Paper Title

Leishmania RNA virus controls the severity of Leishmania infection

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One sentence summary

Recognition of *Leishmania* RNA virus by TLR3 modulates the host immune response after *Leishmania guyanensis* infection

Abstract

Mucocutaneous leishmaniasis (MCL) is caused by infections with intracellular parasites of the *Leishmania Viannia* subgenus, including *L. guyanensis* (*L.g.*). MCL develops following parasitic dissemination to the nasopharyngeal tissues forming destructive metastatic lesions with chronic inflammation. Currently, the mechanisms involved in MCL development are poorly understood. Here we show that metastasizing, but not non-metastatic parasites have high *Leishmania RNA Virus*-1 (LRV1) burden that is recognized by the host TLR3 to induce pro-inflammatory cytokines and chemokines. Paradoxically, these TLR3 mediated immune responses render mice more susceptible to infection, with increased footpad swelling and parasitemia. Thus, LRV1 in the metastasizing parasites subverts the host immune response to *Leishmania* thereby promoting parasite persistence.

Experimental Design

Three biologically independent experiments were performed. For each experiment transcript levels were compared from RNA preparations of uninfected BMM∅'s or BMM∅'s infected with either *L.g.* M+ (Lg13) or *L.g.* M-(Lg17) parasites. In addition, a dye-swap hybridization was performed for each comparison. RNA was purified by RNAeasy Mini Kit (Qiagen™), and the quality and quantity were verified by the Agilent Technologies (Germany) 2100 bioanalyzer and RNA 6000 Nano Assay LabChip→ kit. Mouse cDNA was produced and printed on glass-slide microarrays by the DNA Array Facility Lausanne (DNA Array Facility Lausanne, Switzerland). The 17k mouse cDNA microarray was made using the 15'000 gene clone set (NIA 15k cDNA set) available from the National Institute on Aging (NIA, USA). These cDNA clones are derived from embryonic and fetal mouse tissues. Additional 1400 cDNA clones were added from genes not contained in the NIA collection, containing both known genes and ESTs. Briefly, cDNA was synthesized from 5 ºg of RNA by direct incorporation of Cy3 or Cy5 fluorophore-labeled DCTP using random primers (Invitrogen) mediated by the Superscript II reverse transcriptase. For each labeling reaction, reference control RNA (2ºl Alien spikes pool and 2ºl Arabidopsis spikes pool obtained from the DAFL) was added for data normalization. The labeled probes were purified using the MiniElutel PCR Purification kit (Qiagen), and mixed then concentrated using Millipore Microcon YM-30 columns. For hybridization, Cy3 and Cy5 labeled cDNA were mixed together, and loaded onto the glass-slides. Glass-slides were then scanned using an Agilent Technologies microarray scanner. The resulting TIF images were analyzed using GenePix Pro software (Axon Instruments, USA). Data analysis was performed using R statistics software (http://www.r-project.org/), Cy5 (red) and Cy3 (green) signal intensities were used to calculate M and A values for the array spots. Genes that were at least 1.5 fold over or under-expressed and with a p-value <0.05 were considered as differentially expressed. Statistical significance was calculated after standardization between the slides using the Limma statistical software package. Data analysis, quality assessment and normalization were performed by the DAFL. These resulting differentially expressed genes were then further analyzed using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com).