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Priming by recombinant chicken interleukin-2 induces selective expression of IL-8 and IL-18 mRNA in chicken heterophils during receptor-mediated phagocytosis of opsonized and nonopsonized *Salmonella enterica* serovar enteritidis

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Abstract

Heterophils, the principal avian polymorphonuclear leukocytes (PMNs) equivalent to the mammalian neutrophil, function as professional phagocytes against bacterial infections, mediate acute inflammation, and respond to cytokine stimulation to aid in regulation of innate host defenses. Interleukin-2 (IL-2) has been found to exercise an array of biological effects on other cell types besides T lymphocytes, including NK cells, B cells, monocytes, and neutrophils. In the present experiments, using real-time quantitative RT-PCR, we evaluated the role of rChIL-2 as a priming mediator controlling heterophil responses at the level of gene transcription by examining the expression of mRNA for pro-inflammatory (IL-1β, IL-6, IL-8) and Th1 (IL-18 and IFN-γ) cytokine genes following stimulation with phagocytosis agonists; i.e., opsonized and nonopsonized *Salmonella enteritidis*. Peripheral blood heterophils were isolated and incubated with rChIL-2 from transfected COS cells. rChIL-2 selectively primed the heterophils for an increase in transcription of the pro-inflammatory cytokine IL-8 and of theTh1 cytokine IL-18 induced by all three phagocytic agonists. Although rChIL-2 priming modulated the expression of specific cytokine mRNA in heterophils stimulated by different phagocytic agonists, the rChIL-2 by itself did not directly induce gene expression of either the pro-inflammatory or Th1 cytokines. We propose that rChIL-2 could be priming heterophils solely to function as more efficient innate effector cells to limit bacterial growth through the selective increase of IL-8 and IL-18 gene expression.
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Keywords: Heterophils; Chickens; IL-2; Phagocytosis; Receptors; Priming; IL-8; IL-18

1. Introduction

The phylogenetically conserved phagocytic process is a critical event in the initiation of innate immunity, both as an effector occurrence and as a connection between innate and acquired immune responses. Phagocytosis is triggered by two distinct cell surface receptors, one involving the recognition of the carboxy-terminus of immunoglobulin molecules by the Fc receptor and the other where complement receptors recognize complement fragments (Aderem and Underhill, 1999; Underhill and Ozinsky, 2002). Recognition of potential pathogenic microbes by the innate immune system is the function of a restricted class of cellular receptors known as the nonclonal pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs) which recognize evolutionar-

ily conserved molecular motifs (pathogen-associated molecular patterns (PAMPs)) of infectious microbes (Medzhitov and Janeway, 1997; Janeway and Medzhitov, 2002). The end product of microbial recognition and phagocytosis by cells of the innate response is the activation of intracellular signaling pathways that initiate cellular processes such as activation of microbicidal killing mechanisms, the production of pro- and/or anti-inflammatory cytokines, and production of co-stimulatory molecules required for antigen presentation to the acquired immune system (Kobayashi et al., 2002; Nau et al., 2002).

Polymorphonuclear leukocytes (PMNs) are critical cellular components of innate immunity. In poultry, the predominant PMN is the heterophil. Like the mammalian neutrophil, avian heterophils are involved in the phagocytosis of invading microbes and foreign particles. In neutrophils, various stimuli have been shown to induce gene transcription and translation of inflammatory mediators

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(Hachicha et al., 1998; Lloyd and Oppenheim, 1992; Swain et al., 2002). In contrast, mediators that control heterophil responses at the level of gene transcription and expression are virtually unknown. We recently demonstrated that following receptor-mediated phagocytosis of opsonized and nonopsonized *Salmonella enteritidis* (SE) avian heterophils differentially express pro-inflammatory and Th1 cytokine mRNA transcripts (Kogut et al., 2003). Therefore, heterophils can respond to different phagocytic stimuli and, by controlling cytokine gene expression, can function in regulating inflammatory and subsequent acquired immune responses. The production of cytokines by heterophils suggests that these cells may be able to play a significant role in orchestrating not only pro- or anti-inflammatory activities, but also immunoregulatory actions. Since heterophils represent the first cell type encountering and interacting with an inflammatory or etiologic agent, the ability to synthesize and release an array of cytokines provides evidence for their role in both immunoregulation and pathophysiology of disease.

