Abstract

Previous light-microscopic studies have shown a unique population of mast cells in lymphatic sinuses of lymph nodes located in the head, neck, axillary fossa and inguinal region of the opossum. In the present work, scanning and transmission electron-microscopic studies in the opossum mandibular and superficial axillar lymph nodes have strengthened the differences between connective-tissue mast cells (CTMC) and the lymphatic-sinus mast cells (LSMC). Further, close appositions of mast cells to other cells were described. At the nodal capsule, CTMC contacted fibroblast and granulocytes. In the lymphatic sinuses a few CTMC contacted LSMC, macrophages and reticular cells. The LSMC contacted macrophages, reticular cells and other LSMC. A few LSMC could be located in the medullary cord in close contact with plasma cells or other lymphoid cells, keeping the same ultrastructural features of those found in the lymphatic sinuses. An important new finding was provided by light-microscopic studies in nine abdominal lymph nodes. Most of them (para-aortic, common iliac, cardial, cecocolic and those of the body and tail of the pancreas) displayed numerous LSMC with the same distribution and histological features described herein. However, the mesenteric, pyloric and head-of-pancreas lymph nodes were virtually devoid of LSMC. Instead, their mast cells occurred mainly at the medullary cords and were very similar to the CTMC. Ultrastructural studies at the mesenteric lymph nodes confirmed the CTMC character of the mast cells located at both medullary cords and sinuses, and disclosed interactions with macrophages and lymphoid cells.

Key words
Macrophages · Neutrophils · Lymphocytes · Mast cell subtypes · Marsupials ·

Introduction

Several studies developed by Kitamura and co-workers (reviewed by Kitamura 1989) in mice revealed that mast cells originate from bone marrow precursors. Circulating precursors occur in mouse blood as nongranulated cells that migrate to different tissues in which proliferation and differentiation to granulated mast cells take place under the influence of several growth factors. The mast cell heterogeneity could result from the microenvironment peculiarities (reviewed by Galli 1990; Metcalfe et al. 1997; Lin and Befus 1999). In rodents two populations of mast cells have been clearly distinguished by morphological, biochemical and physiological criteria: the connective-tissue mast cells (CTMC) and the mucosal mast cells (MMC) as described by Enerback (1986) and reviewed by Galli (1990). In the opossum, CTMC and MMC could also be distinguished by light- and electron-microscopic studies, the latter being found in the intestinal mucosa and submucosa (Santos and Machado 1994). Unexpectedly, light-microscopic studies in opossum lymph nodes located in the head, neck and limbs showed numerous mast cells, clearly different from CTMC and MMC, restricted to the lymphatic sinuses. The opossum lymphatic-sinus mast cells (LSMC) differed from the opossum CTMC by their larger size and enlarged cytoplasmic granules that were also more heterogeneous in shape and staining properties (Chiarini-Garcia and Machado 1992). The presence of a unique population of mast cells in the opossum lymph nodes is an interesting finding since no dissimilarity with CTMC has been reported for mast cells of eutherian lymph nodes (Sainte-Marie and Peng 1990; Lozzi et al. 1996). The abundance of LSMC in opossum lymph nodes contrasts with the usually smaller proportion of mast cells in lymph nodes of eutherian mammals. However, in rodents, their number increases with age (Sainte-Marie
and Peng 1990) and after both antigenic and non-antigenic stimulation, probably by a process of draining from the stimulation site (Sainte-Marie and Peng 1990; Lozzi et al. 1996 and references therein).

The present study aims at establishing the ultrastructural features of mast cells of opossum lymph nodes located in the cervical and axillary regions, emphasizing the interaction with other cells. By studying both parietal and visceral lymph nodes located in the abdominal cavity of the opossum, we also intend to verify the extent of the occurrence and distribution of LSMC.

