

# Unique Genetic Differences in Responses of Chicken Immune Cells to an Inflammatory Stimulus and Heat Stress

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### Summary and Implications

Bone marrow antigen presenting cells (BM-APC), from Fayoumis (disease resistant and heat tolerant) and Leghorn (disease susceptible) chicken lines were evaluated for response to an inflammatory stimulus and heat stress. BM-APC from Fayoumis produced more nitric oxide (NO) and had higher Major Histocompatibility Complex (MHC) class II cell surface expression compared to those from Leghorn, indicating that BM-APC studied *in vitro* may be a useful tool to evaluate molecular effects of disease and/or heat tolerance in chickens.

### Introduction

Chickens are regularly exposed to pathogens and environmental challenges. These challenges and their interaction may influence immune responses of an individual. One of the immune cell types that takes part in innate (naive) immune response is called antigen presenting cells (APC). They function in pathogen destruction and presentation of non-host proteins (antigens). Antigens are presented in conjunction with the MHC-II cellular surface proteins. Here, we elucidate the response of BM-APC, from different inbred chicken lines divergent for disease resistance. The goal is to determine if cellular responses *in vitro* can identify genetic differences. We measured NO production and MHC-II protein expression in BM-APC. NO is an important host defense against invading pathogens, and primarily functions to inhibit bacterial growth. We also measured surface expression of MHC-II proteins as an intrinsic characteristic of APC, which allows them to present non-host proteins to other immune cells and activate the adaptive immune response. The ability of APC to rapidly increase the surface expression of MHC-II is a favourable response to pathogen challenge. We hypothesize that BM-APC from Fayoumis will display favourable responses to stimulations compared to cells from Leghorn chickens. Such response of BM-APC will reflect better disease resistance of Fayoumis.

### Materials and Methods

Bone marrow was obtained from embryonic day 18 Fayoumi and Leghorn inbred chicken lines and cultured with purified recombinant cytokines (GM-CSF and IL-4) to differentiate the naive cells into BM-APC. We stimulated BM-APC using a full factorial design with the factors: breed (Fayoumi and Leghorn), Inflammatory stimulus [200 ng/ml

lipopolysaccharide (LPS) or saline], and heat stress (2 hours 45°C or 41.5°C). BM-APC were evaluated for response to treatments by their NO production (24 hours post-stimulation) using the Griess reaction, and MHC-II surface expression (4 hours post-stimulation) using antibody staining in conjunction with flow cytometry.

### Results and Discussion

BM-APC from both chicken lines increased NO production in response to treatments that included an inflammatory stimulus (LPS and LPS+heat), whereas cells from neither line responded to heat treatment alone (Figure 1a). Upon stimulation with LPS, BM-APC from the resistant Fayoumi produced more NO compared to the cells from susceptible Leghorn. NO may be one mechanism Fayoumis uses to be more disease resistant compared to Leghorn *in vivo*.

MHC-II surface expression in BM-APC from the Fayoumi increased upon stimulation with LPS and LPS+heat, whereas those from the Leghorn were not responsive to any treatment (Figure 1b). Cells from the Fayoumi had statistically higher MHC-II surface expression in all treatment groups compared to Leghorn. The discovery that Fayoumis have inherently higher MHC-II surface expression compared to Leghorns may be an indicator of a more primed immune system. The ability to increase surface expression upon stimulation may prove that this assay is a good indicator of disease resistance.

Taken together, the results of the NO production and MHC-II surface expression support the hypothesis that studying BM-APC *in vitro* can indeed be a good indicator for disease resistance in chickens *in vivo*.

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**Figure 1: BM-APC from Fayoumi and Leghorn chicken lines were stimulated with an inflammatory stimulus (LPS), heat, and the combination. Cells were evaluated for NO production (a) and MHC-II surface expression (b). Data are depicted as mean  $\pm$  SEM; N=6-9 from 3 independent experiments. Different letters indicate significance with multiple testing correction  $P < 0.05$ .**

