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ScienceDirect
Comparative Biochemistry and Physiology, Part D 1 (2006) 292–299
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Early host gene expression responses to a *Salmonella* infection in the intestine of chickens with different genetic background examined with cDNA and oligonucleotide microarrays

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Received 20 December 2005; received in revised form 9 May 2006; accepted 14 May 2006
Available online 3 June 2006

Abstract

So far the responses of chickens to *Salmonella* have not been studied *in vivo* on a whole genome-wide scale. Furthermore, the influence of the host genetic background on gene expression responses is unknown. In this study gene expression profiles in the chicken (*Gallus gallus*) intestine of two genetically different chicken lines were compared, 24 h after a *Salmonella enteritidis* inoculation in 1-day-old chicks. The two chicken lines differed in the severity of the systemic infection. For gene expression profiles, a whole genome oligonucleotide array and a cDNA microarray were used to compare both platforms. Genes upregulated in both chicken lines after the *Salmonella* infection had a function in the innate immune system or in wound healing. Genes regulated after the *Salmonella* infection in one chicken line encoded proteins involved in inflammation, or with unknown functions. In the other chicken line upregulated genes encoded proteins involved in acute phase response, the fibrinogen system, actin polymerisation, or with unknown functions. Some of the host gene responses found in this study are not described before as response to a bacterial infection in the intestine. The two chicken lines reacted with different intestinal gene responses to the *Salmonella* infection, implying that it is important to use chickens with different genetic background to study gene expression responses.

Keywords: Chicken; Gene expression; Genomics; Host response; Immune system; Microarray; *Salmonella*; Small intestine

1. Introduction

Salmonella enterica is one of the most common causes of food poisoning in humans, mostly caused by poultry products infected by *S. enterica* serovars Typhimurium or Enteritidis (Rabsch et al., 2001). Following oral ingestion, *Salmonella* colonize the intestines and invade the intestinal mucosa. In addition to the enteric disease in humans *Salmonella* serovars Typhimurium and Enteritidis are also capable of causing severe systemic disease in newly hatched chicks and in birds under extreme stress conditions. The infection seldom causes mortality in birds more than 1 month old (Suzuki, 1994). In young chickens infection with *Salmonella* leads to diarrhea and intestinal lesions and to an influx of heterophils into the gut accompanied by inflammation and damage to villi (Barrow et al., 1987). Heterophils are the avian equivalent of mammalian neutrophils and play a key role in protecting chickens from the development of systemic disease following infection with *Salmonella* serovar Enteritidis by largely restricting the bacteria to the gut (Kogut et al., 1994).

Host gene expression responses to a *Salmonella* infection have widely been studied. One of the methods to investigate gene expression responses is the use of microarrays that allow the analysis of the expression of a large number of genes in a single experiment. Indeed microarrays have been used to study gene expression responses to *Salmonella* (reviewed in (Rosenberger et al., 2001)). With this approach it was found that in human epithelial cells cultures *Salmonella typhimurium* induce a classical proinflammatory gene expression pathway with upregulation of several cytokines, kinases and transcription factors (Eckmann et al., 2000; Zeng et al., 2003). Also in human macrophages

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doi:10.1016/j.cbd.2006.05.001

Salmonella induces a set of gene products, many of which are proinflammatory (Detweiler et al., 2001). As in other organisms, *Salmonella* is capable to alter gene expression levels in chickens; however, mainly cytokine levels have been studied in response to *Salmonella* infections. In the intestine and liver interleukin (IL) 8, K60 (a CXC chemokine), macrophage inflammatory protein 1β, and IL-1β levels were upregulated in response to *Salmonella* (Withanage et al., 2004), suggesting inflammation in those tissues. Also in heterophils, upregulation of pro-inflammatory cytokines IL-6, IL-8 and IL18 was detected after exposure to *Salmonella* as well as a downregulation of transforming growth factor-β4, an anti-inflammatory cytokine (Swaggerty et al., 2004), indicating inflammatory processes to be important after *Salmonella* exposure. The role of IFNγ in the response to *Salmonella* is less clear, up and downregulation as well as no regulation in response to *Salmonella* has been reported (Kaiser et al., 2000; Sijben et al., 2003; Sadeyen et al., 2004). In addition to the cytokines also cationic liver-expressed antimicrobial peptide 2 was shown to be upregulated in the chicken intestine and liver in response to *Salmonella* (Townes et al., 2004).