IL-2 is one cytokine that plays an active role in the activation and maintenance of both acquired and innate immune defenses (Waldmann et al., 1984; Henney et al., 1981; Djeu et al., 1993; Wahl et al., 1987; Rand et al., 1991). IL-2 has been shown to activate human monocytes and neutrophils to generate tumoricidal and microbicidal activities, produce an oxidative burst, and secrete several cytokines and bioactive lipids (Djeu et al., 1993; Wei et al., 1993; Espinosa-Delgado et al., 1990; Wahl et al., 1987; Streiter et al., 1989). Likewise, we have previously established that recombinant chicken IL-2 (rChIL-2) was capable of priming the phagocytic and bactericidal activities of heterophils from young chickens (Kogut et al., 2002), but had no direct stimulatory effect on the respiratory burst or degranulation of the heterophils.

In the present experiments, using real-time quantitative RT-PCR, our objectives were twofold: first, we evaluated the role of rChIL-2 as a direct activator of heterophil cytokine gene transcription and second, the role of rCHIL-2 as a priming mediator of heterophil mRNA expression of pro-inflammatory (IL-1β, IL-6, IL-8) and Th1 (IL-18 and IFN-γ) cytokines following stimulation with phagocytosis agonists opsonized and nonopsonized SE. These cytokine mRNA, generated in response to microbial challenges, are important in activating and coordinating innate immunity and Th1 responses.

2. Materials and methods

2.1. Experimental animals

Outbred Rhode Island Red chickens were bred at the Institute for Animal Health (Compton, UK). Newly-hatched chickens were housed in wire-floor cages with free access to food and water.

2.2. Isolation of peripheral blood heterophils

Avian heterophils were isolated from the peripheral blood of day-old Rhode Island Red chickens as described previously (Kogut et al., 1995, 1998). Briefly, disodium ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood was mixed with 1% methylcellulose (25 centiposes; Sigma, St. Louis, MO) at a 1.5:1 ratio and centrifuged at $25 \times g$ for 30 min. The serum and buffy coat layers were retained and suspended in Ca^{++}, Mg^{++}-free Hanks' balanced salt solution (HBSS, 1:1; Sigma, St. Louis, MO). This suspension was layered over a discontinuous Ficoll-Hypaque (Sigma, St. Louis, MO) gradient (specific gravity 1.077 over specific gravity 1.119). The gradient was then centrifuged at $250 \times g$ for 60 min. After centrifugation, the 1.077/1.119 interface and 1.119 band containing the heterophils were collected and washed twice in RPMI 1640 medium (Sigma, St. Louis, MO) and resuspended in fresh RPMI 1640. Cell viability was determined by trypan blue exclusion. The purity of the heterophil suspensions was assessed by microscopic examination of Hema-3 stained (Curtin Mathison Scientific, Dallas, TX) cytospin (Shandon Scientific, Pittsburgh, PA) smears. Heterophil preparations obtained by this method were typically >95% pure and >95% viable. On average, the other 2% comprised monocytes (at most 0.5%), lymphocytes (0.8%), and thrombocytes (at most 0.7%) (Kogut et al., 1995, 1998). The cell concentration was adjusted to 4×10^6 heterophils/ml and stored on ice until used.

2.3. Bacteria

SE P125589, phage type 4, is highly virulent in newly-hatched chicks and colonises the alimentary tract of chickens well (Barrow, 1991). It is invasive in laying hens (Barrow and Lovell, 1991). A spontaneous mutant, resistant to nalidixic acid was used throughout the study. The strain was maintained in LB broth (Difco, East Molesey, Surrey, UK) containing 30% glycerol stored at $-70\,^\circ$C. SE used for opsonization were prepared in sterile phosphate−buffered saline (pH 7.2) and adjusted to a concentration of 10^9 cfu/ml using a spectrophotometer with a 625 nm reference wavelength. The viable cell concentration of SE was determined by colony counts on brilliant green agar (BGA) + C–N (Oxoid Ltd., Basingstoke, UK) plates.

