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Short communication

Identification of immunologically relevant genes in mare and foal dendritic cells responding to infection by *Rhodococcus equi*

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ABSTRACT

Rhodococcus equi is a facultative intracellular bacterial pathogen of horses; infected foals develop pyogranulomatous pneumonia, however adult horses are largely unaffected. *R. equi* infects and proliferates within host macrophages and dendritic cells (DCs). DCs initiate the appropriate adaptive immune response, thereby playing a critical role in determining the outcome of infection. Our aim was to identify genes that are differentially expressed in *R. equi* infected monocyte-derived DCs (mdDCs). Peripheral blood monocytes from mares and foals were used to derive mdDCs by culturing with recombinant equine IL-4 and recombinant human GM-CSF. RNA harvested 24 h after infection with *R. equi* (ATCC 33701+) was used to perform suppression subtractive hybridization (SSH) experiments. Approximately 38 unique sequences were obtained from these experiments. Differential expression of 19 immunologically relevant genes was validated by PCR. These genes are characterized by the following functions: cell adhesion, chemotaxis/migration, immune/inflammatory response, ion transport, signal transduction, T-cell regulation, and vesicular transport. In summary, we identified several novel genes that are differentially expressed in foal and adult mdDCs in response to *R. equi* infection. These genes provide promising targets for further research into the host response to *R. equi*, and the susceptibility of foals to this disease.

1. Introduction

Rhodococcus equi is a gram-positive facultative intracellular organism and is one of the most important bacterial pathogens of foals less than 6 months of age (Prescott, 1987). *R. equi* most commonly manifests as a severe pyogranulomatous pneumonia in foals, whereas adult horses are largely unaffected by this disease. The disease has a worldwide distribution and in many countries is a leading cause of mortality in foals; there is no effective vaccine for its prevention. In recent years *R. equi* has emerged as an opportunistic human pathogen in immunocompromised people, in whom it causes a similar pyogranulomatous pneumonia. The pathogen is a soil-dwelling organism that is ubiquitous in the environment, inhalation of contaminated dust is thought to be the most common route of infection. The pathogen can become endemic on breeding farms because it replicates within the gastrointestinal tract of infected foals and is shed in large quantities in their feces (Takai, 1997).

R. equi is a nocardioform actinomycete and is closely related to other intracellular pathogens like *mycobacteria* spp., *corynebacterium* spp. and *nocardia* spp. *R. equi* infects and proliferates within host antigen presenting cells, most

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commonly pulmonary alveolar macrophages within the lungs of infected foals. It is able to survive within these cells by evading phagolysosome fusion, however the exact mechanism is not yet fully understood. *R. equi* enters the cell via Fc and non-Fc receptors, and intracellular survival is enhanced by entry via non-Fc receptors, specifically complement receptors (Hondalus et al., 1993). Virulence of *R. equi* is determined by an 80–90 kb plasmid encoding virulence associated proteins A-H (VapA-H), expression of VapA is necessary for survival and replication within host macrophages (Jain et al., 2003). Expression of the plasmid is triggered by a drop in pH, decreased iron availability, increase in oxidative stress and increased temperature, all of which would occur upon uptake by an antigen presenting cell (Benoit et al., 2002; Prescott, 1991; Takai et al., 1996). Expression of the Vap proteins results in altered endosomal maturation and prevention of phagolysosome formation.

Dendritic cells play a key role as the driver of the adaptive immune response, due to their specialized ability to present antigen and stimulate T cells. After pathogen uptake and antigen processing they undergo maturation while migrating to regional lymph nodes. Mature DCs increase surface expression of MHC class II and costimulatory molecules such as CD80 and CD86, which allow them to interact with naive T-cells in the lymph node. Depending on the type of antigenic stimulus, DCs activate appropriate T-cell mediated response (de Jong et al., 2005). A Th1 type response is desirable for the clearance of intracellular pathogens, and the same is true for *R. equi*. A study in adult horses challenged with virulent *R. equi* showed that they mounted a local Th1 response to clear infection (Hines et al., 2003). In mice, adoptive transfer of Th1 IFNγ expressing T cells into *R. equi* infected immunodeficient mice led to bacterial clearance while mice receiving Th2 IL-4 producing cells did not clear infection and developed pulmonary granulomas (Kanaly et al., 1996).

