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Importance of Mast Cells in Human Periapical Inflammatory Lesions

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The role of mast cells (MCs) in periapical inflammatory lesions is not well understood. The objective of this work was to quantify MC numbers in human periapical lesions with the aim to clarify their role in the pathogenesis of these lesions. We analyzed the slides of 64 human periapical inflammatory lesions stained with pH 8.0 toluidine blue technique, quantified the number of MCs, and evaluated any correlation with age, gender, size, and location. The results of this study suggest that MCs were more numerous in females ($p < 0.01$); MC numbers were higher in biopsies from granulomas with proliferating epithelium and lower in biopsies from chronic apical abscesses; MC counts did not correlate with patients' age or size. MCs were observed more commonly in areas containing inflammatory infiltrate and degranulation was a frequent finding in these zones. Our results suggest that MCs play an active role in the pathogenesis of the periapical inflammatory lesions. The potential role of MCs related with the initiation, development, and persistence of the periapical inflammatory process are discussed.

The periapical inflammatory lesion is the response of the periapical tissues to microbial and chemical stimuli coming from the pulp through the root canal systems. These harmful agents include viable bacteria, bacterial disintegration, or metabolic products and altered or necrotic pulpal tissue; these agents are potential antigens that are able to elicit an immunologic reaction. Torabinejad and Bakland (1) described periapical lesions as "areas of inflammatory response against the content of the radicular duct system."

Macrophages, lymphocytes, plasma cells, fibroblasts, endothelial cells, and mast cells (MCs) are involved in the pathogenesis of the inflammatory periapical lesions (2,3). Kontianen et al (3) reported that T lymphocytes are the predominant cell in these lesions, and several works quantified the inflammatory cell content in periapical lesions (3–8). By means of transmission electron microscopy, Perrini and Fonzi (2) demonstrated the presence of numerous MCs in human periapical granulomas (PGs) but no quantification was made. However, Kontianen et al. (3) reported that 2% of cells in periapical granulomas and cysts were MCs. It should be noted that the role of almost all the inflammatory cells present in the periapical reactions and their role in the pathogenesis of the periapical granulomas has been well studied (1–12), but the role of the MC in the development of the human periapical inflammatory lesions (HPIL) has been less studied.

Torabinejad and Bakland (1) proposed that IgE-mediated reactions could play an important role in the initiation and perpetuation of the periapical lesion if MCs and plasma cells containing IgE are present. Perrini and Fonzi (2) suggested that IgE has an active role in the pathogenesis of the PG, and that the anaphylactic and hypersensibility reactions represent an active immunologic phenomenon in the pathogenesis of these lesions.

MCs are commonly found around capillaries and some of their functions are related with their heparin, histamine, and proteoglycan content (9). Another function of the MC is related with the anaphylactic reaction because their cytoplasmic affinity for IgE antibodies (9). Valent et al (10) reported that under certain conditions, MC expressed selected cell surface molecules as receptors for IgE, IL-4, SCF, LFA-1, CD8, CD4, leukosialin, and integrins $\beta 1$ and $\beta 2$.

Mast cells secrete several substances, including leukotrienes (LTC-4 and LTB-4) and prostaglandins, mainly PGD-2 (9). On stimulation, MC are able to secrete proinflammatory or mitogenic cytokines, including interleukins 1, 3, 4, 5, 6, 8, and 10; macrophage inflammatory protein 1α and 1β, macrophage chemotactic protein-1 and T cell activating antigen-3, interferon-γ, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor-α, and transforming growth factor-β (11). Other studies on different properties of the products secreted by the MC had been published (13–19).

Despite the existence of reports quantitating the number of inflammatory cells in the PG (3–8,12,20), only 4 reports quantified the number of MC in the periapical inflammatory reaction (4,20–22). The former (4) presented numbers of MC found in both PGs and cysts. In 1994, our 2 previous works quantified the number of MCs in human PGs (21,22) and in 1995, Walsh et al. quantified the MC number in different tissues or the oral cavity including human PGs (20). From this point of view, the knowledge related with the role of the MC in the pathogenesis of the periapical reactions is very scanty.

The aim of this study was to identify and quantitate the MC numbers present in different types of human periapical inflammatory lesions and clarify their function in these processes.

MATERIALS AND METHODS

The files of the Oral and Maxillofacial Pathology Diagnosis Service of our institution (Facultad de Odontología, UNAM) were reviewed, and the most recently diagnosed cases of human periapical inflammatory lesions were selected and based on microscopic findings, 64 cases of clinical PG were studied. All specimens were cases of periapical radiolucent lesions associated with the radicular areas of carious teeth. They were surgically excised in the Department of Endodontics. After surgery, all specimens were immediately fixed in neutral-buffered formalin solution and routinely processed to obtain 5 μ paraffin-embedded slides; no demineralization was performed. Two new adjacent slides were stained with hematoxylin and eosin to confirm the PG diagnoses and to evaluate the different areas composing the granuloma, and other subsequent slides was stained with Johnson's technique for mast cells (23) modified to pH 8.0. Slides of neurofibroma were used as controls.

