Kinetic Profile of Chicken Macrophage Immune Response upon exposure to *Salmonella*-derived Endotoxin

A.S. Leaflet R2481

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Summary and Implications

Large scale gene expression studies expedite the discovery process and provide a comprehensive view of host immune response. We used the Affymetrix GeneChip chicken genome array to determine the nature and breadth of the gene activation elicited by endotoxin from *Salmonella typhimirium* (ST) 798. The data obtained from this type of research are important to improve vaccine development efficacy and to enhance animal health and food safety. Our findings may contribute to elucidation of disease response pathways.

Introduction

Macrophages are white blood cells whose role is to engulf and digest pathogens and dead cell residues. They stimulate other immune system cells by producing regulatory molecules such as cytokines to mount a counter attack once pathogens enter host cells. *Salmonella* bacterium is one of the most frequently reported causes of food-borne gastroenteritis in humans. Ingestion of contaminated water or food usually poultry or beef products is the main cause of diseases. Therefore, investigating the effects of endotoxin from *Salmonella* in chicken macrophages is important and appropriate to explore the cytokine profile in the context of chicken host defense.

Materials and Methods

The chicken macrophage cell line HD11 was used as a model and cultured at 41°C and 5% CO_2. Cells were treated with 0.0, 0.1, 1.0, and 10.0 µg/ml ST-798 endotoxin for 1, 2, 4, and 8 hours. Expression of IL6, IL8, IL10, IL1β, IFNγ, TLR15, and 28s genes were measured by quantitative PCR. The standard curves for all tested genes were prepared using serial dilutions of templates. C(t) values were calculated by normalizing to 28s housekeeping gene. Comparisons within dose and time were ranked by Tukey HSD test to define the optimum concentration for ST-798 endotoxin to induce an immune response. *P*-values considered significant at $P \leq 0.05$.

Further analysis of chicken immune response was performed with Affymetrix genechip that contains 38,535 probes to determine the kinetic profile of chicken immune response.

Results and Discussion

The present study reports that 1.0 µg/ml ST-798 endotoxin is sufficient to elicit an immune response in chicken macrophages. Exposure to endotoxin significantly affected the expression levels of IL1β ($P < 0.0001$), IL6 ($P = 0.03$), IL8 ($P < 0.0001$) and TLR15 ($P = 0.002$) Table 1. *Affymetrix* GeneChip chicken genome array analysis showed that 13, 33, 1761, 61genes were significantly influenced by endotoxin at 1, 2, 4, and 8 hours; respectively (Figure 1). Therefore, 4 hours exposure was the critical time point for HD11 cells, since the maximum number of differentially expressed genes was reached at this time (Figure 2).

Next, we compared the gene networks for each time point using Ingenuity Pathway Analysis. Results demonstrated that 10% of the total differentially expressed genes were involved in only inflammatory response. Three, 8, 80, and 9% of inflammatory response genes at 1, 2, 4 and 8 hours were significantly affected; respectively (Figure 3). The NFκBIA, IL1B, IL8, CCL4 genes were consistently induced at all time points after endotoxin treatment, showing their important role in response to *Salmonella.*

Gene profiling, in a timecourse experiment, allowed us to monitor chicken immune response. Our results have provided a detailed look at transcriptional regulation of genes that are involved in chicken macrophage response and showed how complex the genetic regulation of host defense is.

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Table 1. ANOVA model effects on HD11 gene expression levels (*p*-values).

Genes	Time	Dose	Interaction
TLR15	0.03	0.002	0.69
IL8	<0.0001	<0.0001	0.54
IL1β	<0.0001	<0.0001	0.67
IFNγ	<0.0001	0.38	0.80
IL6	0.014	0.03	0.02
IL10	<0.0001	0.43	0.78

Figure 1. Differentially expressed genes by time, during stimulation with *S*T-798 ($q < 0.05$) compared to non-stimulated chicken HD11 cells.