There is increasing evidence that the difference in susceptibility between foals and adult horses to *R. equi* may be due to differences in the maturity of their immune response, specifically the ability to produce interferon gamma (IFNγ) (Breathnach et al., 2006). There are also maturational differences between DCs of foals and adults, including a reduced ability of DCs of young foals to produce TNFα in response to LPS stimulation (Merant et al., 2009). While *R. equi* challenged foal DCs produce equivalent amounts of IL-12, a cytokine which promotes Th1 response and IFNγ production, when compared to adult DCs, they fail to upregulate MHCII expression. This could lead to impaired ability to interact with and stimulate naïve T-cells (Flaminio et al., 2009). Because DCs are vitally important in the initial stages of infection to produce a successful and efficient immune response, and there is evidence for maturational differences between foal and adult DCs, we chose to examine the response of these cells to *R. equi* infection. Previous studies in our laboratory have examined cytokine expression by infected DCs and have found only minor differences between DCs derived from foals and adult horses (Watson et al., 2008). Our experimental objective was to identify novel genes that are up- or down-regulated in response to *R. equi* infection in monocyte-derived dendritic cells from foals and adult horses.

2. Materials and methods

2.1. Animals

All horses were housed in dry lots at the Center for Equine Health, University of California, Davis. Peripheral blood from three mare foal pairs, were used for culturing monocyte-derived dendritic cells for the subtractive suppressive hybridization study. Foals were 2 months of age at time of sampling. Peripheral blood from three additional adult horses and three unrelated 2-month-old foals from the same facility was used to culture monocyte-derived dendritic cells for RT-PCR experiments. Approximately 150 ml and 250 ml of whole blood was collected from foals and adult horses respectively. Blood was collected by jugular venipuncture into anticoagulant (adenine citrate dextrose phosphate solution) containing bags (Baxter, Deerfield, IL). All procedures were approved by the Institutional Animal Care and Use Committee of the University of California.

2.2. Cell culture and infection

Peripheral blood mononuclear cells were isolated from whole blood by density gradient centrifugation (Histopaque 1.077). Cells were resuspended in freezing media (10%DMSO, 90% FCS) and stored at −80 °C.

Equine monocyte-derived dendritic cells were cultured as previously described (Hammond et al., 1999). Briefly, PBMCs were thawed and washed in RPMI media. Cells were resuspended in CRPMI (RPMI containing 10% fetal calf serum) and plated on tissue culture plates. Plates were incubated at 37 °C for 2 h. Adherent cells were washed three times to remove non-adherent cells, and DC media was added (RPMI with 100ng/ml recombinant human GM-CSF (Peprotech Inc., Rocky Hill, NJ) and 150 U/ml recombinant equine IL-4) (Jackson et al., 2004). On day 3 of culture 1/3 of the media was replaced with fresh DC media. On day 5, half of the cells were infected with virulent *R. equi* ATCC strain 33701+ at an MOI of 1. One hour after infection 10 μg/ml gentamicin was added to cell cultures to kill extracellular bacteria. Twenty-four hours post-infection, cells were lysed and RNA harvested in RLT buffer (Qiagen) containing 10 μg/ml βME.

2.3. Suppression subtractive hybridization

Total RNA was extracted from the infected and control mdDCs using the RNeasy isolation system (Qiagen). RNA from 3 individuals in each experimental group was pooled, and used to synthesize full-length cDNA (SMART™ PCR cDNA Synthesis Kit, Clontech, Mountain View, CA) (Diatchenko et al., 1996). Subtracted cDNA libraries were created using the PCR-Select cDNA subtraction kit (Clontech). The following subtraction experiments were carried out: mare infected vs. mare control, foal infected vs. foal control, mare infected vs. foal infected. The forward and reverse subtractive libraries were ligated into pCR 4-TOPO®