Mast cell in dental pulp: does it have a role?

Mastócitos na polpa dental: eles têm uma função?

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ABSTRACT

Mast cells play an important role in a variety of biological processes and actively participate in the inflammatory response. There is a controversy in the literature whether mast cells are present in dental pulp. In this investigation we sought to answer the question concerning the presence of mast cells in human and rat dental pulp tissues, under normal and inflammatory conditions. Human and rat dental pulp under normal and inflammatory conditions were analyzed using toluidine blue histochemistry and immunohistochemistry techniques. Our results showed that inflamed and non-inflamed dental pulps neither from humans nor from rats presented mast cells. The role of mast cells in the inflammatory dental pulp response is not clear. Growth factors and cytokines involved in their migration, development and survival could be absent in this tissue and need further investigations.

INTRODUCTION

It has been a matter of controversy in the literature whether mast cells are present in dental pulp. Studies using human samples have shown that such cells are present in dental pulps¹,² whilst others have demonstrated the absence of those cells³,⁴,⁵. In addition, there is a lack of information regarding the presence of mast cells in dental pulp of rats, which are animals used to study important aspects of the pathophysiology of dental pulp in vivo⁶.

Mast cells play an important role in a variety of biological processes including allergic reactions, atherosclerosis and inflammation⁷,⁸. The activation of these cells leads to the release of chemical mediators such as histamine and arachidonic acid metabolites, which increase vascular permeability and tissue swelling⁹.

On the other hand, dental pulp is a connective tissue surrounded by hard walls of mineralized tissue. Such characteristic decreases its possibilities of swelling in response to injuries⁴,¹⁰. Since mast cells are active during inflammatory responses it becomes crucial to find out if these cells are actually present and play a role in dental pulp under normal and inflammatory conditions either in humans or in rats.

Therefore, the purpose of the present work was, under the same experimental conditions, to identify mast cells in healthy and inflamed dental pulp from rats and humans using toluidine blue histochemistry and immunohistochemistry techniques.

MATERIAL AND METHODS

Rat Tissue Samples

Male Holtzman rats (260-320g) were anesthetized (ketamine-xylazine 90-15mg/kg, i.m.); dental pulp from the upper first molar (n=6) was exposed and inflammation was locally induced by bacterial lipopolysaccharide (LPS, Escherichia coli; batch B111; 1.2µg/site, 1µl, Sigma Chemi-
Mast cell in dental pulp: does it have a role?
Queiroz-Júnior CM et al.

Human Tissue Samples
Human pulp tissues were obtained from teeth of patients attending Dental Surgery, Orthodontic and Endodontic clinics of Universidade Federal de Minas Gerais Dental School. Following diagnosis and indication for tooth extraction, patients that signed the informed consent were enrolled in the study. This study was conducted according to the ethical guidelines of the Institutional Ethical Committee, which approved the protocols described throughout the text (Protocol Number 497/07).

Inflamed human pulp samples (n=12) were obtained from teeth with clinical signs and symptoms of acute pulpitis and with clinical dentine caries, but with no exposed pulp. Samples were immediately immersed in 10% formaldehyde for 48h and then demineralized in 10% ethylenediaminetetraacetic acid (EDTA, Synth, Diadema, SP, Brazil) solution (pH 6.0) for 4 months. The specimens were dehydrated in ethanol, immersed in xylene and embedded in paraffin for further analysis. Healthy pulps (n=5) were obtained from third molars and pre-molar extracted for orthodontic reasons. These pulps were used as non-inflamed controls, and the gingival tissue (n=12) from periodontal surgery was used as a positive control for mast cells.

Both rat and human tissue samples were stained for mast cell detection. All experiments were conducted in accordance with the ethical guidelines of the Institutional Ethical Committee.

RESULTS
The presence and role of mast cells in many tissues under different conditions, such as inflammation, have been consistently unraveled. Nevertheless, their recruitment to dental pulp remains an open question. The present study aimed to answer such question. Firstly, analysis of hematoxilin/eosin stained slides of inflamed dental pulps from either humans or rats showed a mixed polymorphonuclear inflammatory infiltrate, with a predominance of neutrophils.

As can be observed in figure 1, TB histochemistry did not identify mast cells in non-inflamed and inflamed pulps from either rats (A, B) or humans (G, H); nevertheless, in the same experimental conditions this technique stained mast cells in rat and human gingival tissues (C, I). Likewise, immunohistochemistry for tryptase did not identify mast cells in dental pulps from rats and humans either under normal or inflamed conditions (D, E, J, K).

