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Tick saliva inhibits the chemotactic function of MIP-1α and selectively impairs chemotaxis of immature dendritic cells by down-regulating cell-surface CCR5

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Abstract

Ticks are blood-feeding arthropods that secrete immunomodulatory molecules through their saliva to antagonize host inflammatory and immune responses. As dendritic cells (DCs) play a major role in host immune responses, we studied the effects of *Rhipicephalus sanguineus* tick saliva on DC migration and function. Bone marrow-derived immature DCs pre-exposed to tick saliva showed reduced migration towards macrophage inflammatory protein (MIP)-1α, MIP-1β and regulated upon activation, normal T cell expressed and secreted (RANTES) chemokines in a Boyden microchamber assay. This inhibition was mediated by saliva which significantly reduced the percentage and the average cell-surface expression of CC chemokine receptor CCR5. In contrast, saliva did not alter migration of DCs towards MIP-3β, not even if the cells were induced for maturation. Next, we evaluated the effect of tick saliva on the activity of chemokines related to DC migration and showed that tick saliva per se inhibits the chemotactic function of MIP-1α, while it did not affect RANTES, MIP-1β and MIP-3β. These data suggest that saliva possibly reduces immature DC migration, while mature DC chemotaxis remains unaffected. In support of this, we have analyzed the percentage of DCs on mice 48 h after intradermal inoculation with saliva and found that the DC turnover in the skin was reduced compared with controls. Finally, to test the biological activity of the saliva-exposed DCs, we transferred DCs pre-cultured with saliva and loaded with the keyhole limpet haemocyanin (KLH) antigen to mice and measured their capacity to induce specific T cell cytokines. Data showed that saliva reduced the synthesis of both T helper (Th)1 and Th2 cytokines, suggesting the induction of a non-polarised T cell response. These findings propose that the inhibition of DCs migratory ability and function may be a relevant mechanism used by ticks to subvert the immune response of the host.

Keywords: Dendritic cells; Saliva; Migration; T cell response; *Rhipicephalus sanguineus*

1. Introduction

Ticks (Acari: Ixodidae) are bloodsucking, non-permanent ectoparasitic arthropods of human and veterinary medicine importance. Approximately 10% of the currently known 867 tick species act as vectors of a broad range of pathogens of domestic animals and humans and are also responsible for damage directly due to their feeding behav-

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iour (Anderson, 2002; Jongejan and Uilenberg, 2004). The economic losses to animal production alone are in the order of US $13 billion annually (Anonymous, 1995).

The skin site at which ticks attach to feed is the critical interface between the tick and its host. At this site, ticks stimulate host defenses that can determine whether or not they are rejected (Wikel et al., 1994). Once attached, over several hours or days ticks inoculate, via saliva, potent anti-inflammatory and immunomodulatory components that may prevent or retard deleterious host responses or pharmacologically counteract their host's defenses (Ribeiro, 1995; Ribeiro and Francischetti, 2003). Saliva compounds have also been found to modulate humoral and cellular pathways involving antibodies, T cells, cytokines, chemokines and antigen-presenting cells, such as dendritic cells (DCs) (Ribeiro, 1995; Wikel, 1996, 1999; Hajnická et al., 2001; Cavassani et al., 2005; Vancová et al., 2007).

DCs belong to a group of functionally homogeneous cell types that act to stimulate antigen-specific T cells after migrating to secondary lymphoid organs (Banchereau et al., 2000). DCs reside in an immature state in peripheral tissues, where they provide a sentinel function for incoming antigens (Banchereau and Steinman, 1998; Banchereau et al., 2000). Upon microbial contact or stimulation by inflammatory cytokines, DCs initiate the acquired immune response uptaking antigens and moving, via the afferent lymphatics, into the T cell area of the draining lymph nodes (Cyster, 1999; Sallusto et al., 1999). Many studies have shown that, to reach peripheral tissues, immature DCs migrate towards several inflammatory CC and CXC chemokines by ligands to chemokine receptors (e.g. macrophage inflammatory protein (MIP)-1α via CCR1 and CCR5, MIP-1β via CCR5 only and regulated upon activation, normal T cell expressed and secreted (RANTES) via CCR1, CCR3 and CCR5 receptors) (Dieu et al., 1998; Vecchi et al., 1999). In contrast, to migrate from peripheral tissues to the lymph nodes, mature DCs lose their responsiveness to most of these inflammatory chemokines and acquire sensitivity to MIP-3β via receptor CCR7, a chemokine strongly expressed in lymph nodes (Kellermann et al., 1999).