The quantitation method was the same system used in our 2 previous studies (21,22). We used a specially designed sheet to build a map of each studied lesion, in this sheet, a series of circles were printed and each circle represented a 400× microscopic field and inside each circle the number of MCs was recorded. During the review of the slides, we were very careful to take as reference specific structures to avoid imprecise sampling and draw up the boundaries of each studied high-power field as accurately as possible. A previous calibration between both reviewers was done (κ = 93%). Discrepancies among the authors were solved by agreement and consensus. The retrieved clinical data were age, gender, location of the lesions, size and involved tooth or teeth. Data were analyzed by the Student *t* test and $p \leq 0.05$ was considered significant.

RESULTS

From the 64 examined human periapical inflammatory lesions, 37 (58.7%) were in male patients; 26 (41.3%) in females and in 1 case no gender was recorded. Of these 64 cases, 33 (58%) were located in the anterior area; 10 (17.5%) in the canine-premolar zone; 14 (24.5%) in the molar region and in 7 cases no location was specified. Age range was between 14 and 74 years (mean, 29.5 yrs). Age distribution is seen in Table 1. Size of the studied cases varied from 0.15 to 2.6 cm (mean, 1.48 cm).

MC number varied in the different specimens examined and even among the different high-power fields (HPF) analyzed in the same specimen. Positive (MC-containing) fields in the studied granulomas varied from 2 to 1316. In our study, we found MC in different areas of the periapical lesion and some MCs were found degranulated.

MCs were found forming groups and distributed in several forms: regional fashion, isolated groups, and isolated cells. In the 64 studied specimens, a total of 9789 HPF were counted. Mast cells were found in 2820 microscopic fields (29%) and were not detected in 6969 microscopic fields (71%). The results are presented in Table 1 to Table 4 as the mean of MC per specimen.

Surgical biopsies from females contained more MC per field compared with those from males (females, 271; males, 99 respectively; $p < 0.01$). As shown in Table 1, mean MC number was not directly related with the age of the patients and as can be seen in Table 2, their number tended to be greater in posterior locations. Data in Table 2 shows that higher numbers of MC were found in molar and anterior areas compared with canine-premolar zone and mean MC numbers rose from anterior to posterior locations. Table 4 shows the findings of MC number in relation with the involved teeth.

TABLE 1. Number of mast cells and age of the patients

Age (yrs)	No. of Cases	No. of MCs	Mean ± SD
9–19	17	3640	214.1 ± 45.9
20–39	30	3587	119.6 ± 78.1
40–59	11	3475	315.9 ± 163.7
60+	1	28	28
Nonspecified	5	242	48.4
Total	64	10,792	168.6 ± 61.7

MC, mast cells; SD, standard deviation.

We observed that these MC were more commonly found forming compact areas of variable size and rarely as isolated cells. Also, we could observe them more frequently in areas with chronic inflammatory infiltrate (Fig. 1), less frequently in fibrotic zones and rarely in edematous areas. The granules of the MC were more abundant and had a bigger size in cells located within the inflamed areas and also, cellular degranulation was regularly seen in zones with inflammatory infiltrates (Fig. 2).

Microscopic review of the 64 HPIL cases revealed that there were 4 different histologic classes of lesions. Of the studied cases, 46 were dental granulomas (72%); 38 presented proliferating epithelium (82.6%) and 8 did not (17.4%). Other diagnoses were 16 chronic abscesses (25%) and 2 acute periapical abscesses (3.1%). Their MC numbers are shown in Table 4. No statistical relationship was found among size of the specimens and their MC content. As can be seen in Table 4, we found higher MC numbers in granulomas with proliferating epithelium compared with those granulomas without epithelium. This difference was statistically significant ($p < 0.01$).

DISCUSSION

It was previously suggested that MCs are present and active in chronic inflammatory periapical reactions (21). Perrini and Fonzi (2) studied PG and argued that MCs were difficult to locate. They reported that numerous MC were present in their specimens, but cellular quantification was not made. In this study and in 2 previous studies (21), we used the Johnson's technique modified to pH 8 with good results, this technique made mast cells easily recognizable.

Several studies reported the presence of MC in the periapical inflammatory reactions (2,12,13,20–22,24,25), but only the Ledesma and Garcés, Ledesma, and Walsh et al. studies quantitated MC levels (20–22). Ledesma and Ledesma and Garcés quantified MC numbers in human PGs and suggested that MCs play an active role in the initiation and development of the periapical inflammatory lesions (21,22).

