International Journal of Sciences

Research Article Volume 2 - September 2013 (9)

Early Development of Mast Cell System in Lingual Tissues of Intrauterine and Newborn Rats

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Abstract: Mast cells (MC) are predominantly localized at the interface between host and environment such as skin and mucosal surfaces. They are able to perceive a variety of allergens and invading pathogens. In oral tissues, degranulation of mast cells has been a consistent feature of inflammatory lesion like liquen planus, gingivitis, periapical process and tumoral responsive. The aim of the present study is to describe the morphology and to establish the homing time in the tongue's connective tissue of rats. Tongue sections were collected from Wistar rats processed and included in paraffin wax, cut and stained with toluide blue and alcian blue-safranin and immunoenzyme staining procedure with Monoclonal Antibody to mast cell Tryptase. The total number of mast cells was counted and the area was measured to estimate the population density (mm^2) and the individual cell morphology. Results showed morphology changes and a series of variations between the first and the second week samples after birth ($p>0.001$), but there were not changes between intrauterus and newborn period. Mast cells were detected from the fifteenth day of intrauterine life in closed relation with skeletal muscle cells. These data suggest a quick MT adaptation and a strategic location that allow them to react against different oral antigens. More studies are going to be necessary to elucidate this phenomenon.

Keywords: tongue, oral immunity, mast cells

Introduction

Mast cells (MT) are connective tissue cells, derived from a pluripotent cell CD34 +, located in the bone marrow [1]. Mast cells are distributed preferentially in the skin and in a various mucous membranes from the digestive, urinary and respiratory systems, and although the amount and density are higher at the interface between external and internal environments, where they can respond to foreign antigens [2]. They have basophilic granules in their cytoplasm that are surrounded by a metachromatic membrane when stained with basic dyes, which allows their identification in histological samples [3].

Mast cells are known for their role in a hypersensitivity cutaneous reaction, as well as in their participation of different diseases, including pathologies of the oral mucosa such as lichen planus or periodontal disease [4-8]. Today is assessed the participation of these cells in the tissue repair mechanisms as well as in a tumor progression [9, 10]. In mice have been described two subpopulations of mast cells: connective tissue mast cells (CTMC's) and mucosal-type mast cells (MMC's). Both types of cells have cytoplasmic granules that contain tryptase, an enzyme that is released during activation and degranulation of mast cells [11].

Several studies indicate that the enzymatic activity of tryptase can be used as a marker during the mast cell differentiation into the embryonic development, since it is early expressed in erythropoiesis sites such as the yolk sac and dermis, at later stages [12, 13]. In the reviewed literature, we found no descriptions of must cells morphological features characteristics in different stages of embryonic development and early postnatal days, in the tongue. Considering the importance of MT in the mucosal immune system, we face this study whose main objective is to establish the period of settlement of MT in lingual tissues of newborn rats and describe its distinctive morphology at each stage.

Materials and Methods:

Tissue samples were taken from fourteen "Wistar" rats from lingual tissues of both rats sexes and divided in two groups. The first group included, from the thirteenth day of intrauterine life until birth. Each animal was designated as E13, E15, E17, E19 and E21, depending on the day of intrauterine life. In a second group samples were processed from the first

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day of birth until the fourteenth day. Samples were classified in the first week (days 1 to 7) and second week (day 8 to 14) of postnatal life. The animals were sacrificed, respecting protocols of care and use of laboratory animals suggested by the Argentine Association of Science and Technology of Lab Animals (AACyTAL) [14]. Each of the pieces was immersed in a solution containing 4% laboratory formaldehyde. The specimens were kept in fixative for 12 hours. Then the samples were dehydrated in increasing concentrations of alcohols and embedded in paraffin.

Histology and histochemistry

a) Paraffin sections of fixed tissue were cut at a thickness of 5 µm and were stained with the following techniques: (a1) haematoxylin and eosin to confirm the identity of tissue constituents; (a2) Alcian blue (AB)-safranin carried out as described by Bancroft and Stevens (1982) (4).

b) As a second procedure, the mast cells were detected immunohistochemicaly by marker as the anti-mast cell tryptase (Monoclonal Antibody to mast cell Tryptase, clone AA1, IMGENEX).

