces pro-inflammatory gene transcription that mediates host innate responses and modulates acquired responses [20, 24–26]. In chickens, the penetration of the intestine by *Salmonella enteritidis* (SE) and other *Salmonellae* is known to initiate an inflammatory response characterized by a large influx of heterophils to the site of initial infection [27].

Traditionally, the association between genetics and disease resistance in chickens has focused on the acquired immune response [28–31]. Given the ability of the innate immune system to recognize a variety of pathogens, we hypothesize that innate immunity could be a more useful marker when genetically selecting chickens for disease resistance. Instead of identifying chickens resistant to a single pathogen, our laboratory is interested in identifying a biomarker(s) for poultry that could indicate which lines have the potential to mount the most effective immune response against multiple microorganisms.

Previous studies in our laboratory have shown differences in *in vitro* heterophil function between two parental lines (A and B) of broiler chicks and their F1 reciprocal crosses (line C = male B × female A; line D = male A × female B) [32]. Specifically, heterophils from line A were more functionally efficient compared to heterophils from line B, and heterophils from line D were more efficient than heterophils from line C, thereby suggesting functional efficiency of the heterophil is an inherited trait and associated with the male parent line [32]. Therefore, the objectives of the current study were: (1) to determine if the previously reported *in vitro* differences in heterophil function between the lines translated to an increase in resistance or susceptibility to *in vivo* infection with SE and (2) to quantitate pro-inflammatory cytokine mRNA expression levels in heterophils isolated from SE-infected chicks from the different lines.

MATERIALS AND METHODS

Experimental birds

Broiler chickens used in this study were obtained from a commercial breeder. To maintain confidentiality, the lines were designated A, B, C and D, where lines A and B are parental lines and C and D are F1 reciprocal crosses of the two parent lines (C = male B × female A; D = male A × female B). Fertilized eggs were set in incubators (GQF Manufacturing Company, Savannah, GA, USA or Jamesway Incubator Company Inc., Ontario, Canada) and maintained at wet and dry bulb temperatures of 32 and 37 °C respectively. After 10 days of incubation, the eggs were candled and non-fertile and non-viable eggs discarded. The viable eggs were returned to the incubator until day 18, then transferred to hatchers (Humidaire Incubator Company, New Madison, OH, USA) and maintained under the aforementioned temperature and humidity conditions until day of hatch. Upon hatching, 115 chicks per line (A–D) were placed in floor pens containing wood shavings and provided with 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 of metabolizable energy (ME)/kg of diet, and all other nutrient rations met or exceeded the standards established by the National Research Council [33].

Bacteria

A poultry isolate of SE (no. 97-11771) was obtained from the National Veterinary Services Laboratory (Ames, IA, USA) and approved by the United States Department of Agriculture Animal and Plant Health Inspection Service for use in our facilities. SE was selected for resistance to novobiocin and carbenicillin and was maintained in tryptic soy broth containing antibiotics (25 μg/ml novobiocin and 100 μg/ml carbenicillin; Sigma Chemical Co., St. Louis, MO, USA). A stock culture of SE was prepared in sterile PBS and adjusted to a concentration of 1×10^9 c.f.u./ml using a spectrophotometer at a reference wavelength of 625 nm (Spectronic 20D, Milton Roy Co., Golden, CO, USA). The viable cell concentration of the challenge dose was determined by colony counts

on Brilliant Green agar plates containing carbenicillin and novobiocin (BGA + CN; Difco Laboratories, Detroit, MI, USA).

Organ invasion by SE

Thirty 1-day-old chicks from lines A–D were challenged orally with 7×10^3 c.f.u./ml SE per chick or 5×10^6 c.f.u./ml SE per chick. A challenge dose of 5×10^6 c.f.u./ml SE was administered to ensure the chicks used as heterophil donors were infected. At 24 h post-challenge chicks were euthanized and necropsied. At necropsy, livers and spleens were aseptically removed and incubated overnight at 41 °C in tetrathionate enrichment broth. From each enrichment, 10 μl were streaked onto BGA + CN plates for isolation of SE and incubated overnight at 41 °C. Subsequently, BGA + CN plates were examined for the presence of non-lactose fermenting CN-resistant *Salmonella* colonies. Representative colonies were confirmed SE-positive by plate agglutination using specific Group D_1 antisera (Difco Laboratories). Ten non-challenged chicks from lines A–D were included as negative controls. Four experimental trials were conducted and data from these replicate experiments were pooled for presentation and statistical analysis.