In contrast to other species, no genome-wide expression profiles in the chicken in response to *Salmonella* have been measured. Therefore several processes in the chicken host in response to *Salmonella* may be unidentified. Furthermore validation of the *in vitro* gene expression observations by *in vivo* data are scarce. In addition it is not known why some chicken lines are more susceptible to *Salmonella* infections than others (Barrow et al., 1987; Guillot et al., 1995). In the present study we describe the gene expression response in the intestine of young chickens after a *Salmonella* infection. The data obtained with a whole genome oligonucleotide array were compared to those obtained with a tissue specific cDNA array. Also two different chicken lines were used and the results were compared with each other to determine the role of the genetic background in the host response.

2. Materials and methods

2.1. Array fabrication

The oligonucleotide arrays were obtained from Affymetrix, the GeneChip Chicken Genome Array. These arrays contained 38,449 *Gallus gallus* probe sets with 11 probe pairs of 25-mers per sequence.

The cDNA microarrays were constructed as described earlier (van Hemert et al., 2003). The latter microarrays contained 3072 cDNAs from a subtracted and normalized intestinal library and 1152 cDNAs from a subtracted and normalized concanavalin A stimulated spleen library. All cDNAs were spotted in triplicate on each cDNA microarray.

2.2. Chicken lines

The following study was approved by the institutional Animal Experiment Commission in accordance with the Dutch regulations on animal experimentation. Two meat type chicken lines (*G. gallus*), the fast growing line A and the slow growing line B were used in the present study (Nutreco®). These chicken lines differ in response to *Salmonella*. Line A had a more severe systemic infection, as after a *Salmonella* infection more CFU in the liver were found and the clearance was slower than for line B. In addition the chickens from line A had a higher weight gain depression after a *Salmonella* infection compared to the chickens from line B (van Hemert et al., in press).

2.3. Experimental infection

Ten one-day old chickens of each line (A and B) were randomly divided into 2 groups. After hatching, it was determined that birds were free of *Salmonella*. Of each chicken

Table 1
Genes more than two-fold regulated in both chicken lines in response to *Salmonella* infection, with $p<0.01$ for the microarray data

Accession no.	Locus ID[a]	Description	Fold-change	
			Line A	Line B
GeneChip array				
NM_205320	396260	Mature avidin	64.3	3.9
CF250837	418700	Similar to lysozyme (EC 3.2.1.17) g	8.4	19.2
	418700	Similar to lysozyme (EC 3.2.1.17) g	6.2	12.8
X61198	395708	lysozyme	7.2	4.7
BU435658	HLA-DRB1	MHC class II beta chain (B-LB) mRNA, B-LB-B21 allele	2.1	55.9
BU260479	423432	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10	7.8	10.6
NM_204989	395837	Fibrinogen gamma chain (FGG), mRNA	6.9	100
BX932101	396241	Ovotransferrin	2.2	15.1
BU220239	416546	Similar to NADPH oxidase organizer 1 isoform b; regulatory protein P41NOX; Nox organizer 1	18.6	3.7
AJ721110	421702	Similar to Vascular non-inflammatory molecule 3 precursor (Vanin 3)	10.5	3.1
NM_205213	396135	Hepatocyte growth factor-like/ macrophage stimulating protein (HGF1/MSP), mRNA	8.9	3.3
BM425681	417345	Hypothetical gene supported by CR391572	6.3	2.8
XM_418660	420559	Similar to KIAA2005 protein	2.0	2.5
cDNA array				
NM_204933	395773	Cytidine deaminase (CDD), mRNA	2.4	2.4
NM_205125	396023	Dickkopf homolog 3	5.3	6.8
XM_420282	422305	Similar to DNA segment, Chr 10, Johns Hopkins University 81 expressed	6.1	7.1
XM_416896	418700	Similar to lysozyme (EC 3.2.1.17) g	2.3	6.2
XM_418586	420484	Similar to fatty acid synthase	2.3	2.4

[a] The locus ID refers to the LocusLink and Entrez Gene databases from the NCBI.