ABSTRACT

Sand flies (Diptera: Phlebotominae) are bloodfeeding insects serving as vectors of *Leishmania* parasites (Kinetoplastida). Sand flies possess salivary glands with pharmacologically active molecules that provide them with an effective weapon against the host defence and that play an important role in *Leishmania* infection development. During the bloodfeeding, sand fly saliva is inoculated into the feeding site. Repeated exposures induce saliva-specific immune response, both humoral and cell-mediated. While anti-saliva antibody response correlates with the intensity of exposure and can be used as a marker of exposure, specific cellular immunity provide protection against leishmaniasis in some vector-parasite-host combinations. Sand flies differ in composition of the saliva and thus the elicited immunity is species-specific. This species-specific variability makes difficult the development of one saliva-based vaccine applicable to different *Leishmania*-vector combinations.

However, saliva composition is more conserved among closely-related vector species, which may evoke cross-protection in bitten and subsequently infected hosts. We focused on cross-reactive properties of saliva from *Phlebotomus papatasi* and *Phlebotomus duboscqi*, the two natural vectors of *Leishmania major*. We demonstrated that protection against *Leishmania* infection was observed not only in *P. papatasi*-exposed mice challenged with homologous saliva but also in the group challenged with *P. duboscqi* saliva. These groups did not differ significantly in parasite load, macrophage activity or in the levels of anti-*L. major* and anti-*P. papatasi*/*P. duboscqi* antibodies which indicates cross-protection caused by salivary antigens of these two *Phlebotomus* species.

Although cross-reactivity of salivary proteins among species is advantegous for aforementioned saliva-based vaccine development, it is a disadvantage for their utilization in sand flies exposure testing; cross-reaction among sympatrically occuring species could lead to false positive results. In our study focusing on sero-epidemiology characterization of three endemic foci in Ethiopia, I used murine model to test the specificity of *P. orientalis* SGH against anti-saliva antibodies elicited by sympatrically ocurring sand fly species. Antigen from *P. orientalis* reacted strongly only with anti-*P. orientalis* antibodies, while reactivity with heterologous anti-sand fly saliva antibodies were comparable to those from non-exposed mice. The observed high species-specificity of the reaction indicates similar specificity also for anti-*P. orientalis* antibodies from other host species. Therefore, anti-*P.orientalis* antibody response supports the hypothesis about possible role of domestic animals in the epidemiology of visceral leishmaniasis caused by *L. donovani*.

Studies dealing with the utilization of anti-saliva antibody response for estimating sand fly exposure usually exploit whole salivary gland homogenate as the antigen, however recombinant proteins were suggested as convenient alternative for salivary gland homogenate. In two extensive epidemiological studies, we validated recombinant form of *P. perniciosus* yellow-related protein as a marker of sand fly exposure based on convincingly high correlation between antibodies recognizing recombinant protein and the whole salivary antigen. Moreover, it was shown that this protein depicts the dynamics of antibodies comparatively with saliva and may even be applied as antigen in the distant regions where *P. perniciosus* is the unique or principal vector species.