2.4. Opsonization of Salmonella enteritidis

Normal chicken serum (CS) was used as a source of complement for opsonization of SE as described previously (Kogut et al., 1995). The SE (10^8 bacteria/ml) were suspended in pooled CS, opsonized for 30 min at $39\,^\circ$C on a rotary shaker, washed twice with Ca^{++}, Mg^{++}-free HBSS, and stored at $4\,^\circ$C in HBSS (10^8 bacteria/ml) until used.

A hyperimmune serum against the homologous serovar of SE used in the experiments was prepared in 42-week-old

layer hens (Hy-Line W-36) (Kogut et al., 1998). The hyperimmune sera were collected from the blood of these hens and pooled. The pooled sera were octanic acid fractionated and the supernatant submitted to DEAE chromatography (Bethyl Lab, Montgomery, TX). The predominantly IgY fractions were pooled and concentrated. SE (10^8 bacteria/ml) was suspended in a subagglutinating concentration of IgY for 30 min at 39 °C on a rotary shaker, washed twice with Ca^{++}, Mg^{++}-free HBSS, and stored at 4 °C in HBSS (10^8 bacteria/ml) until used.

2.5. Production of recombinant ChIL-2

Supernatants of COS cells containing rChIL-2 were produced as described (Lawson et al., 2001). Briefly, primers were designed to ChIL-2 cDNA clones isolated by Sundick and Gill-Dixon (1997). RT-PCR was performed using these primers and the resulting PCR products were cloned into pGEM-T Easy. The cDNA inserts were then ligated into the NotI site of the expression vector pCIneo. COS cells were then transfected by incubating the cells in serum-free DMEM medium containing 7.5 µg/ml DNA (pCIneo containing ChIL-2 or no plasmid) for 3 h at 37 °C, 5% CO_2. The transfecting medium was removed and growth medium replaced with serum-free DMEM and the cells incubated for 72 h when the supernatants were collected and stored. The dilution of rChIL-2 used in all experiments was 1:1000 as was previously reported (Kogut et al., 2002).

2.6. Priming of heterophils

To determine the effect of rChIL-2 on the expression of mRNA for pro-inflammatory and Th1 cytokines by heterophils, the cells were incubated for 4 h at 39 °C with either rChIL-2 (1:1000 dilution of stock) or supernatants from mock-transfected COS cells and then subsequently stimulated with either nonopsonized, CS-opsonized, or IgY-opsonized SE for 30 min at 39 °C.

2.7. Real-time quantitative RT-PCR

Cytokine mRNA levels in control heterophils and heterophils following phagocytosis with opsonized or nonopsonized SE were quantitated using a method based on that of Kaiser and colleagues (2000, 2002, 2003).

Total RNA was prepared from heterophils using the RNeasy mini kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Purified RNA was eluted in 50 µl of RNase-free water and stored at −70 °C.

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). 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 labeled with the fluorescent reporter dye 5-carboxyfluorescein at the 5′ end and with

Table 1
Real-time quantitative RT-PCR probes and primers

RNA target		Probe/primer sequence	Exon boundary	Accession number[a]
28S	Probe	5′-(VIC)-AGGACCGCTACGGACCTCCACCA-(TAMRA)-3′		X59733
	F[b]	5′-GGCGAAGCCAGAGGAAACT-3′		
	R[c]	5′-GACGACCGATTGCACGTC-3′		
IL-1β	Probe	5′-(FAM[d])-CCACACTGCAGCTGGAGGAAGCC-(TAMRA)-3′	5/6	AJ245728
	F	5′-GCTCTACATGTCGTGTGTGATGAG-3′		
	R	5′-TGTCGATGTCCCGCATGA-3′		
IL-6	Probe	5′-(FAM)-AGGAGAAATGCCTGACGAAGCTCTCCA-(TAMRA)-3′	3/4	AJ250838
	F	5′-GCTCGCCGGCTTCGA-3′		
	R	5′-GGTAGGTCTGAAAGGCGAACAG-3′		
IL-8	Probe	5′-(FAM)CTTTACCAGCGCGTCCTACCTTGCGACA-(TAMRA)-3′	1/2	AJ009800
	F	5′-GCCCTCCTCCTGGTTTCA G-3′		
	R	5′-TGGCACCGCCAGCTCATT-3′		
IL-18	Probe	5′-(FAM)-CCGCGCCTTCAGCAGGGATG-(TAMRA)-3′	4/5	AJ416937
	F	5′-AGGTGAAATCTGGCAGTGGAAT-3′		
	R	5′-ACCTGGACGCTGAATGCAA-3′		
IFN-γ	Probe	5′-(FAM)-TGGCCAAGCTCCCGATGAACGA-(TAMRA)-3′	3/4	Y07922
	F	5′-GTGAAGAAGGTGAAAGATATATCATGGA-3′		
	R	5′-GCTTTGCGCTGGATTCTCA-3′		