Procedure for Immunoenzyme Staining

1. Sections were rinsed in PBS for 2x2 min and then incubated in normal serum block.
2. After this, sections were incubated in the primary antibody for overnight.
3. Rinsed in PBS buffer and then incubated in 1% hydrogen peroxidase for peroxidase blocking.
4. Secondary Antibody: sections were incubated in biotinylated secondary antibody (Anti-mouse Ig G, Rockland MagTag Histo Kit).
5. Rinsed in PBS buffer for 3x2 min and incubated in streptavidin.
6- Rinsed in PBS buffer for 3x2 min.
7. Sections were incubated in peroxidase Chromagen/Substrate solution of diaminobenzidine (DAB).
8. Rinsed in PBS buffer for 3x2 min and immersed en haematoxylin to contrast the nuclei.
9. Finally was covered with coverslip and mounting medium

The positive reaction was identified as a brown precipitate in the cytoplasm (mast cells granules),and then contrasted with haematoxylin.

The samples were observed under a microscope Nikon Optiphot-2 and photographed with a Nikon CCD digital camera. To analyze the cellular distribution, images were digitized using the program Motic Images plus 2.0. Finally, to determine the characteristics of the mast cells population per mm 2, six areas were randomly taken from each of the cuts (three superficial and three deep), where there were counted and averaged an amount of mast cells per mm2 to evaluate their size(diameter and surface) and the particular details of morphology.

Statistical analysis. The results are expressed as mean ± standard error. Comparisons between different means were performed using ANOVA test and the comparison between the two sectors studied used the Student test with a significance level of 0.05%.

Results

Cell quantitation: The first positive mast cells tryptase, were detected from day 15 of the intrauterine life. Their number, scarce and difficult to quantify, remained constant during pregnancy. By comparing the samples from the first week with the second week of birth, during the early postnatal period, the results allowed to detect a change in the number of MT per mm2 in aggregate. . In the first days after birth the number of mast cells remained unchanged when compared with intrauterine cells: 2.20±0.86 (mean+SEM). A progressive and marked increase reaching significant differential values of 16.2 ± 2.31, was detected during the second week ($p < 0.001$). The values found during the second week were consistent with those described previously for lingual tissues of adult animals ($p < 0.05$) [15]. Data is summarized in Table 1.

Morphological aspects: to analyze the morphological characteristics of mast cells found from day 15 of intrauterine life, it was observed that these had, initially, a rounded appearance similar to a lymphocyte, with few granules concentrated around the nucleus and with a predilection for a sector cell as observed in the finest cuts. Activated mast cells, or degranulated cells, were not detected. The average size was 12 ± 2 microns in diameter and a cell-surface average of 122± 15 um^2. These morphological characteristics remained without any remarkable change during the first postnatal week (Figures Nº 1.2 and 3). From the second week, the size of the mast cells grew, becoming with a more evident ovoid appearance. At the same time, it was accompanied by an increase in the number of cytoplasmic granules. The ovoid shape and the presence of abundant granules, partly covering the core, showed an appearance comparable to cells found in adult animals (Figure Nº4). The number of activated mast cells was higher. In terms of size, the average was 25± 4 um in diameter (major axis) with a cell surface of 150 ± 20um^2.

Mast cell granules stained positively with toluidine blue and Alcian blue -safranin technique, in all sections observed. Interestingly, the reaction of the granules, using immunostaining with monoclonal

anti-mast cell tryptase, begins at the level of perinuclear region, but only on one extreme of the cell, while the granules are more evenly distributed from the eighth day, after which the reaction was of similar intensity to that seen in adult lingual tissues.

Discussion

Matsson L,in 1993 described the presence of mast cells in different sites of the oral mucosa, gums and tongue including juvenile rats,1 month and 6 months old [16]. On the other hand, D. Abraham et al., (2007), detected activity of several proteases, including tryptase, in the dermis and some oral tissues of rats from day 17 to 18 of intrauterine life [17].

Following this line, our findings indicate that MT are present in tongue tissue, from the fifteenth day of intrauterine life and remain with little change during the early postnatal days within a connective tissue and muscle undeveloped.

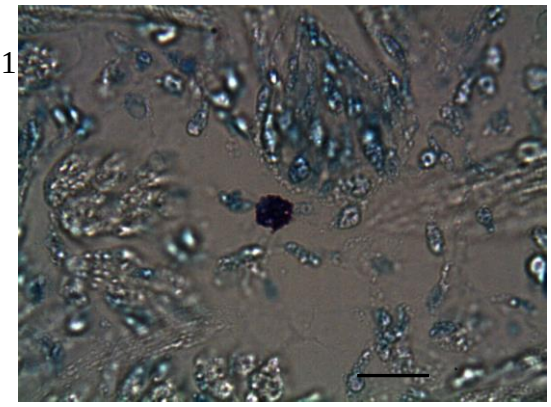
Although immature MT precursors from blood, reach connective lingual tissue early on, they only may be able to offer a complete functional response when,they develop the entire chemical mediators that could harbor their granules, in the presence of local tissue factors.This is evidenced by the near absence of degranulated cells found in uterus.