Rhipicephalus sanguineus, known as the brown dog tick, is a worldwide species and has been linked to tick-borne diseases such as spotted and boutonneuse fever and ehrlichioses in humans, babesiosis and ehrlichioses in dogs (Flechtmann, 1973; Walker et al., 2000; Demma et al., 2006). Natural *R. sanguineus* tick hosts (dogs) are unable to develop appreciable resistance, even after repeated feedings (Chabaud, 1950; Szabó et al., 1995). Likewise, tick-infested mice also do not develop resistance to further infestations with this tick species (Ferreira and Silva, 1998). In contrast, guinea pigs develop resistance to secondary infestations with *R. sanguineus* ticks (Chabaud, 1950; Szabó et al., 1995).

There is circumstantial evidence to support the suggestion that DCs may play a role in the acquisition and expression of tick resistance (Nithiuthai and Allen, 1984, 1985; Cavassani et al., 2005). It has been demonstrated that tick antigens injected into the skin through tick saliva are taken up by Langerhans cells and presented to T lymphocytes in lymph nodes draining the bite site (Allen et al., 1979). Exposure of murine bone marrow (BM)-derived DCs to tick saliva inhibits their process of differentiation and diminishes the population of DCs in an immature stage. Moreover, *R. sanguineus* tick saliva significantly blocks the terminal maturation of DCs and reduces the production of IL-12 (Cavassani et al., 2005).

Hajnická et al. (2001) demonstrated the presence of an anti-CXCL8 (IL-8) activity in the salivary gland extracts (SGE) from several *Ixodid* tick species and have recently shown that saliva isolated from several different species of ticks contain a variety of inhibitory activities directed against chemokines, such as CCL2 (MCP-1), CCL3 (MIP-1α, CCL5 (RANTES) and CCL11 (Eotaxin) (Vancová et al., 2007).

The aim of this study was to investigate whether *R. sanguineus* tick saliva affects DC migration, a process pivotal to immune surveillance and T cell activation. We demonstrate that saliva from *R. sanguineus* ticks selectively inhibits chemotaxis of immature DCs by down-regulating CCR5. In addition, tick saliva per se inhibits the chemotactic function of MIP-1α. Assessment of the function of migrated-DC cultured with tick saliva revealed them to be poor stimulators of cytokine production by antigen-specific T cells. Therefore, inhibition of DC migration and function provides additional mechanistic explanation for tick saliva immunosuppressive effects.

2. Materials and methods

2.1. Animals

Experimental C57BL/6 mice (6–8 weeks of age) and mongrel dogs (1–3 years old) were bred and maintained under standard conditions in the animal facilities of the Department of Biochemistry and Immunology, School of Medicine, University of São Paulo (USP), Ribeirão Preto-SP, Brazil. All animal experiments were performed in accordance with protocols approved by the School of Medicine of Ribeirão Preto Institutional Animal Care and Use Committee.

2.2. Saliva collection

Rhipicephalus sanguineus ticks were laboratory-reared, as previously described by Ferreira et al. (1996). All ticks used for infestations were 1–3-month-old adults. To obtain engorged ticks for saliva collection, dogs ($n = 20$) were infested with 70 pairs of adult *R. sanguineus* ticks contained in plastic feeding chambers fixed to their backs. The saliva-collection procedure was performed using fully engorged female ticks (after 5–7 days of feeding) by inoculation of 10–15 μl of a 0.2% (v/v) solution of dopamine in PBS, pH 7.4, using a 12.7 × 0.33 mm needle (Becton–Dickinson,