[a] Genomic DNA sequence.
[b] Forward.
[c] Reverse.
[d] 5-Carboxyfluorescein.

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.

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 (Kaiser et al., 2000). 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.

2.8. Statistical analysis

The anti-coagulated blood from 50 chickens was pooled and the heterophils were isolated from each treatment group as described above. The RT-PCR assay was conducted three times over a 2-month period with pooled heterophils (heterophils pooled from 50 chickens for each preparation; i.e.,

150 chickens in total were used as cell donors). At least three replicates were conducted for each assay with the heterophils from each pool of chickens. The data from these three repeated experiments were pooled for presentation and statistical analysis.

The mean and standard error of the mean were calculated for each of the treatment groups. Differences between the nonstimulated heterophils and the phagocytic stimuli-activated heterophils were determined by one-way analysis of variance on the pooled data of the three replicate experiments. Significant differences were further separated using Duncan's multiple range test. The data obtained using heterophils stimulated with the nonopsonized and opsonized SE were compared to nonstimulated control heterophils.

3. Results

For the TaqMan experiments, replicate experiments on different days were highly repeatable, with a coefficient of variation for four replicate RT-PCR assays of $\log_{10}$ serially diluted RNA for the different reactions as shown in Table 2. There was a linear relationship between the amount of input RNA and the C_t values for the various reactions (Table 2). Regression analysis of the C_t values generated by the $\log_{10}$ dilution series gave R^2 values for all reactions in excess of 0.98 (Table 2). The increase in cycles per $\log_{10}$ decrease in input RNA for each specific reaction, as calculated from the slope of the respective regression line, is given in Table 2.

To account for the variation in sampling and RNA preparations, the C_t values for cytokine-specific product for each sample were standardized using the C_t value for 28S rRNA product for the same sample from the reaction run simultaneously. To normalize RNA levels between samples within an experiment, the mean C_t value for 28S rRNA-specific product was calculated by pooling values from all samples in that experiment. Tube-to-tube variations in 28S rRNA C_t values about the experimental mean were calculated. Using slopes of the respective cytokine and 28S rRNA $\log_{10}$ dilution series regression lines, the difference in input total RNA, as represented by the 28S rRNA, was then used to adjust cytokine-specific C_t values. Standardization does not dramatically alter the distribution of the results as a whole.

3.1. Pro-inflammatory cytokines

For the priming studies, heterophils were preincubated with or without rChIL-2 for 4 h at $39\,°C$ and then stimulated with either nonopsonized, NCS-opsonized, or IgG-opsonized SE for 30 min at $39\,°C$. rChIL-2 did not directly stimulate an up- or down-regulation of mRNA expression for IL-8 (Fig. 1, panel A) nor any other pro-inflammatory cytokine (data not shown). Pretreatment of heterophils with rChIL-2 had no effect on the expression of IL-1β nor IL-6 mRNA (data not shown) following receptor-mediated phagocytosis of the nonopsonized,

Table 2
Standard curve data from real-time quantitative RT-PCRs on total RNA extracted from stimulated heterophils

	ΔR_n [a]	log Dilutions	C_t [b]	R^2 [c]	Slope
28S	0.05	10^{-1} to 10^{-5}	8–22	0.9937	2.6551
IL-1B	0.02	10^{-1} to 10^{-5}	26–32	0.992	2.9569
IL-6	0.02	10^{-1} to 10^{-5}	21–36	0.9931	2.689
IL-8	0.02	10^{-1} to 10^{-5}	14–28	0.9869	3.3163
IL-18	0.02	10^{-1} to 10^{-5}	20–32	0.9891	4.1632
IFN-γ	0.02	10^{-1} to 10^{-5}	14–33	0.9948	2.6218
TGF-β4	0.02	10^{-1} to 10^{-5}	20–36	0.989	2.4663

[a] ΔR_n: 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 ΔR.

