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The Intradermal Leishmanin Reaction Induces Antigen-specific Maturation of Canine Dendritic Cells with Up-regulation of MHCII Synthesis and Expression

L. Sacchi, L. E. Calvi*, L. H. Kramer, E. Ferroglio†, G. Grandi*, E. Clementi and S. Corona‡

*Department of Animal Biology, University of Pavia, *Department of Animal Production, University of Parma, †Department of Epidemiology and Ecology, University of Turin, and ‡Laboratory of Electron Microscopy, Infectious Disease Unit, Policlinic S. Matteo, Pavia, Italy*

Summary

Dendritic cells (DCs) are professional antigen-presenting cells that reside in many tissues, including the skin. This study showed that intradermal injection of leishmanin in *Leishmania infantum*-infected dogs induced the "up-regulation" of surface MHCII expression, associated with progressive ultrastrucutural changes characteristic of DC maturation, including the formation of multilaminar MHC class II-containing compartments and arrays of tubulo-vesicular structures. These changes were not observed in control dogs from *L. infantum* non-endemic areas. The results indicated that canine DCs were effector cells in delayed-type hypersensitivity, that the leishmanin reaction was specific for a cell-mediated reaction to *L. infantum* in infected dogs, and that canine DCs possessed ultrastructural organelles reminiscent of those in activated human DCs.

Keywords: dendritic cells; dog; *Leishmania infantum*; leishmanin; MHCII; parasitic infection

Introduction

Dendritic cells (DCs) are the most potent antigen-presenting cells known and represent the link between innate and adaptive immune responses to invading pathogens (Banchereau and Steinman, 1998; Guermonprez *et al.*, 2002). They originate from the bone marrow and their precursors travel via the blood stream to almost all organs, where they can be found as sentinels, in an immature state, with high endocytic and phagocytic capacity. Upon contact with invading pathogens or inflammatory cytokines or on interaction with activated T cells, DCs migrate to regional lymph nodes where they present, to resting or naïve T cells, antigens captured in the periphery, thus initiating adaptive immune responses. Contact with and subsequent uptake of antigens by DCs are associated with marked alterations in function and phenotype (Steinman *et al.*, 1995; Banchereau and Steinman, 1998). In most tissues, DCs are present in a so-called "immature" state, unable to stimulate T cells, with high expression of the surface molecule CD1a. Although these DCs lack the requisite accessory signals for T-cell activation, they are well equipped to capture antigens able to induce full maturation and mobilization of DCs, the latter being a key event in the induction of immunity. Once the DC has internalized an antigen, the cell begins to assemble antigen–major histocompatability class II (MHCII) complexes, which are expressed on the cell surface. MHCII expression is usually "up-regulated" within 24 h of exposure to antigens (Steinman *et al.*, 1995). In the presence of mature DCs, CD4-expressing T-helper cells turn into gamma interferon

Correspondence to: L.H. Kramer, Dipartimento do Produzioni Animali, Università di Parma, Via del Taglio 8, 43100 Parma, Italy (*e-mail:* kramerlh@unipr.it).

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(IFNγ)-producing Th1 cells, necessary for protective immune responses against many micro-organisms, including *Leishmania* spp. (Liew and O'Donnell, 1993).