Other authors reported the presence of MC in periapical reactions (2–8,12,21,22). Smith et al (7) studied their presence in odontogenic cysts, suggesting that MC can be the source of mucopolysaccharide deposition and that MC can play an important role in the growth of these lesions.

TABLE 2. Number of mast cells related to gender and location of the studied lesions

Location	Females			Males		
	No. of cases	No. of MCs	Mean	No. of cases	No. of MCs	Mean
Anterior	12	2248	187	21	1856	88
Prem-canine	7	1822	260	3	111	37
Molars	5	2913	583	9	1151	128
Nonspecified	2	72	36	4	547	137
Total*	26	7055	271	37	3665	99

* One case was not included since it had no gender and no location data.
MCs, mast cells.

TABLE 3. Number of mast cells and involved teeth

Teeth	No. of cases	MCs	Mean
Centrals	12	1853	154.4
Laterals	21	2251	107.2
Canines	2	32	32
Bicuspids	8	1901	212
1st molars	10	1911	191.1
2nd molars	3	2127	709
3rd molars	1	26	26
Nonspecified	7	691	38.7
Total	64	10,792	168.6

MCs, mast cells.

Our results showed that differences in MC number exist among gender and location and type of the studied human periapical inflammatory lesion, these findings agree with those previously reported (21,22). MC numbers varied from 1 biopsy to another, this can be the reason because MC numbers varied in the different anatomic locations studied. A reasonable mechanism to explain gender differences cannot be offered in this moment and it is a matter for future research.

MCs were randomly distributed within the studied lesions and different authors mentioned they were predominately found in different sites (2,12,13,20–22,25,26). Several authors claimed that their results showed that MC were mainly located in inflammatory areas, others reported they were chiefly situated in a perivascular position and only Kontianen et al. found MC situated in fibrotic areas mainly (2,12,13,20–22,25,26). Results of these previous studies on location of the MC in the different zones of the periapical inflammatory reactions can help in obtaining knowledge about their role in the initiation, development, and progression of the lesion because they have been associated with vasodilatation (1,9), proteoglycan production (9), angiogenic response (26), collagen synthesis (4,12,25), regulation of inflammation (2,13,21), bone resorption (22,27), and ECM destruction (7,28–30).

Perrini and Fonzi (2) suggested that hypersensibility is an active phenomenon in the PG. Their opinion was based in that these lesions contain IgE and that possibly MC degranulate. In our study, we found MC in different areas of the lesion, forming groups or as isolated cells. MC quantities varied greatly among the studied cases and even between different zones of the same specimen. These results suggest that they have a role in the pathogenesis of the HPIL. Another finding of the present study that supports this assumption was that MC were more numerous in areas formed mainly by chronic inflammatory infiltrate and that degranulated cells were found more frequently associated to chronic inflammation. Torabinejad and Bakland (1) mentioned that the IgE-mediated reaction could play an important role in the initiation and perpetuation of the periapical lesions if mast cells and plasma cells containing IgE are present in the PG. Our observation in this study that MC were found in variable distribution: regional fashion, isolated groups, and isolated cells suggest that MC are active cells and that they participate in the pathogenesis of the inflammatory reaction of the HPIL.

Our finding that MC were more common in areas with chronic inflammatory infiltrate, coupled with the presence of abundant degranulated cells in these areas, suggest that MC have an active role in these lesions. These findings disagree with data reported by Kontianen et al (4); they reported that MCs were more numerous in fibrotic areas.

It has been demonstrated that an interaction between MC and T lymphocytes exists (14,15), suggesting that liberation of histamine from mast cells inhibit the T lymphocyte activity against mitogens or antigens. Dohlsten et al. (16) suggested that histamine prevents IL-2 and gamma-interferon production. In 1993, Piatelli et al. (13) found MC positive to IL-2 receptor and suggested that these cells can play a role in a negative feedback mechanism in the control of the immunologic reaction; they proposed that MC fixed IL-2 in their membranes preventing IL-2 stimulation. Our results could support this theory because in this study, MC were more frequently seen in areas of chronic inflammatory infiltrate, and the presence of degranulated MC detected in these areas were a constant feature.

Frandji et al. (17) described another role of MC; they were able to present antigens to immune cells. Additional data supporting this assertion were presented by Czarnetzky et al. and Czarnetzky and Wüllenweber (18,19), who showed that MC derive from macrophage-like cells and that they are capable of phagocytosis. All these findings suggest that the MC could play a dual regulatory function: to inhibit T lymphocytes and to present the antigen to the immune system. Both theories are sustained by the presence of numerous MC in inflamed areas. Our results and those reported previously suggest that MC could play an important role in the initiation, development, and persistence of the inflammatory process associated with HPIL.