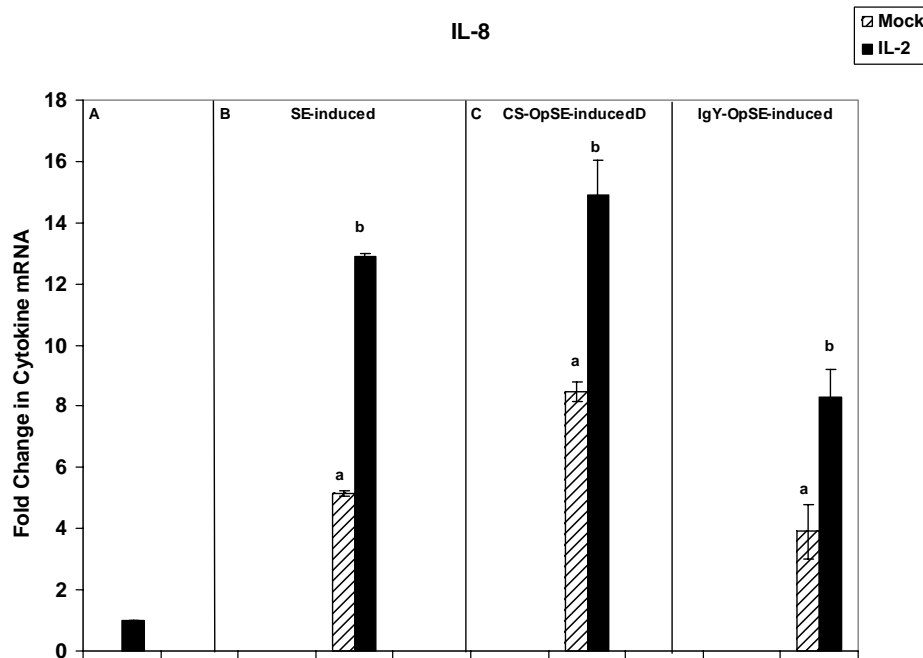[c] R^2: coefficient of regression.

Fig. 1. Quantification of IL-8 mRNA in heterophils after priming with either medium alone or rCh-IL-2 then stimulation with either nonopsonized *S. enteritidis* (SE) or opsonized SE (CS-OpSE or IgY-OpSE). Data are expressed as fold-change in cytokine mRNA levels in stimulated heterophils when compared to those from age-matched non-primed, non-stimulated heterophils (set as 1). No difference in mRNA levels, therefore, is shown as a fold-change of 1. Error bars show SEM from triplicate samples from three separate real-time quantitative RT-PCR experiments. Bars with different lower case letters are significantly different ($P < 0.05$). Panel A: direct stimulation of non-primed heterophils by rChIL-2; panel B: rChIL-2-primed, nonopsonized SE-induced; panel C: rChIL-2-primed, CS-OpSE-induced and panel D: rChIL-2-primed, IgY-OpSE-induced.

CS-opsonized, and IgY-opsonized SE. However, pretreatment of the chicken heterophils with rChIL-2-primed the cells for a significant upregulation of mRNA expression of IL-8 (Fig. 1, panels B–D) following stimulation with either nonopsonized or opsonized SE when compared to the heterophils not treated with rChIL-2 (mock-transfected supernatants, Fig. 1, panels B–D).

3.2. Th1 cytokines

rChIL-2 did not directly stimulate an up- or down-regulation of mRNA expression for IL-18 (Fig. 2, panel A). Pretreatment of the heterophils with rChIL-2 had no effect on the expression of IFN-γ mRNA (data not shown) following receptor-mediated phagocytosis of the nonopsonized, CS-opsonized, and IgY-opsonized SE.