Leishmania infantum is the aetiological agent of canine leishmaniosis and human visceral leishmaniosis in the Mediterranean area. In general, infection with *Leishmania* spp. can either resolve through an effective cell-mediated immune response (Th1) or can lead to chronic and progressive disease. Almost 40% of *L. infantum*-infected dogs are symptomless and many of these animals are able to mount a specific cell-mediated response to infection through the production of IFNγ by Th1 lymphocytes and subsequent activation of macrophages. Several research groups are currently studying the role of DCs in the immune response to *Leishmania* infection to determine whether interaction between such cells and the parasite plays a role in the development of protective immune responses, in particular in murine models of disease. Epidermal Langerhans cells (LCs) can phagocytize *Leishmania major* and serve as host cells for the organisms both *in vitro* and *in vivo* (Blank *et al.*, 1993). Furthermore, LCs are potent stimulators of *L. major*-specific Th1-cell proliferation and cytokine production *in vitro* (Will *et al.*, 1992) and are able to transport parasites from the infected skin to the draining lymph node (Moll *et al.*, 1993), where they may persist for several months (Moll *et al.*, 1995). Recently, Marchal *et al.* (1997) demonstrated that CD1a-positive cells in the skin of dogs infected with *L. infantum* can harbour amastigotes of the parasite. Delayed-type hypersensitivity (DTH) reactions are typical manifestations of cell-mediated immunity generated by DCs (see Schuler, 1991, for a review) and recently several authors have suggested that the leishmanin reaction, a DTH reaction to *L. infantum* promastigotes, can specifically identify dogs with a cell-mediated response to *L. infantum* infection (Pinelli *et al.*, 1994; Solano-Gallego *et al.*, 2000, 2001, 2005; Fernandez-Bellon *et al.*, 2005). The present study showed that the canine leishmanin reaction was associated with alterations in canine DC morphology indicative of antigen-specific activation and maturation of canine DCs and demonstrated for the first time *in vivo* that DCs are effector cells in DTH reactions in dogs.

Materials and Methods

Animals

Eight dogs with natural *L. infantum* infection and three healthy dogs (controls) from a non-endemic area were studied. All dogs were from local animal shelters and research was performed in conformity with the University of Parma Ethics Committee for Animal Experimentation.

The eight infected dogs were shown to be positive for the leishmanin reaction, according to Solano-Gallego *et al.* (2001). Briefly, inactivated promastigotes of *L. infantum* were suspended in a 0.4% phenol solution at a concentration of 3×10^8 promastigotes/ml. Approximately 0·1 ml was injected intradermally in the groin of each dog. The same amount of a 0·4% phenol solution alone was used as a negative control. At 72 h post-injection (p.i.) the sites were evaluated for erythema and wheal formation, a wheal of $\geqslant 5$ mm being considered positive. The same procedure was carried out in the three control dogs, in which, as expected, leishmanin gave negative results. Subsequently, approximately 15 days later, the intradermal injections were repeated in all dogs and 6-mm punch biopsy samples were obtained from the injection sites at 12 and 24 h p.i. Biopsy samples were halved, one half being embedded in cryostat medium and snap-frozen for immunohistochemistry, and the other cut into 1-mm slices and processed for transmission electron microscopy (TEM).

TEM

Skin biopsy samples were fixed in Karnovsky's fixative in 0·1 M cacodylate buffer (pH 7.2), postfixed for 2 h in OsO_4 1% in the same buffer, dehydrated through a graded ethanol series and propylene oxide, and then embedded in Epon 812 medium. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 900 electron microscope.

Immunohistochemistry (IHC)

Frozen biopsy samples were sectioned at 5 μm and processed for immunohistochemistry. Monoclonal antibodies against MHCII (CA2.1C12), CD1a (CA9.AG5) and CD1c (CA13.9H11) were obtained from Prof. P.F. Moore, University of California Davis (Moore *et al.*, 1998). After endogenous peroxidase quenching with 2% H_2O_2, sections were incubated for 30 min with primary antibodies at a 1 in 10 dilution. An avidin–biotin complex with horseradish peroxidase (ABC-HRP) system (LSAB kit, Dako, Milan, Italy) was applied and amino-3-3′ ethylcarbamazine (AEC; Dako) was used as a chromogen. Sections were counterstained with Mayer's haematoxylin. Positive cells were counted in 10 fields (×40) of each biopsy sample, including epidermis, dermis and subcutaneous tissue. The results were expressed as mean ± standard deviation (SD).

Results

TEM

In *L. infantum*-negative control dogs, dendritic cells (DCs) were located in the stratum spinosum. These