**Materials and methods**

Eight South American opossums, *Didelphis albiventris* (Marsupialia, Didelphidae) were captured in Belo Horizonte, Brazil, under a permit provided by the Brazilian Institute for the Environment (IBAMA-MG). The animals looked healthy and were maintained in individual cages for less than 24 h with water ad libitum. They weighed 450–1100 g on the day of sacrifice and were free of cutaneous wounds. Care of the animals and euthanasia were in accordance with the guidelines for laboratory animals established by the National Institute of Health, USA.

Transmission electron microscopy (TEM)

Three adult animals (two females and one male) under Nembutal anesthesia (30 mg/kg of body weight, i.p.) were perfused from the left ventricle to the right atrium with Ringer solution followed by a modified Karnovsky’s fixative (2.5% gluteraldehyde-2% paraformaldehyde in 0.1 M cacodylate buffer at pH 7.2). The perfusion pressure for both solutions ranged from 70 to 80 mm Hg and the volumes depended on the animal weight. In two other opossums (one male and one female), the right mandibular lymph nodes were removed before the perfusion procedure, in which the controlled pressure ranged from 110 to 120 mm Hg. After perfusion, sagittal slices of superior lips, and fragments of mandibular, superficial axillary, and mesenteric lymph nodes were immersed in the fixative for 4–6 h, then post-fixed in reduced osmium (Russell and Burguet 1978) for 2 h, dehydrated in graded series of ethanol and embedded in Epon 812. After staining with uranyl acetate and lead citrate, the sections of nodal CTMC, their average diameter was estimated at 3.49±0.07 µm, displaying surface folds ending far from the cell surface contacting the surrounding collagen fibrils (Fig. 2A). However, paired mast cells were frequent in the dermis. This mast-cell-mast cell interaction involved membrane interdigitations (Fig. 1C).

In all animals, the capsule of the mandibular and superficial cervical lymph nodes exhibited several CTMC and some mononuclear leukocytes and neutrophils sparsely distributed among the numerous fibroblasts and collagen fibrils. Several CTMC were less buried in collagen fibrils, exhibiting large surface areas in direct contact with granulocytes and fibroblasts (Fig. 1E). In the latter, pinocytic vesicles could accumulate in the contacting fibroblast membrane (Fig. 1D). In the nodal capsule, degranulating CTMC seemed more frequent than in the lip dermis and some of them were in close apposition to neutrophils (Fig. 1F). Mast cells with bizarre forms or displaying surface folds ending far from the cell surface or even lamelliform extensions were also found. By measuring 109 homogeneous-appearing cytoplasmic granules of nodal CTMC, their average diameter was estimated at 0.49±0.07 µm.

In SEM, the CTMC could be identified with certainly only after fracture that showed up their cytoplasmic granules. Probably the difficulty in recognizing non-fractured CTMC was due to their smooth surfaces, besides the surrounding collagen fibrils (Fig. 2A).

**Results**

**Connective-tissue mast cells**

At TEM, dermal CTMC presented narrow surface processes curved toward the cell surface and displayed a discontinuous granular coat (external lamina) in which collagen fibrils stuck in (Fig. 1A). However, direct contact with fibrillar collagen was also apparent (Fig. 1B). Although dermal CTMC could lie near fibroblasts or their long processes, no close contact between mast cells and any type of connective-tissue cells were observed. However, paired mast cells were frequent in the dermis. This mast-cell-mast cell interaction involved membrane interdigitations (Fig. 1C).

Lymphatic-sinus mast cells (LSMC) in the mandibular and axillary lymph nodes

The mandibular and superficial axillary lymph nodes exhibited LSMC in all lymphatic sinuses. They were rather numerous in the medullary sinuses (Figs. 2B, 3A). However, the LSMC were observed only when a pressure of 70–80 mm Hg was maintained throughout the intracardiac perfusion with the fixative. A perfusing pressure of 110–120 mm Hg washed out virtually all LSMC. At SEM, the LSMC were easily identified by their black-