In the priming studies, as seen previously (Kogut et al., 2003), the expression of mRNA of IL-18 was dramatically down-regulated following the receptor-mediated phagocytosis of all three phagocytic stimuli when compared to the nonstimulated control heterophils (Fig. 2, panels B–D). However, pretreatment of the chicken heterophils with rChIL-2-primed the cells for a significant upregulation of mRNA expression of IL-18 (Fig. 2, panels B–D) following stimulation with either nonopsonized or opsonized SE when compared to the heterophils not treated with rChIL-2 (mock-transfected supernatants, Fig. 2, panels B–D).

4. Discussion

Previous experimental results have demonstrated the priming activity of rChIL-2 on chicken heterophils (Kogut et al., 2002). The priming ability of rChIL-2 on chicken heterophils was verified in the present experiments. Not only did rChIL-2 prime the heterophils for an increase in transcription of IL-8 mRNA induced by phagocytic agonists, but it also upregulated expression of mRNA of the Th1 cytokine, IL-18. Although rChIL-2 priming modulated the expression of certain cytokine mRNA in heterophils stimulated by different phagocytic agonists, rChIL-2 by itself did not directly induce gene expression of either the pro-inflammatory or Th1 cytokines. By definition, this represents true "priming" of the heterophils.

The real-time quantitative RT-PCR results from the present studies demonstrate for the first time that cytokine-primed chicken heterophils can be induced, via receptor-mediated phagocytosis, to express increased levels of mRNA of selective cytokines. The enhanced expression of IL-8 and IL-18 mRNA in IL-2-primed heterophils does not appear to be differentially dependent on the receptor stimulated during phagocytosis since the activation of all three receptors during phagocytosis of the opsonized and nonopsonized SE induced the specific upregulation of IL-8 and IL-18 mRNA. These results are in contrast to those observed during phagocytosis by rChIFN-γ-primed
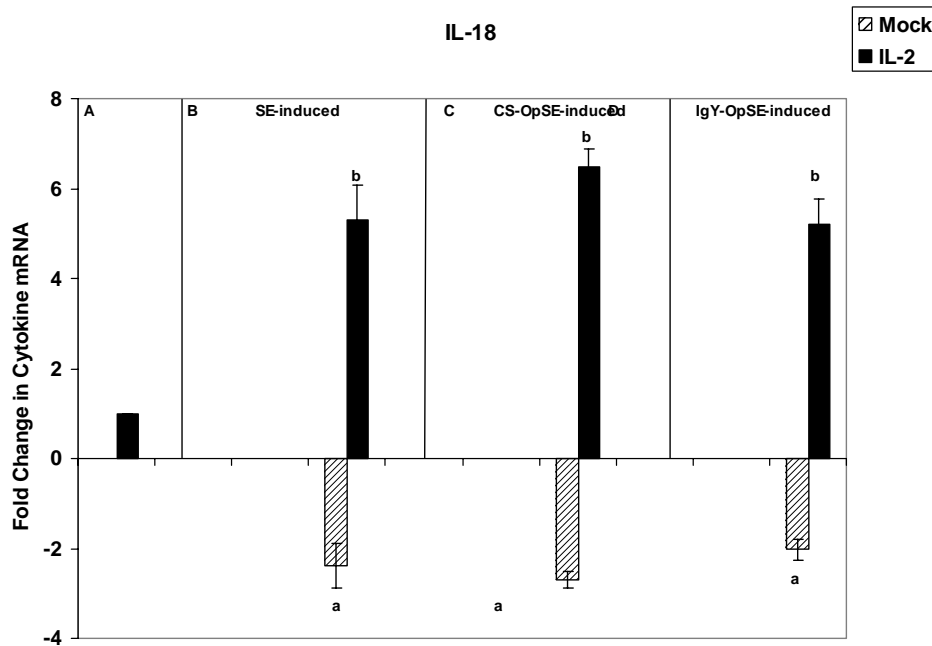
Fig. 2. Quantification of IL-18 mRNA in heterophils after priming with either medium alone or rCh-IL-2 then stimulation with either nonopsonized *S. enteritidis* (SE) or opsonized SE (CS-OpSE or IgY-OpSE). Data are expressed as fold-change in cytokine mRNA levels in stimulated heterophils when compared to those from age-matched non-primed, non-stimulated heterophils (set as 1). No difference in mRNA levels, therefore, is shown as a fold-change of 1. Error bars show SEM from triplicate samples from three separate real-time quantitative RT-PCR experiments. Bars with different lower case letters are significantly different ($P < 0.05$). Panel A: direct stimulation of non-primed heterophils by rChIL-2; panel B: rChIL-2-primed, nonopsonized SE-induced; panel C: rChIL-2-primed, CS-OpSE-induced and panel D: rChIL-2-primed, IgY-OpSE-induced.