Heterophil isolation for analysis of cytokine expression

Twenty-five 1-day-old chicks from lines A–D were orally challenged with 5×10^6 c.f.u./ml SE and 25 non-challenged 1-day-old chicks from lines A–D were used as controls. At 4 h post-challenge [34], heterophils were isolated from the chicks as previously described [35]. Briefly, blood was collected in vacutainer tubes containing disodium ethylenediaminetetraacetic acid (EDTA) – an anticoagulant. The blood was pooled and diluted 1:1 with RPMI 1640 media containing 1% methylcellulose and centrifuged at 60 ***g*** for 15 min. The supernatant was transferred to a new conical tube, diluted with Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (1:1), layered onto discontinuous Histopaque® gradients (specific gravity 1·077 over 1·119), and then centrifuged for 1 h at 300 ***g***, 5 °C. The Histopaque layers were collected, washed with RPMI 1640 (1:1) and centrifuged at 500 ***g***, 5 °C for 15 min. The supernatants were discarded and the cell pellets resuspended in fresh RPMI 1640, counted on a haemacytometer, and diluted to the desired concentration (1×10^7 cells/ml) for subsequent RNA isolation. All tissue culture reagents and chemicals were obtained from the Sigma Chemical Company.

RNA isolation from SE-infected and non-infected chicks

Heterophils (1×10^7 cells) from control and SE-infected chicks were pelleted (500 ***g*** for 15 min at 4 °C), supernatants discarded, and heterophils resuspended in 350 μl lysis buffer (Qiagen RNeasy mini RNA extraction kit, Qiagen Inc., Valencia, CA, USA). The lysed cells were transferred to QIAshredder homogenizer columns (Qiagen Inc.), and centrifuged for 2 min at $\geqslant$8000 ***g***. Total RNA was extracted from the homogenized lysate according to the manufacturer's instructions. Purified RNA was eluted with 50 μl RNase-free water and stored at −80 °C until analysed for cytokine mRNA expression.

Real-time quantitative RT–PCR

Cytokine mRNA levels in control heterophils (heterophils isolated from uninfected chicks) and heterophils isolated from SE-infected chicks were quantitated as previously described [36].

For both cytokine and 28S rRNA-specific amplification, primers and probes were designed using the Primer Express software program (PE Applied Biosystems, Foster City, CA, USA). Details of the probes and primers are given in Table 1. All cytokine probes were designed, from the sequence of the relevant genes, to lie across intron–exon boundaries. Cytokine and 28S rRNA probes were labelled with the fluorescent reporter dye 5-carboxyfluorescein (FAM) at the 5′-end and the quencher *N*,*N*,*N*′,*N*′-tetramethyl-6-carboxyrhodamine (TAMRA) at the 3′-end.

RT–PCR was performed using the reverse transcriptase qPCR Master Mix RT–PCR kit (Eurogentec, Seraing, Belgium). Amplification and detection of specific products were performed using the ABI PRISM 7700 sequence detection system (PE Applied Biosystems) with the following cycle profile: one cycle of 50 °C for 2 min, 96 °C for 5 min, 60 °C for 30 min, and 95 °C for 5 min, and 40 cycles of 94 °C for 20 s, 59 °C for 1 min. All amplification reactions were one-step reactions. Final primer concentrations were as follows: 28S, 0·6 μM (15 pmol/μl); IL-1β, 0·4 μM (10 pmol/μl); IL-6, 0·2 μM (5 pmol/μl); IL-8, 0·6 μM (10 pmol/μl); and all probes were used at a final concentration of 200 nmol/μl.

