2015

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Recommended Citation

Available at: [https://lib.dr.iastate.edu/ans_air/vol661/iss1/55](https://lib.dr.iastate.edu/ans_air/vol661/iss1/55)
Thymus Transcriptome Response to Avian Pathogenic E. coli (APEC) Infection

A.S. Leaflet R2992

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Summary and Implications
Colibacillosis, caused by avian pathogenic Escherichia coli (APEC), is responsible for multi-million dollar losses in the poultry industry every year in the United States. Therefore, it is important to understand the functional genomics of avian response to APEC, to find effective control strategies. The transcriptome characterizes genetic elements that are expressed. The thymus transcriptomes of 24 birds with known challenge status, necropsy day, and pathology level were sequenced using RNA-Seq. Many innate immune response pathways were significantly changed. Differentially expressed (DE) genes analysis showed APEC infection affected immune homeostasis through impairing the engagement of regulatory signal transduction pathways for T cell development. This study gives insight into T cell development and immune response under APEC infection. The DE gene analysis revealed gene networks that affect T cell development as well as immune homeostasis following APEC infection. These results add knowledge of host immune response to APEC infection.

Introduction
Colibacillosis is an acute and largely systemic disease resulting in massive financial losses to the poultry industry. This infectious disease is initiated in the respiratory tract by inhalation of fecal dust. This may be followed by invasion of the bloodstream and immune organs, causing sudden death, septicemia, and pericarditis. APEC-contaminated poultry products may pose a challenge to human health. Moreover, consumers are increasingly demanding higher food safety and less antimicrobial usage in animal production. Although vaccines offer an attractive route to control APEC, many vaccines are only effective against homologous APEC. Consequently, breeding for genetic resistance is likely to become increasingly important. Therefore, it is important to understand the functional genomic of avian response to APEC in order to find an effective control strategy.

The transcriptome is a reservoir of all types of RNA molecules, including mRNA, tRNA, rRNA etc., which is a contemporary research area to study gene function.

Genome-wide study of gene expression profiling of immune organs or cells is a major method to simultaneously compare the expression levels of hundreds of thousands genes under different conditions. The thymus, a primary lymphoid organ, plays a vital role in lymphocyte development and T cell receptor gene rearrangement. This study aims to detect the transcriptomic response of genes involved in the earliest phases of immune response associated with APEC infection through thymus. Our goal is to obtain greater understanding of the host genomic response to APEC.

Material and Methods

Animals and samples
At four weeks age, 288 commercial male broilers were inoculated with 0.1 APEC O1 via the air sac and 72 male broilers were injected with the same dose of phosphate buffered saline (PBS). Birds were euthanized and samples collected at post-infection day 1 and day 5. Then, challenged birds were assigned into mild (resistant) and severe (susceptible) categories based upon scoring of lesions in liver, air sacs, and pericardium.

RNAseq Analysis
Total RNA was isolated from each thymus sample. Each RNA sample was constructed into a cDNA library and sent to the ISU DNA facility to sequence. After raw reads were trimmed and aligned, gene expression was analyzed.

Quantitative Real Time PCR Validation
The expression level of 8 genes differentially expressed by RNAseq were analyzed in triplicate by qRT-PCR. The adjusted cycle threshold (Ct) value of each sample was analyzed using ANOVA and JMP pro 11 software.

Results and Discussion

Most differentially expressed genes were clustered in susceptible birds vs. resistant birds at day 5, susceptible birds vs. non-challenged birds at day 5, and susceptible birds at day 5 vs. day 1. The genes, RAG2, LIG4, CD3D, and CD3Z, which are involved in early T cell development, were significantly down-regulated in susceptible birds compared to resistant and non-challenged birds at day 5. Genes in the T cell receptor pathway were also decreased in expression in susceptible birds, including CD4, Lck, ZAP70, and VAV1. Collectively, APEC infection affected T cell lymphopoiesis through weakening the engagement of both positive and negative regulatory signal transduction pathways for T cell development.

The innate immune pathways, such as the toll-like receptor pathway and NOD-like receptor pathway were significantly activated during APEC infection (figure 1).
Most genes of the innate immune response were up-regulated in susceptible birds compared to resistant birds and non-challenged birds, which indicates that susceptible birds tried to enhance their innate immune response by generating inflammatory cytokines under APEC infection. Cytokines produced by the innate immune response will trigger the adaptive immune response (figure 1). For example, IL18 assists γδ T cells to produce IFNγ, which can stimulate naïve T cell differentiation to Th1 cells. In the current study, the expression of IL18, IL18R, IFNγ, IFNγR and TLR are increased in susceptible birds vs. non-challenged birds at day 5, and in susceptible birds day 5 vs. day 1, revealing adaptive immune response was strengthened through γδ T cells and Th1 cells for susceptible birds.

These findings contribute to the knowledge of transcriptomic response of cells involved in the earliest phases of immune response, including ones that drive the subsequent immune reaction during APEC infection.

**Acknowledgement**

This work is supported by the United States Department of Agriculture National Institute of Food and Agriculture, Hatch Project Number #5203.

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**Figure 1. Innate and adaptive immune response pathway**

*Immune response of thymus transcriptome under APEC infection. (A). The innate immune response including NOD-like receptor signaling and toll-like receptor signaling pathway following APEC infection. Additional, NOD-like receptor signaling and toll-like receptor signaling pathway share partial pathway from MAPK Kinase (MKK). Phagosome and lysosome pathway also assisted NOD-like receptor signaling and toll-like receptor signaling pathways. (B). Adaptive immune response. The cytokines IL18 and IFNG generated by innate immune response can trigger γδ T cell and naïve T cell (αβ T cell) activation. Up-regulated genes were those with red color while down-regulated genes were in blue color. Significant changed pathways were drown magenta color. a: challenged day 5 severe vs. challenged day 1 severe; b: challenged day 5 severe vs. challenged day 5 mild; c: challenged day 5 severe vs. non-challenged day 5.*