Local factors that induce migration of MT to specific settlement sites seem to be very important, as suggested by some studies that indicate that the surface molecules that mediate binding of mast cells to extracellular matrix protein and other tissues related to resident cells are important during differentiation, migration and localization [18,19]. Kobayashi et al., 1986 y Otsu et al., 1987, conclude that the type of mast cell is not necessarily predetermined, but strongly influenced by the microenvironment [11,21].

In a recent paper published by Chang-Cheng Xie et al., (2007) found that mast cells appeared in cultured skeletal muscle cells, suggesting a probable interaction between mast cells and the proliferation and differentiation of skeletal muscle cells [21]. In our work it is possible to detect a joint maturation of tongue skeletal muscle cells and mast cells surrounding, although we cannot ensure that a paracrine interaction type, or otherwise, may be occurring between both cell types.

Data analysis suggest a rapid adaptation of the MT to the new settlement and a distribution strategy, allowing them to respond to various antigens that entering the oral cavity. More studies are needed to determine the factors causing this phenomenon.

FIGURES

1 

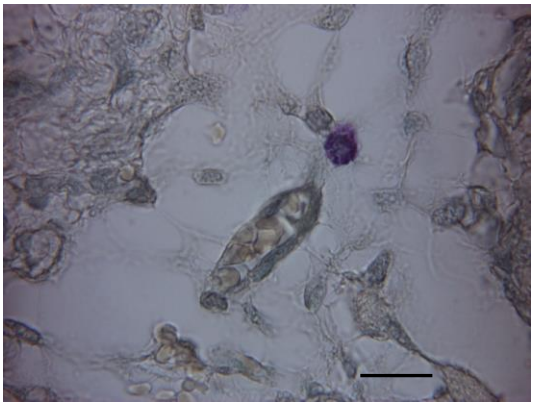
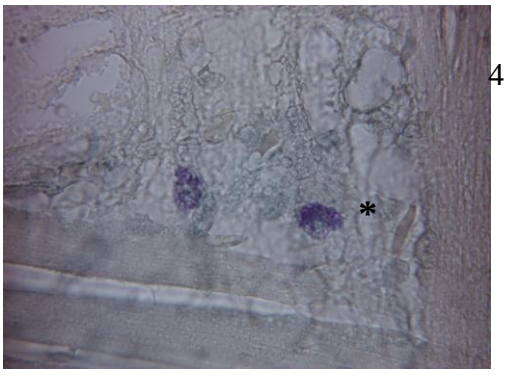
2 

Figure 1: Embryo of 15 days. Rounded isolated Mt (8.5 μ m). Immunohistochemistry. Scale bar: 10 μ m. (400x)

Figure 2: First day postnatal. There is still a similarity in size and number. Immunohistochemistry. Scale bar: 10 μ m. (400x)

3 

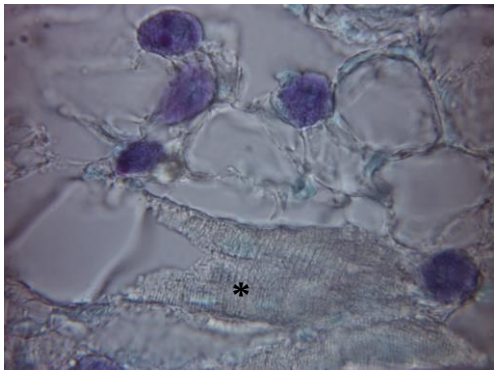
4 

Figure 3: eighth postnatal day, Mt larger (12 μm) and more numerous. Note the developing skeletal muscle fibers (*).Immunohistochemistry. Scale bar: 10 μ m. (400x)
Figure 4: adult rat. Numerous mast cells with large amounts of intracytoplasmicgranules. (*)Striated muscle cells. Immunohistochemistry. Scale bar: 10 μ m. (400x)

Table 1
Average number of cells(mastcell/mm2) in rats: A) prenatal, B) first week, C) second week, and C)adult
Tukey Test: Pairwise Comparisons for One-Way Layout Design

	A) prenatal	B) first week pn	C) second week pn	D) adult
Mean	1,86	2,21	16,23	18,75
S.E.M.	0,21	0,22	0,65	0,3

Probability	
A-B	N.S. (P>0.05)
A-C, A-D	*** (P<=0.001)
B-C, B-D	*** (P<=0.001)

Acknowledgement
We would like to thank Ms. Juana Dora Dávila for her technical assistance of excellence. This paper was supported by Secretaria de Ciencia, Técnica y Postgrado, and Facultad Odontología, Universidad Nacional de Cuyo, Mendoza, Argentina (resol 058 CD, proyecto n° 06/K064).

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