Gene Expression Responses to Infection with Avian Pathogenic *Escherichia coli* in Chicken Spleen

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Summary and Implications

Mortality and other production losses due to infection with avian pathogenic *Escherichia coli* (APEC) are a worldwide problem for the poultry industry. In this study, changes in gene expression during APEC infection were characterized in the chicken spleen using RNA-sequencing. Over 400 genes had significant expression changes in response to APEC, with greater changes at 1 day post infection (DPI) than 2 DPI. This decrease was consistent with bacterial clearance from the spleen. Immune signaling, complement, and antimicrobial genes (such as *AVD* and *IL22*) were highly upregulated in response to APEC and predicted to increase phagocyte migration and leukocyte activation. These pathways could contribute to chicken resistance to APEC and provide targets for future research.

Introduction

Colibacillosis is an endemic bacterial disease that impacts poultry health and production in the US and worldwide. Caused by infection of poultry with APEC, colibacillosis often begins in the respiratory tract, and once systemic, can lead to septicemia and death. Current vaccines provide partial (primarily homologous) protection against infection, but are not effective against all the diverse strains of APEC. Therefore, novel methods to decrease colibacillosis are needed. This study identified chicken responses to APEC infection using global gene expression levels in the spleen, which is an immune tissue critical for fighting infectious diseases. Understanding the expression changes induced by APEC will provide genes and pathways to target in future efforts to improve chicken resistance.

Materials and Methods

Animals and Bacterial Challenge

Chicks were obtained from reciprocal F_1 crosses of a disease-resistant Fayoumi line and a disease-susceptible broiler line (broiler ♂ x Fayoumi ♀ and Fayoumi ♂ x broiler ♀). At 14 days of age, chickens in the APEC group (n = 32) were challenged by injection of $1x10^6$ colony forming units (CFU) of APEC O1:K1:H7 into the right air sac. Control birds (n = 32) received phosphate buffered saline (PBS) via the same route. Birds were euthanized at 1 or 2 DPI and spleen samples were collected.

Bacteriology

Bacterial load in each spleen sample was determined by plating homogenized tissue (10-fold serial dilutions) on MacConkey agar, incubating overnight at 37 °C, and then counting APEC colonies on each plate. Statistical analysis was performed in JMP Pro 12.

RNA-sequencing

cDNA libraries (n = 48; 12 libraries/treatment/DPI) were made from high quality splenic RNA; both F_1 crosses were represented equally for each treatment/DPI. Libraries were sequenced on the Illumina HiSeq 3000, the resulting short sequence reads were mapped on the chicken genome, and the number of reads aligned to each gene was counted. Using edgeR, gene expression at each DPI was compared between APEC and PBS groups to identify genes with significant differential expression (DE; q-value < 0.05, $\log_2$ fold change ≥ 1). This DE analysis combined the responses of both F_1 crosses. Potential functional effects of DE genes were investigated using Ingenuity Pathway Analysis (IPA).

Results and Discussion

Bacteriology verified infection of the APEC challenged birds and revealed that bacterial load in the spleen decreased significantly from 1 to 2 DPI (Figure 1). DE analysis identified 418 genes that changed expression in the spleen in response to APEC. The majority of significant genes (365) were identified at 1 DPI. Among these, immune signaling (*AVD*, *IL22*, *IRG1*, and *IL6*), complement (*PTX3*), and antimicrobial (*EXFABP*) genes were the most highly upregulated by infection with APEC. These likely reflect initiation of immune responses, such as “differentiation of phagocytes”, “activation of mononuclear leukocytes” and “migration of phagocytes”. Consistent with declining bacterial load, fewer genes (88) were significant at 2 DPI. *AVD* and *IL22* remain highly upregulated, while most immune-related genes had smaller expression changes at 2 DPI (Figure 2). Overall, this expression pattern suggests that APEC drives a rapid immune response in the spleen that decreases as the bacteria are cleared. The identified cytokines, complement factors, and antimicrobial proteins could impact chicken resistance to APEC infection and warrant further investigation.

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Figure 1. Bacterial load decreases from 1 DPI to 2 DPI. Bacterial levels in APEC challenged spleens were measured as CFU per gram of tissue, log transformed (log_{10}(CFU/g)), and used to calculate least squares means at 1 and 2 DPI.

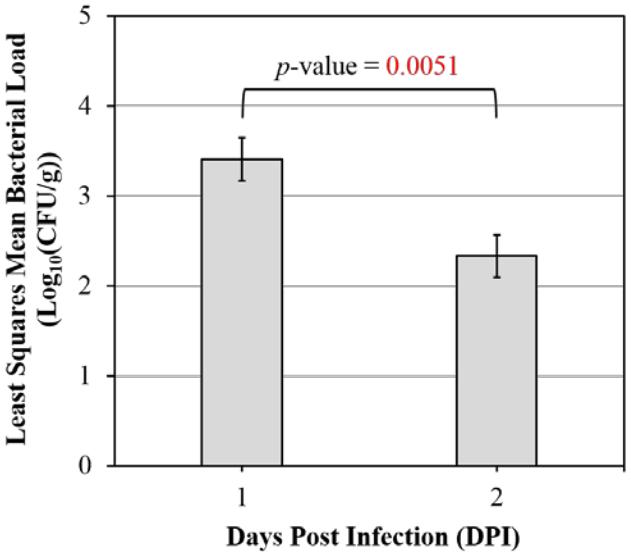

Figure 2. APEC infection induces greater expression changes in immune-related genes at 1 DPI than 2 DPI. Genes shown were identified by IPA as associated with both bacterial infections and mononuclear leukocyte activation. IPA predicted (z-score ≥ |2|) that the DE in these genes could inhibit bacterial infections (blue) and/or activate mononuclear leukocytes (orange). Intensity of the red color for each gene represents the degree of upregulation at 1 and 2 DPI. These genes are representative of the trend over time for other immune-related genes and functions.

Gene	APEC to PBS[1]		Predicted Functional Effects of DE[2]
	1 DPI	2 DPI	
FN1			⬆ mononuclear leukocytes
LGALS3			⬇ bacterial infections
C3			⬇ bacterial infections ⬆ mononuclear leukocytes
CCL4			⬆ mononuclear leukocytes
S100A9			⬆ mononuclear leukocytes
IL22			⬇ bacterial infections ⬆ mononuclear leukocytes
IL6			⬇ bacterial infections ⬆ mononuclear leukocytes
CCR2			⬇ bacterial infections ⬆ mononuclear leukocytes
STAT1			⬇ bacterial infections ⬆ mononuclear leukocytes
SLC11A1			⬇ bacterial infections ⬆ mononuclear leukocytes

[1] log_2 fold change: 0.1 – 5.4

[2] inhibition (z-score ≤ -2; ⬇)
activation (z-score ≥ 2; ⬆)