heterophils (Kogut et al., 2003). rChIFN-γ primed the heterophils for an increase in transcription of IL-1β, IL-6, and IL-8 induced by phagocytic agonists plus upregulated the expression of Th1 IL-18 and IFN-γ mRNA. Taken together, these results demonstrate that cytokine gene expression in avian heterophils can be regulated by the priming agent to allow the cell to respond in a qualitative manner to the nature of a stimulus (Hallet and Lloyds, 1995; Condliffe et al., 1998). For example, the results from the present studies imply that IL-2 may play a very specialized role in the effector innate responses of avian heterophils. The increased expression of IL-8 shows that heterophils direct their own recruitment that leads to an influx of heterophils to the site of infection, thus increasing the ability of the host to limit the infection. Accordingly, heterophils are proficient in amplifying the local acute inflammatory response. This self-activation, amplification, and recruitment of cells is characteristic of mammalian PMNs during phagocytosis (Hachicha et al., 1995, 1998). Furthermore, the selective expression of IL-18 in the IL-2-primed heterophils would further lead to enhancing bacterial clearance. IL-18 has been shown to be more potent than either IFN-γ or IL-12 in inducing host protection and enhancing bacterial clearance of *Listeria, Salmonella*, *Shigella*, and *Mycobacterium tuberculosis* (Neighbors et al., 2001, Biet et al., 2002). We can speculate that, unlike heterophils primed with rChIFN-γ, which enhances both the innate response, by the increased transcription of both pro-inflammatory cytokine

genes, and the acquired immune response, by the increased transcription of Th1 cytokine genes, rChIL-2 could be priming heterophils solely to function as more efficient innate effector cells to limit bacterial growth through the selective increase of IL-8 and IL-18 gene expression. This hypothesis is supported further by our earlier observations where rChIL-2 significantly enhanced the phagocytic and bactericidal activities of avian heterophils (Kogut et al., 2002). Therefore, we propose that the biological effects of IL-2 in birds could be extended to include a supporting role in heterophil-mediated innate immune responses. Similar PMN priming activities of IL-2 have been reported with human neutrophils (Djeu et al., 1993; Wei et al., 1993, 1994).

It should be pointed out that quantitative RT-PCR does not necessarily equate to bioactive protein. For example, to date only IFN-γ and IL-6 mRNA levels have been shown to correlate with protein levels (Kaiser et al., 2000). Only a limited number of reliable bioassays for chicken cytokines are available (IFN-γ, type I IFN, IL-1β, IL-2, IL-6, and IL-18) and even fewer monoclonal antibodies (IL-2, IFN-γ, type I IFN). Therefore, real-time quantitative PCR is currently the most highly sensitive method available to reliably quantify a variety of avian cytokines, particularly in the absence of suitable bioassays for most of them (Kaiser et al., 2000, 2002, 2003).

The innate response plays a critical part in the early response of the avian host to salmonellosis in chickens (Hu et al., 1997; Kaiser et al., 2000; Kogut et al., 1994;

Wigley et al., 2002) with heterophils playing an essential effector cell role in controlling intestinal (Kogut et al., 1994) and extra-intestinal infections of *S. enteritidis* and *Salmonella gallinarum* (Kogut et al., 1996). The stimulation of pro-inflammatory cytokine gene expression by agonists of Toll-like, complement, and Fc receptors also appear to be important facets of the early phase of the host innate response to salmonellosis (Farnell et al., 2003; Kogut et al., 2003). The results from the present studies further indicate that heterophils exposed to opsonized and nonopsonized *S. enteritidis* in the presence of IL-2 enhances the innate response, by the selective increase in the transcription of IL-8 and IL-18 genes. These results illustrate that chicken IL-2 is more pleiotrophic than believed since it can directly activate chicken heterophils to exert innate effector functions.

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