Table 1. *Real-time quantitative RT–PCR probes and primers*

RNA target		Probe/primer sequence	Exon boundary	Amplicon size (bp)	Accession no.[a]
28S	Probe	5′-(FAM)-AGGACCGCTACGGACCTCCACCA-(TAMRA)-3′[b]		62[c]	X59733
	F[c]	5’-GGCGAAGCCAGAGGAAACT-3′			
	R[d]	5′-GACGACCGATTGCACGTC-3′			
IL-1β	Probe	5′-(FAM)-CCACACTGCAGCTGGAGGAAGCC-(TAMRA)-3′	5/6	80	AJ245728
	F	5′-GCTCTACATGTCGTGTGTGATGAG-3′,			
	R	5′-TGTCGATGTCCCGCATGA-3′			
IL-6	Probe	5′-(FAM)-AGGAGAAATGCCTGACGAAGCTCTCCA-(TAMRA)-3′	3/4	71	AJ250838
	F	5′-GCTCGCCGGCTTCGA-3′			
	R	5′-GGTAGGTCTGAAAGGCGAACAG-3′			
IL-8	Probe	5′-(FAM)-CTTTACCAGCGCGTCCTACCTTGCGACA-(TAMRA)-3′	1/2	74	AJ009800
	F	5′-GCCCTCCTCCTGGTTTCAG-3′			
	R	5′-TGGCACCGCCAGCTCATT-3′			

[a] Genomic DNA sequence.
[b] FAM, fluorescent reporter dye 5-carboxyfluorescein; TAMRA, quencher *N*,*N*,*N*′,*N*′-tetramethyl-6-carboxyrhodamine.
[c] Forward.
[d] Reverse.

Table 2. *Standard curve data from real-time quantitative RT–PCR on total RNA extracted from stimulated heterophils*

	ΔRn[a]	Log dilutions	C_t[b]	R^2[c]	Slope
28S	0·05	10^{-1}–10^{-5}	8–22	0·9952	3·1657
IL-1B	0·02	10^{-1}–10^{-5}	26–32	0·9977	2·8451
IL-6	0·02	10^{-1}–10^{-5}	21–36	0·9915	3·7278
IL-8	0·02	10^{-1}–10^{-5}	14–28	0·9907	2·87

[a] ΔRn, change in the reporter dye.
[b] C_t, threshold cycle level: the cycle at which the change in the reporter dye levels detected passes the ΔRn.
[c] R^2, coefficient of regression.

Quantification was based on the increased fluorescence detected by the ABI PRISM 7700 sequence detection system due to hydrolysis of the target-specific probes by the 5′ nuclease activity of the *rTth* DNA polymerase during PCR amplification. The passive reference dye 6-carboxy-χ-rhodamine, which is not involved in amplification, was used to correct for fluorescent fluctuations, resulting from changes in the reaction conditions, for normalization of the reporter signal. Results are expressed in terms of the threshold cycle value (C_t), the cycle at which the change in the reporter dye passes a significance threshold. In this work, the threshold values of the change in the reporter dye are given in Table 2 for all reactions described.

Standard curves for the cytokine and 28S rRNA-specific reactions were generated as previously described [36]. Each RT–PCR experiment contained triplicate no-template controls and test samples, and a $\log_{10}$ dilution series of standard RNA. Each experiment was performed in triplicate, with replicates performed on different days. Regression analysis of the mean values of six replicate RT–PCRs for the $\log_{10}$-diluted RNA was used to generate standard curves.

Statistical analyses

Statistical analyses were performed using the SigmaStat® statistical software (Jandel Corporation, San Rafael, CA, USA). A one-way analysis of variance (ANOVA) was performed to detect differences between mean values. Mean values were further analysed for significance with Tukey's test ($P<0·05$).

RESULTS

Organ invasion with SE

The percentage of SE-positive organ cultures from chicks challenged with 7×10^3 c.f.u./ml SE is presented in Figure 1. Significantly ($P<0·05$) fewer organ cultures from line A chicks were positive for SE compared to liver/spleen cultures from line B chicks (Fig. 1). There were no significant differences between SE-positive organ cultures from line C chicks compared to organ cultures from line D chicks (Fig. 1). When comparing the F1 reciprocal crosses (C and D) to the parent lines (A and B), line B chicks had the

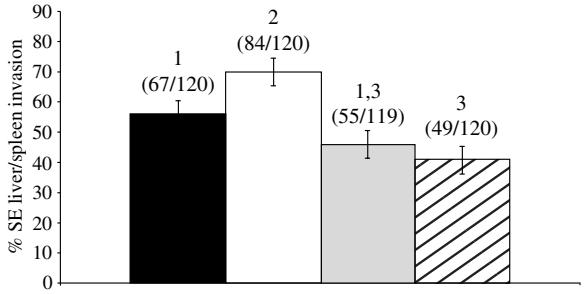

Fig. 1. SE liver/spleen invasion: values are expressed as the mean percent positive for SE organ invasion per group over four experiments. Different numbers indicate significant differences between the four different lines (A–D) of commercial broilers ($P<0{\cdot}05$). Numbers in parentheses represent the number of chicks positive for SE organ invasion/the total number of chicks tested. ■, Line A; □, line B; ▨, line C; ▨, line D.

