Innate immune responses of primary equine respiratory epithelial cells to infection with a modified-live or wild-type equine influenza virus

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ABSTRACT: Due to its short incubation period and ever-changing surface glycoproteins, equine influenza virus (EIV) remains one of the most important respiratory viral pathogens of the horse. In order to design effective EIV control strategies it is important to understand how to invoke protective immunity. Systemic EIV antibody titers are not always accurate predictors of clinical protection. Likewise, an intranasal, cold-adapted, MLV equine influenza vaccine (Flu Avert® I.N.) has been shown to produce solid clinical protection in the presence of low systemic antibody titers.1,2 These observations suggest that factors other than adaptive host immune responses play an important role in EIV protection. The innate immune system recognizes viral RNA using pattern recognition receptors (PRRs) that recognize pathogen associated molecular patterns (PAMPs) on microbes, including EIV. Following PRR activation, intracellular signaling results in secretion of cytokines and other pro-inflammatory mediators. As the first point of contact with infected pathogens, the respiratory epithelium represents the first line of defense.

AIM: The objective of this study was to characterize and compare the innate immune responses of equine respiratory epithelial cell (EREC) cultures following infection with either the MLV vaccine strain contained in Flu Avert I.N.® or a similar wild-type EIV strain, A/equine/Kentucky/91 (Eq/KY). It was our hypothesis that infection with the MLV vaccine strain elicits similar innate immune responses in equine airway cells as observed following infection with wild-type EIV.

METHODS: Equine respiratory epithelial cells (EREC) were collected from the upper and lower respiratory tracts of horses euthanized for non-respiratory related illness. The cells were grown at the air-fluid interface until fully differentiated (Fig. 1). Subsequently, cell cultures were inoculated with either the MLV cold-adapted vaccine strain or Eq/KY and incubated at either 30°C or 37°C. Negative controls included medium-only inoculated cells (mock infection) and cells inoculated with a killed whole virus vaccine (KV), Calvenza® (Boehringer Ingelheim Vetmedica, St. Joseph, MO) that is also labeled for intranasal administration. The KV was diluted 1:100 with inoculation medium prior to addition to cells. Twenty-four and 48 hours post inoculation, cell cultures were collected and assayed for mRNA expression and protein production of IFN-γ (red bars), IL-4 (green bars), and IL-10 (hatched bars) in subnatants collected from the basolateral chamber of ERECs at 48 hours post inoculation and measured with a fluorescent bead-based system (Luminex IS 100 instrument, Luminex Corp., Austin, TX). TLR3 expression was examined using quantitative real-time PCR.

RESULTS: The EREC culture model supported the growth of both EqKY and MLV vaccine strain of EIV. As expected, the cold-adapted MLV (Flu Avert® I.N.) demonstrated superior growth kinetics at lower incubation temperatures (30°C) compared to EqKY. The KV (Calvenza®) exhibited severe cytotoxicity and by 8 hours following cell inoculation, the cell monolayer was destroyed. TLR3 mRNA expression was significantly increased in EqKY infected ERECs when compared to KV or mock inoculated cells at 24 hours post inoculation. EREC inoculation with MLV produced a similar trend in TLR3 mRNA expression.

DISCUSSION AND CONCLUSION: The goal of this study was to compare early innate immune responses at the first point of contact between virus and host, the respiratory epithelium, remains poorly characterized.

The purpose of this study was to use a primary equine respiratory epithelial cell (EREC) culture system to characterize and compare innate immunity to wild-type EIV and the MLV vaccine strain. Four-week old differentiated ERECs were infected with either a wild-type Eq A/equine/Kentucky/91 (Eq/KY) or the MLV influenza vaccine strain contained in Flu Avert I.N.®. Cytokine responses were determined using quantitative real-time polymerase chain reaction (PCR), a fluorescent bead-based system and ELISA. Toll-like receptor (TLR) 3 expression was examined using quantitative real-time PCR. One-way analysis of variance was used to evaluate differences in mRNA cytokine and TLR3 expression between groups, as well as determination of differences in cytokine concentrations between subnatants collected from infected and uninfected ERECs. Differences were considered significant when p ≤ 0.05.

Increased production of IFN-γ was observed following EREC infection with both viruses. Respiratory epithelial cells are not typically thought of as a source of IFN-γ, a cytokine known to play a key role in anti-influenza host defense. These results highlight the fact that immunity to EIV is multi-faceted and involves several arms of the host’s immune response, including innate mucosal immunity. Infection and replication efficacy of a given EIV is likely central to triggering a robust host immune response to the invading pathogen. These results characterize the early immune response to EIV generated by EREC and show that infection with either wild-type EIV or Flu Avert® I.N.® will trigger cell mediated innate immune response that may provide unique EIV protection.

References:

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