# Timing-dependent immunomodulatory effects of sand fly saliva during *Leishmania major* Infection

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**Abstract:** Leishmaniases are tropical diseases caused by Leishmania parasites and transmitted by their vectors, phlebotomine sand flies. A key role in the manifestation of Leishmania infection is played by host exposure to sand fly saliva, to which the host is exposed during both infectious and non-infectious bites. The timing and frequency of exposure to sand fly saliva can affect the host immune response, leading to different outcomes of the infection. In this study, we analyzed the effect of various exposure schemes of BALB/c mice to *Phlebotomus duboscqi* prior and after *Leishmania major* infection, with a specific focus on the parameters underlying the establishment of the protective versus enhancing effect of sand fly saliva on *Leishmania* infection. We showed that continuous exposure to sand fly saliva prior and after the infection led to the delayed onset of lesion development and smaller size of the lesion, in contrast to exposure only after the infection. Moreover, *Phlebotomus duboscqi* saliva attracted eosinophils to the bite site, which positively correlated with host protection. This eosinophil-related protective effect was limited to smaller lesions, while larger lesions were characterized by an increased frequency of neutrophils and M1 macrophages. Taken together, our findings demonstrate that the host immune status at the time of infection determines how post-infectious exposure to sand fly bites influences disease outcome. Immunization by sand fly saliva after infection showed both protective and exacerbating effect on lesion pathology, depending on the timing and character of the ongoing immune response. These results shed light on the conditions shaping *Leishmania* infection in endemic areas with recurrent sand fly exposure.

**Keywords:** sand fly saliva, *Phlebotomus duboscqi*, *Leishmania major*, immune response

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