Host Cellular Response to Multiple Stressors Using a Chicken *in vitro* Model

A.S. Leaflet R3097

Anna Slawinska, Assistant Professor, UTP University of Science and Technology in Bydgoszcz, Poland;
John C.F. Hsieh, Graduate Assistant;
Carl J. Schmidt, Professor, University of Delaware;
Susan J. Lamont, Distinguished Professor, Department of Animal Science

Summary and Implications

Heat stress (HS) is a major environmental stressor to chickens because chickens lack sufficient physical ability to mitigate heat. One of the potential results of heat stress is the "leaky gut syndrome", which allows gut bacteria to escape into the host and release toxins including lipopolysaccharide (LPS). To model the chicken immune response to bacteria toxins under heat stress, a chicken macrophage-like cell line, HD11, was subjected to HS, LPS, or HS + LPS treatments. Expression of a gene panel of heat shock proteins, stress-related molecules, signaling molecules, and immune response molecules were measured and analyzed at 4 time points across the 3 conditions. As expected, heat shock proteins and immune response molecules increased in expression during HS and LPS challenge, respectively. Treatment of HS + LPS increased the expression of these responsive genes even more than either treatment alone. This suggests that heat stress proteins not only mitigate heat stress, but also trigger a higher level of immune response in chickens.

Introduction

As the average global temperature increases, HS is one of the major challenges in poultry production due to chicken's lack of efficient body temperature control. During HS, the tight junctions between cells lining the gut lose connectivity allowing the intestinal content to leak out. In turn, the gut bacteria release toxins such as LPS triggering an inflammatory response of the immune system. To investigate the effects of HS and LPS on chickens, we used an *in vitro* model, HD11 cells, to help identify changes in gene expression in response to HS, LPS, and HS + LPS treatments. We hope to elucidate these changes at the cellular level during multiple environmental stresses.

Materials and Methods

The chicken macrophage-like HD11 cell line was cultured at 41.5°C, the body temperature of chickens. The HD11 cells were stimulated based on 4 treatment groups: (1) HS + LPS, (2) HS, (3) LPS, or (4) untreated. Heat stress was applied to groups 1 and 2 by incubating the cells at 45°C for 2 hours. LPS treatment was applied at the same time as the heat stress for groups 1 and 3. After 2 hours, the cells were returned to 41.5°C and allowed to recover for 0, 2, 4, and 8 hours before harvesting. The experiment was replicated 3 times with 3 biological replicates for every treatment group and time point. Total RNA was isolated from the harvested samples, and gene expression was measured with microfluidic RT-qPCR technology (Fluidigm Corporation, San Francisco, CA) targeting 44 genes of heat shock proteins, stress-related molecules, signaling molecules, and immune response molecules. Raw gene expression (Ct values) was converted to relative expression using a delta Ct method (Real-Time PCR Analysis Software, Fluidigm Corporation, San Francisco, CA) to calculate $\log_2$ fold change. Hierarchical clustering of genes based on expression levels across treatments and time points was performed in R.

Results and Discussion

Hierarchical clustering of the genes with similar expression patterns identified 8 clusters (Figure 1). The first cluster included genes encoding heat shock proteins having high expression for treatments with heat. The second cluster grouped the chemokine genes together with inducible nitric oxide synthase (iNOS) gene, for treatments with LPS. Both clusters 1 and 2 showed expression reinforcement by the synergistic combination of HS + LPS treatments. The third cluster included two cytokines and Ubiquitin B (UBB) gene, and the cluster was induced at 0h and then down-regulated at later time points for treatments with heat. The remaining five clusters grouped the genes with lower expression, the majority of which belonged to the TLR4 signaling, stress response and apoptosis pathways. There are 3 main findings from this study. First, acute heat stress (0h) activated heat shock protein genes that were not as stimulated by LPS alone. Next, HD11 responded to LPS with up-regulation of immune response molecules, such as chemokines and cytokines. Finally, we observed HS + LPS synergistically increased expression of genes, suggesting enhanced immune response during heat stress in chickens.

Acknowledgments

Dr. A. Slawinska was supported by Polish-American Fulbright Commission 2014/2015 Senior Advanced Research Award. This work is supported by USDA Agriculture and Food Research Initiative Competitive Grant #2011-67003-30228 and Hatch project #5358.

Figure 1. Log_2 Fold Change Heat Map. A heat map for the 3 different treatments across the 4 time points. Log_2 fold change calculated based on treatment samples' relative expression values compared to the control samples' relative expression values. Green cells implies increased expression while red cells implies decreased expression. Genes on the right are clustered using a hierarchical clustering method and 8 clusters were found. Cluster 1 = BAG3, HSPA2, HSPH1, DNAJA4, HSP25; Cluster 2 = iNOS, IL1B, CCL5, CCl4, IL8; Cluster 3 = IL12B, IFNG, UBB; Cluster 4 = TGFB2, DNAJB6, JUN, HSP90; Cluster 5 = TLR4, NLRC5, HSF2, HSF5; Cluster 6 = CASP8, IL18, RB1CC1, TRAF6, SMAD6, MyD88, CASP7, CASP1, IRAK4, TP53; Cluster 7 = CASP9, MAP48IP3, CASP3; Cluster 8 = CIRBP, MAPK9, IFNB, TGFB3, HSPA14, LITAF, CD40. Each gene is color coded based on their major functional category: heat shock proteins (red), immune response molecules (green), signaling molecules (yellow), and stress-related molecules (orange).