

## Extracellular vesicles of *Leishmania*-infected-macrophages can modulate dog immune response

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### Background

Recognized as a major veterinary issue and public health concern, canine Leishmaniosis (CanL) is caused by parasites of the genus *Leishmania* [1]. Macrophages (MØs) are key cells of innate immune system and host cells for *Leishmania* parasites. *Leishmania* is well adapted to aggressive conditions of MØ phagolysosome and can modulate MØs' immune activation, favoring parasite replication and spread in the host [2,3]. Extracellular vesicles (EVs) are membrane-bounded vesicles naturally shed by all types of cells that transport molecules with immunomodulatory potential, which may play a key role in host-parasite communication [4]. Thus, this study explores the ability of EVs released from *Leishmania*-infected-MØs to activate naïve immune cells, as a proof-of-concept for cell-to-cell communication via EVs.

### Materials and Methods

Mononuclear cells were isolated from peripheral blood of healthy dogs, cultured and differentiate into macrophages [5]. Adherent MØs were exposed to virulent parasites and EVs produced by the infected cells were isolated and added to naïve MØs. Immune activation of naïve MØs was analyzed by assessing urea production and gene expression of key innate immune receptors (TLR2, TLR4 and TLR9) as well as cytokines [interleukin (IL)-1 $\beta$ , IL-10, IL-12 and transforming growth factor (TGF)- $\beta$ ] by real-time-PCR. EVs generation was observed by scanning electron microscopy.

### Results

Mononuclear cells isolated from blood differentiate into adherent MØs and actively produce EVs either when resting or infected. Virulent promastigotes can easily infect adherent-MØs. Non-stimulated (naïve) MØs exposed to EVs of *Leishmania*-infected MØs react by increasing their production of urea and generation of TLRs and pro-inflammatory cytokines.

### Conclusions

The findings of this study provide new insights into the exploitation of host immune response by *Leishmania*. Canine naïve cells appear to be primed for parasite invasion by EVs shed by parasite-infected-MØs, which may facilitate parasite dissemination, highlighting the deep adaptation of *Leishmania* parasites to the dog.

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