MC also contain tryptase and chymase; they are proteolytic enzymes that take part in degradation of the extracellular matrix (28) taking part in the breakdown of proteoglycan of the connective tissue capsule of jaw cysts during normal and inflammatory conditions (7). Studies on the presence of MC tryptase in odontogenic jaw cysts suggested they are involved in bone tissue remodeling (27). In 1993, a previous study by Ledesma et al. suggested that MC could be associated with bone tissue destruction and growth of human periapical granulomas (22). Heparin is another mast cell-derived protein involved in bone resorption in vitro, and it has been associated with inhibition of collagen synthesis (27,28). IL-1, IL-6, and TNF-α secreted by MC have been shown to

TABLE 4. Presence of mast cells in the studied periapical lesions

Type of lesion	Mean age (yrs)	M/F ratio	Mean size	No. of specimens	Percent of the sample	Range of MC	Mean of MCN ± SD
Acute abscess	35.5	0/2	0.55 cm	2	3.1	126–342	234
Chronic abscess	29	10/6*	0.52 cm	16	25	2–382	96 ± 54.3
Granuloma without epithelium	28.5	22/16	0.88 cm	38	59.4	3–1316	153.8 ± 56.0
Granuloma with proliferating epithelium	28.3	5/2	0.9 cm	8	12.5	40–943	507.8 ± 125.6
Total	29.5	37/26	1.5	64	100	2–1316	168.6 ± 68.7

* One case with unknown gender.
MCN, mast cell number; SD, standard deviation.

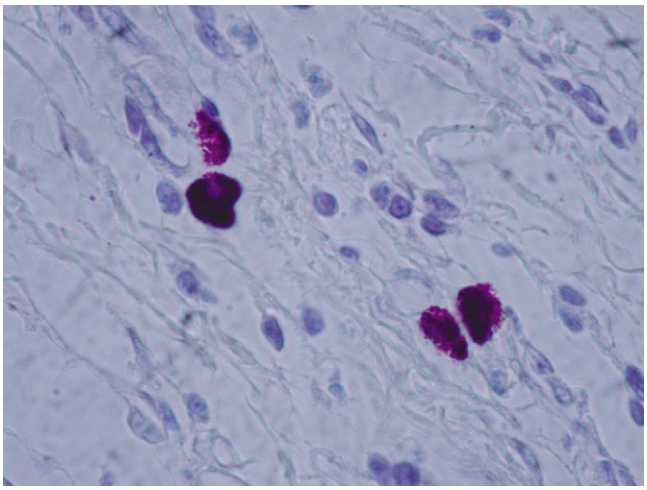

FIG 1. Numerous mast cells (arrows) are observed surrounded by chronic inflammatory cells, edema, capillaries, and scarce collagen. One of them is degranulated. Johnson's technique pH 8 (200×).

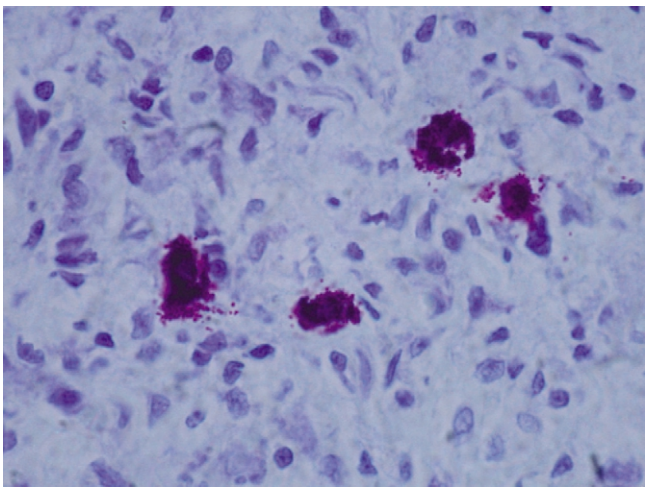

FIG 2. Another field showing degranulated mast cells surrounded by chronic inflammatory infiltrate. Johnson's technique pH 8 (200×).

increase bone resorption intensifying the osteoclastic activity (29). Previous studies showed that mast cell tryptase could activate metalloproteinases 1 and 2 contributing to EEC degradation and bone resorption in cystic growth (30). These findings and our results suggest that one of the main activities of the mast cells in HPIL is seen in the inflammatory process related to bone destruction for enlargement of the lesions.

Taking together our results and those discussed previously, we can suggest that presence of the MC in periapical inflammatory lesions is not mainly related with an immediate hypersensitivity reaction and that the most important role of the MC in these inflammatory lesions is related to development and perpetuation of the periapical inflammatory process.

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