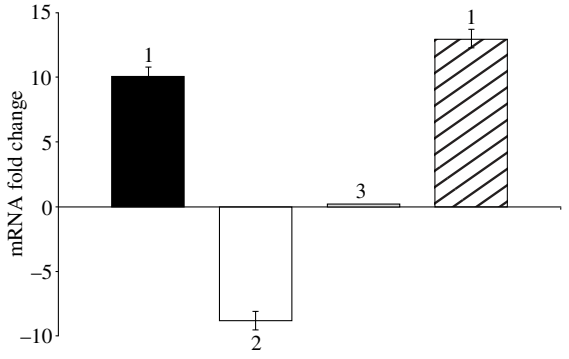

Fig. 2. IL-1β mRNA expression in heterophils isolated from different lines of SE-infected chicks, columns represent fold change from controls. Different numbers indicate significant differences between the four different lines (A–D) of commercial broilers ($P<0{\cdot}05$). ■, Line A; □, line B; ▨, line C; ▨, line D.

most SE-positive organ cultures ($P<0{\cdot}05$) compared to organ cultures from line C or line D chicks (Fig. 1). The number of organ cultures positive for SE organ invasion from line A chicks was not significantly different from the number of SE-positive organ cultures from line C chicks. More organ cultures from line A chicks were positive for SE organ invasion ($P<0{\cdot}05$) compared to organ cultures from line D chicks (Fig. 1).

Organ cultures from all lines (A–D) were 96–100% positive following oral challenge with 5×10^6 c.f.u./ml SE (data not shown).

Pro-inflammatory cytokine mRNA expression

Cytokine mRNA expression levels from heterophils collected from SE-infected chicks and non-infected controls were evaluated. Data are shown as fold

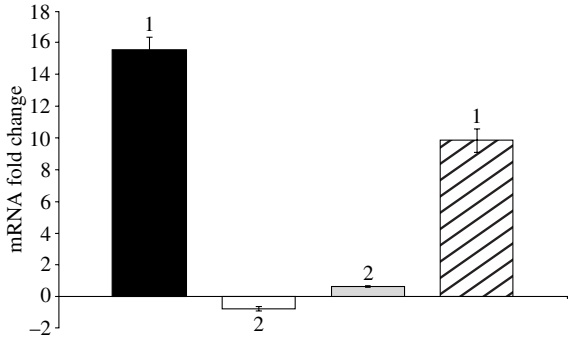

Fig. 3. IL-6 mRNA expression in heterophils isolated from different lines of SE-infected chicks, columns represent fold change from controls. Different numbers indicate significant differences between the four different lines (A–D) of commercial broilers ($P<0{\cdot}05$). ■, Line A; □, line B; ▨, line C; ▨, line D.

increase of cytokine mRNA from heterophils of the SE-infected chicks against control values from heterophils from non-infected chicks (Figs 2–4). A challenge dose of 5×10^6 c.f.u./ml SE was administered to ensure the chicks used as heterophil donors were infected.

Heterophils isolated from SE-infected line A chicks had a significant ($P<0{\cdot}05$) up-regulation of IL-1β mRNA levels compared to heterophils isolated from SE-infected line B chicks (Fig. 2). Expression levels of IL-1β mRNA were also significantly ($P<0{\cdot}05$) up-regulated in heterophils isolated from SE-infected line D chicks when compared to heterophils isolated from SE-infected line C chicks (Fig. 2). When comparing the F1 crosses (C and D) to the parent lines (A and B), mRNA expression levels of IL-1β in heterophils isolated from SE-infected chicks from lines A and D were up-regulated above background levels. However, IL-1β mRNA expression levels in heterophils from line B chicks were down-regulated compared to controls and unchanged from control levels in heterophils from line C chicks (Fig. 2).