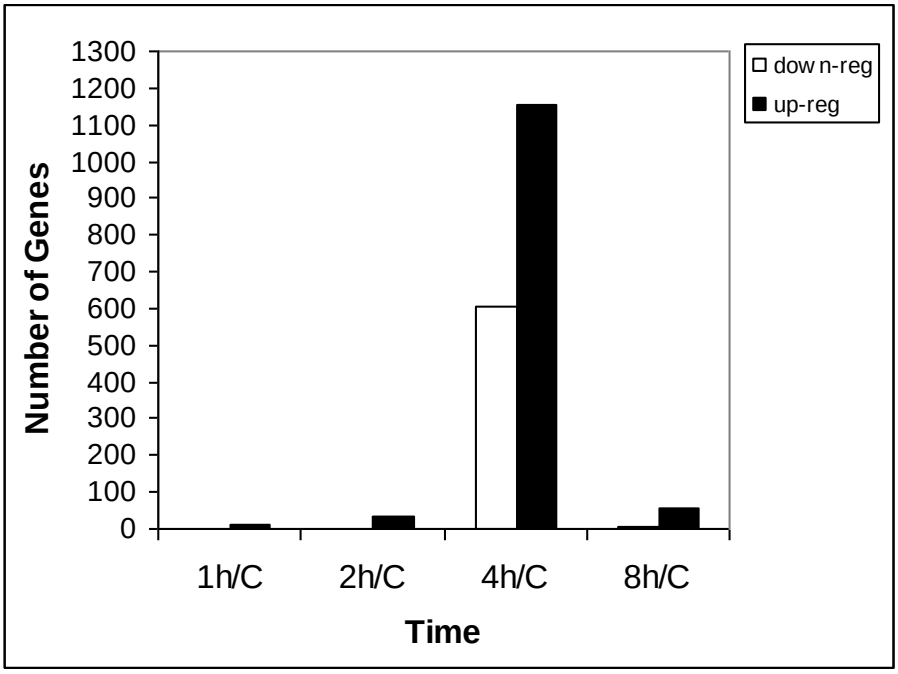

Figure 2. Gene networks at 4 hours post-stimulation. Red and green colors show up-regulation and down-regulation; respectively (IPA). Grey molecules are not differentially expressed. They are included to illustrate how significantly up-regulated genes interact with them.

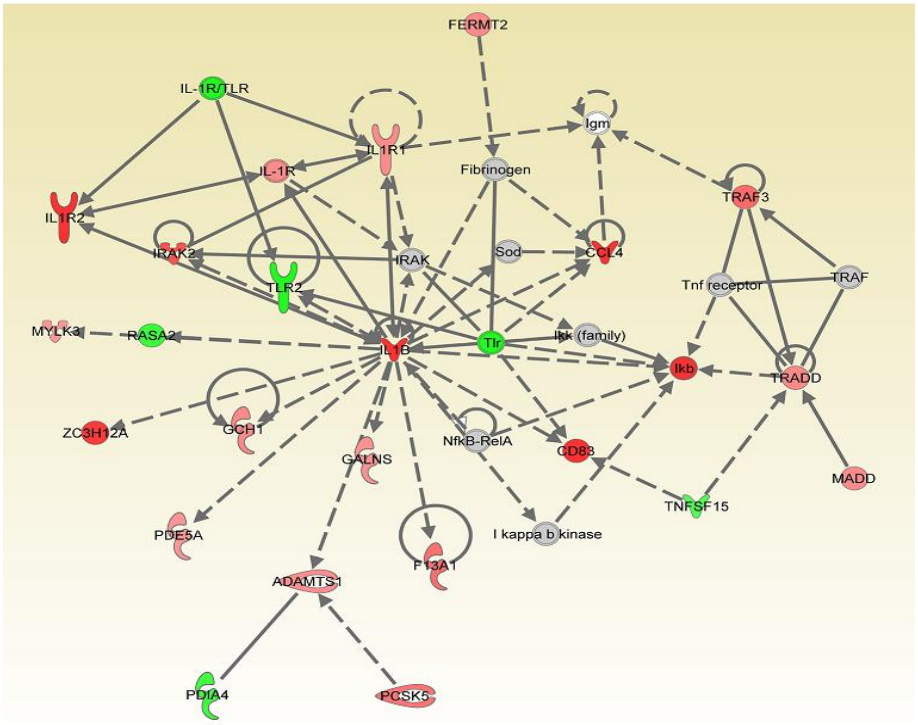

Figure 3. Distribution of inflammatory response genes by time