Rooster's Genetic Response to Immune Stimulation

A.S. Leaflet R2993

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Summary and Implications

Lipopolysaccharide (LPS), commonly used to mimic bacterial infection without using live bacteria, was injected into roosters from three genetically diverse lines and gene expression was measured at two time points post-injection. TLR4, MD-2, and MyD88 are three genes that are responsible for initiating the major immune pathway that responds to LPS. Results showed significant differences in gene expression at different times post-stimulation for both MyD88 and TLR4. The three chicken lines had different expression levels of TLR4 in response to LPS. This shows that there are genetic differences in this immune pathway. Further studies are needed, but it may be possible to use TLR4 expression information in the selection process. Overall, bacterial infections are a serious threat to chickens and a better understanding of this pathway will lead to beneficial, applied approaches in the poultry industry.

Introduction

Lipopolysaccharide (LPS) is a component of the cell wall of certain bacteria. The immune system recognizes LPS as harmful and initiates a defense against it. Stimulating with LPS instead of infecting with bacteria, is a much safer approach, making LPS a useful research tool. When the host immune cells come into contact with LPS, Toll-Like Receptor 4 (TLR4) and myeloid differentiation protein-2(MD-2) are two proteins that work together at the cell membrane to initiate the response. Inside the host cell, another protein, MyD88, is responsible for passing the signal downstream to other cellular components in this pathway.

Iowa State University is home to several unique research lines of chickens, including broiler, Leghorn, and Fayoumi. The inbred Fayoumi line was imported from Egypt and has been shown through multiple studies to have a more resilient immune system. The Leghorn is an inbred line that originated from a cross of commercial egg-laying lines. The outbred broiler came from a commercial male breeding line used for meat production. These three poultry breeds are genetically different and we hypothesize that they will also differ in their response to LPS.

Materials and Methods

In total 36 roosters, 12 birds from each line were used. An LPS injection was given to 6 roosters and a control PBS (saline) injection was given to the other 6 roosters in each line. At two time points, 1 and 3 hours post-injection, 5mL of blood was collected from each bird. White blood cells were isolated from the blood, and then RNA, the intermediate step between DNA and protein, was isolated from those cells. The relative RNA expression level was measured using qPCR, which quantifies the amount of gene-specific RNA present in the sample.

Results and Discussion

All three chicken breeds appear to follow the same general pattern of TLR4 expression. At the first time point, the LPS-stimulated birds expressed less TLR4 than the control birds and by the second time point the TLR4 expression returned to the control level (Figure 1). Genetics had a significant effect on TLR4 expression in that the three lines varied in the strength of the interaction between time post-injection and treatment group (Figure 1).

All three lines of chickens on average had a significantly higher relative amount of MyD88 RNA at hour 3 post-injection, in response to LPS (Figure 2). Compared to TLR4, MyD88 had a delayed response in terms of RNA expression levels. The major difference between the two genes is in the expression levels of the LPS stimulated birds relative to the PBS control birds.

RNA expression levels of MD-2 were not affected by LPS, chicken line, or time post-injection within the context of this study. Because adult birds were used in this study, it is important to note that this is likely not be the first time these chickens came into contact with LPS, because LPS is found everywhere. All three genes varied in relative RNA expression patterns, when comparing the response to treatment, time, and line, which suggests different methods of regulation for each gene. Additionally, this study further demonstrates the distinct immune response profiles exhibited by these three research lines.

Acknowledgements

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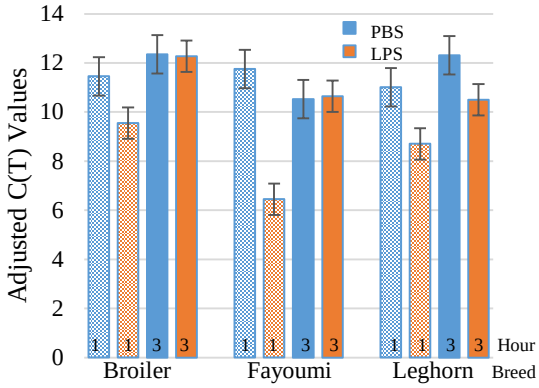

Figure 1: TLR4 relative RNA expression Line*Treatment*Time. The adjusted C(T) values from the three way interaction ($p<0.05$) are separated by breed, hour, treatment. The blue bars represent the PBS control birds, the orange the LPS stimulated birds, both are patterned at the hour 1 time point. The major difference is in the LPS birds at hour 1.

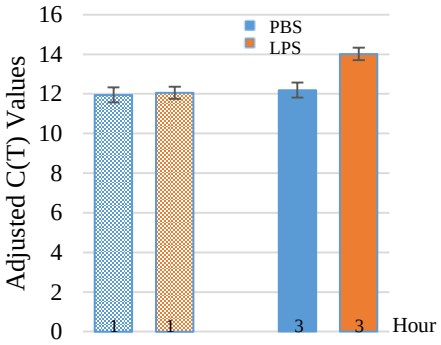

Figure 2: MyD88 relative RNA expression Treatment*Time. This significant ($p<0.05$) two-way interaction shows the differences between the PBS and LPS treated birds at the two time points, averaged over the three lines of chickens. The blue bars represent the PBS control birds, the orange the LPS stimulated birds, both patterned bars represent the hour 1 time point, solid bars the hour 3 time point. At hour 3, the LPS-stimulated birds have a significantly higher relative RNA expression level of MyD88.