Heterophils isolated from SE-infected line A chicks had a significant ($P<0{\cdot}05$) up-regulation of IL-6 mRNA expression compared to heterophils isolated from SE-infected line B chicks (Fig. 3). Expression levels of IL-6 mRNA were also significantly ($P<0{\cdot}05$) increased in heterophils isolated from SE-infected line D chicks when compared to heterophils isolated from SE-infected line C chicks (Fig. 3). When comparing the F1 crosses back to the parents, IL-6 mRNA expression in heterophils isolated from SE-infected chicks from lines A and D were significantly ($P<0{\cdot}05$) up-regulated above control levels. However, IL-6

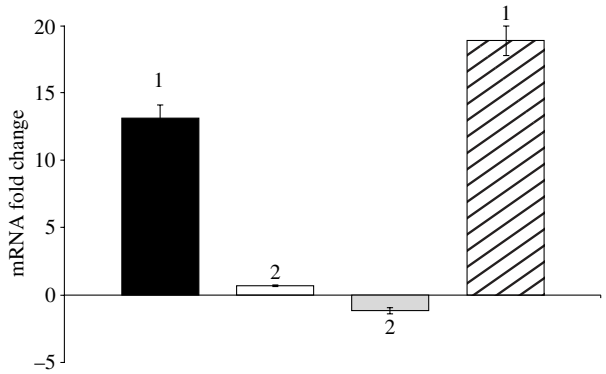

Fig. 4. IL-8 mRNA expression in heterophils isolated from different lines of SE-infected chicks, columns represent fold change from controls. Different numbers indicate significant differences between the four different lines (A–D) of commercial broilers ($P<0{\cdot}05$). ■, Line A; □, line B; ▨, line C; ▨, line D.

mRNA expression was down-regulated in heterophils from SE-infected line B chicks and was comparable to control levels in heterophils from SE-infected line C chicks, yet significantly ($P<0{\cdot}05$) below IL-6 mRNA expression levels of heterophils isolated from SE-infected line A and line D chicks (Fig. 3).

Heterophils isolated from SE-infected line A chicks were significantly ($P<0{\cdot}05$) up-regulated in IL-8 mRNA expression compared to heterophils isolated from SE-infected line B chicks (Fig. 4). Expression levels of IL-8 mRNA were also significantly ($P<0{\cdot}05$) higher in heterophils isolated from SE-infected line D chicks when compared to heterophils isolated from SE-infected line C chicks (Fig. 4). When comparing the F1 crosses to the parent lines, mRNA expression levels of IL-8 in heterophils isolated from line A and line D SE-infected chicks were significantly ($P<0{\cdot}05$) up-regulated above control levels. However, heterophils isolated from SE-infected line B chicks were up-regulated above control levels values while heterophils isolated from SE-infected line C chicks were down-regulated from control levels (Fig. 4).

DISCUSSION

Genetic selection for disease resistance has traditionally focused on aspects of acquired immunity. This has proven difficult and often resulted in adverse effects on growth parameters and other characteristics required by the poultry industry [37–39]. Based on emerging evidence that the innate immune response drives the acquired immune response [3–5], our laboratory is interested in identifying measures of innate immunity applicable to the development of new, more generally disease-resistant, lines of poultry. The heterophil, the avian equivalent of the mammalian neutrophil, is the first cell to migrate to the site of a bacterial infection [27]. Therefore heterophil functional efficiency is of interest when evaluating the potential efficacy of an innate immune response in chickens. Previous studies conducted in our laboratory reported differences in *in vitro* heterophil functional efficiency among parental lines of broilers and F1 reciprocal crosses, indicating heterophil functional efficiency may be genetically associated and heritable [32]. The current studies confirm and extend our earlier study in that the two lines (A and D) reported to have more functionally efficient heterophils were less susceptible to extra-intestinal SE infections whereas the two lines (B and C) reported to have functionally less efficient heterophils were more susceptible to SE organ invasion (Fig. 1).