Toluidine Blue (TB) Histochemistry
TB method was modified from Heaney et al. Briefly, sections (4μm) were deparaffinized in xylene and hydrated with water. TB staining was performed with a 1% TB solution (Synth, Diadema, SP, Brazil) diluted in phosphate buffer (pH 5.7) for 120s. Soon afterward, sections were quickly dehydrated through 96% ethanol and p.a. acetone, after rinsing in phosphate buffer for 1min. They were then immersed in xylene and mounted in synthetic resin.

Immunohistochemistry (IH)
Paraffin-embedded tissues were sectioned (4μm) and collected in serial sections on glass slides coated with 2% 3-aminopropyltriethoxysilane (Sigma Chemicals, St. Louis, MO, USA) for processing by standard IH technique (immunoperoxidase: avidin-biotin-peroxidase). Samples were deparaffinized by immersion in xylene, followed by ethanol and then immersion in citrate buffer (pH 6.0; Sigma-Aldrich Co., St Louis, MO, USA) for 20min at 95°C for antigen retrieval, except for sections that would be examined with the mouse anti-human mast cell tryptase monoclonal antibody (M7052). Soon afterward, sections were incubated with 3% hydrogen peroxide diluted in Tris-buffered saline (TBS; pH 7.4) for 30min. Thereafter, sections were incubated at 4°C overnight in a humidified chamber with one of the following primary antibodies: rabbit polyclonal anti-rat mast cell tryptase (clone FL-275, Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted at 1:2 000 and mouse monoclonal anti-human mast cell tryptase (clone M7052, Dako, Glostrup, Denmark), diluted at 1:2 000. All antibodies were diluted in 1% PBS bovine serum albumin. Following the incubation with the primary antibodies, sections were washed in TBS with Triton X-100 p.a. (H282, Mallinckrodt, Phillipsburg, NJ, USA) and treated with the labeled streptavidin-biotin kit (K0492, Dako). Samples were then incubated in a 3,3'-diaminobenzidine (DAB) chromogen solution (K3468, Dako) for 3min at room temperature. Finally, after washing with distilled water, the slides were counterstained with Mayer’s hematoxylin and were covered. Negative controls consisted of sections in which primary antibodies were omitted and replaced with non-immune rabbit (X0910, Dako) or mouse (X0910, Dako) serum.
pointed out herein could explain the poor results previously found when SP was used to induce vascular permeability in rat dental pulp, and also could have a positive role in this environment, not allowing an enormous swelling which could lead to a quickly necrosis of pulp tissue. Our findings corroborate those from a recent work using IH in human samples, which only identified mast cells in pulp polyps, a specific condition in which dental pulp is able to swell; but the same study did not find mast cells in encased pulp tissues even when they were inflamed. Although this lack of mast cells in dental pulp could be a positive characteristic to this tissue, such absence could also interfere with the defense mechanisms of dental pulp since mast cells have recently gained new importance as immunoregulatory cells and as a source of cytokines and chemokines.

The mechanisms underlying the absence of mast cells in dental pulp are still a matter of speculation. It is known that Stem Cell Factor (SCF) and its receptor c-kit are essential for mast cell development and growth in a tissue. However, SCF-mediated mast cell development is regulated by other factors including cytokines like IL-3, IL-9, and IL-10 and growth factors like nerve growth factor (NGF). These cytokines work directly stimulating proliferation of uncommitted progenitors or as cofactor for mast cell proliferation. NGF stimulates the differentiation and proliferation of mouse bone marrow derived mast cells and it works synergistically with SCF suppressing mast cell apoptosis in humans.

Since SCF and c-kit seems to be present in dental pulp some of these other factors, which are crucial for final mast cell development and which work modulating SCF activity, might be lacking in dental pulp. Conversely, this tissue may harbor important inhibitory factors for mast cell development and/or survival such as the granulocyte-macrophage colony-stimulating factor (GM-CSF), a well-known inhibitor of mast cell development in both rodents and human systems.

CONCLUSION

In conclusion our results demonstrated, through different standard techniques for mast cells detection, that dental pulps from either humans or rats did not present such cells. Regarding the absence of those cells in rat pulps such finding contributes to validate the in vivo studies using those animals, especially those in which inflammation is induced in pulp tissues. The mechanisms involved in explaining the absence of mast cells in dental pulp are still a matter of speculation. Thus, further studies are required to investigate the growth factors, cytokines and other mediators involved in mast cell chemotaxis, maturation and survival that could be absent in dental pulp.

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