In mammals, neutrophil activation, following receptor-mediated phagocytosis, induces transcription of pro-inflammatory cytokines that mediate the host innate responses and modulate acquired responses [20, 25, 26, 40]. Recent studies conducted in our laboratory indicate heterophils, like neutrophils, are capable of pro-inflammatory gene expression upon infection [41]. In the present studies, pro-inflammatory cytokine gene expression in heterophils was evaluated in order to increase knowledge and understanding of the mechanism involved in SE resistance. We found significant up-regulation in pro-inflammatory cytokine (IL-1β, IL-6, IL-8) mRNA expression in heterophils isolated from chicks that were more resistant (lines A and D) to SE organ invasion when compared to levels in heterophils isolated from the more susceptible lines (lines B and C) (Figs 2–4). Pro-inflammatory cytokines are important in the recruitment of immune cells to the site of infection. Therefore, an increase in mRNA expression of pro-inflammatory cytokines by heterophils could result in a more efficient and potentially effective immune response.

IL-6 has been shown to have multiple biological activities against many cellular targets [42]. For example, one study where researchers examined lung inflammation in mice and showed IL-6 had both inflammatory and anti-inflammatory activities depending on the stimulus used [43]. IL-6 also has an anti-inflammatory role in both local and systemic acute inflammatory responses by controlling the level of pro-inflammatory cytokines [44]. Preliminary

studies conducted in our laboratories, using recombinant chicken IL-6, indicate IL-6 has no direct effect on heterophils, neither activating nor priming heterophil functional activity (P. J. Ferro and M. H. Kogut, unpublished observations). Therefore, IL-6 produced by heterophils probably affects other cells involved in an immune response. In mammals, recent studies show IL-6 is capable of inhibiting the suppression of effector T cells (T_E) by regulatory T cells (T_R) thereby allowing efficient generation of T_E against a pathogen [45, 46]. Recognition of PAMPs by Toll-like receptors (TLR) leads to the activation of antigen-presenting cells (APC) resulting in the production of IL-6 and other soluble factors [46]. The presence and functional activity of TLRs on chicken heterophils has been recently reported [47]. Perhaps the activation of the appropriate signalling pathway(s) in the heterophil, in part, contributes to the line differences in susceptibility to organ invasion by SE observed in the current study.

In mammals, IL-1β is considered an early response pro-inflammatory cytokine produced by many cells in response to microbial challenges. The expression of IL-1β by heterophils is consistent with findings in mammals that neutrophils are a source of locally produced IL-1β [40]. IL-1β is important in the induction of innate response mediators such as acute phase proteins and the chemokine IL-8 [40]. Therefore, an increase in IL-1β is likely to contribute to an improved innate immune response, as supported by the current study where heterophils isolated from chicks more resistant to SE extra-intestinal infection showed an increase in IL-1β mRNA expression, and a decrease in IL-1β mRNA expression was observed in heterophils isolated from chicks more susceptible to SE organ invasion.

IL-8 is a chemokine important in recruiting neutrophils to sites of inflammation and infection [18]. Neutrophils are capable of secreting IL-8 thereby autoamplifying an acute inflammatory response to infection [18]. Kogut [48] reported IL-8-like activities in chickens during an inflammatory reaction which also functioned to recruit heterophils to the site of SE infection. The recruitment of heterophils to the site of an infection is important in the clearance of a pathogen due to the ability of the heterophil to phagocytize and kill SE [49]. Therefore, heterophils isolated from chickens that express higher levels of IL-8 would recruit immune cells to the site of infection more efficiently thereby increasing a chick's resistance to a pathogen. As was previously reported, heterophils from line A and line D chicks phagocytized SE more efficiently than heterophils from line B and line C chicks [32]. Additionally, heterophils from the lines that were more resistant (A and D) to SE organ invasion expressed more IL-8 mRNA than heterophils from the more susceptible lines (B and C). These data, in conjunction with the previous study [32], provide possible factors (heterophil functional efficiency and pro-inflammatory cytokine expression) whereby lines A and D are more resistant to extra-intestinal infection by SE than lines B and C.

To our knowledge this is the first report to quantitate IL-1β, IL-6, and IL-8 mRNA expression levels in heterophils isolated from 1-day-old chicks orally challenged with SE. These results show a relationship between *in vitro* heterophil function [32] and resistance to organ invasion by SE. These data also indicate that pro-inflammatory cytokine mRNA expression by heterophils contributes, at least in part, to resistance/susceptibility of 1-day-old chicks to extra-intestinal infection by SE.

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