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Ultrastructural aspects of two different mast cell populations in human healthy gingival tissue

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SUMMARY

The results of our recent microscopy studies clearly have demonstrated the constant presence of numerous metachromatic cells in healthy human gingival connective tissue. Despite the great number of studies on mast cell population in many human organs (lung, skin, uterus, and bowel), at the present time few are the studies regarding the morphostructural aspects of mast cells in the human gingiva. The aim of this study was to assess by transmission electron microscopy the presence of mast cells in the healthy human gingiva and to characterize the ultrastructural aspects of mast cells populations. 30 specimens of human gingival tissue were collected from 30 patients with informed consent. The samples were prepared for T.E.M. examination. In all the ultrathin sections observed we detected numerous and ubiquitarious mast cells. These exhibited several morphological types of cytoplasmic granules with characteristic subgranular architectural variety in shape and density. This allowed us to divide mast cells into two groups: cells with granules consisted of compact coiled scrolls, fine granular material and lattice – grating configuration, and cells containing granules with discrete scrolls formed by more concentric lamellae and particulate structure. The two ultrastructural aspects observed correspond to McTC and McT of the international litterature. Therefore in the human gingival connective tissue, like in other organs, two types of mast cells are clearly present. Surprisingly, the human gingival tissue shows, like the lung, McT as the prevailing subpopulation, in contrast to the skin, uterus and gastrointestinal submucosa where McTC prevail.

KEY WORDS:

Gingival tissue, mast cells, cytokines, chemotaxis, TNF-alfa.

RÉSUMÉ

Dans le cadre d'une étude sur la population cellulaire du tissu conjonctif gingival humain nous avons constaté, en microscopie optique, la présence constante de nombreuses cellules metachromatiques. Pour définir la nature de telles cellules et pour en déterminer les aspects ultra-structuraux, nous avons étudié au microscope électronique à transmission 30 biopsies du tissu gingival humain, cliniquement sain. Dans tous les échantillons examinés nous avons observé de nombreux mastocytes dont le contenu granulaire nous est apparu caractérisé par un aspect «à particules» et «en rouleaux» ou bien, dans d'autres éléments cellulaires, par un aspect «en grillage». Les deux aspects ultrastructuraux décrits nous permettent de distinguer

les mastocytes gingivaux en deux sous-populations, différentes comme l'ont confirmé plusieurs auteurs, selon la localisation anatomique, selon la structure intérieure et le contenu enzymatique des granules, et, enfin, selon la réaction à des substances sécrétagogues.

MOTS CLÉS:
Tissu gingival, mastocytes, cytokines, chimiotactisme, TNF-alfa.

INTRODUCTION

Mast cells are secretory cells distributed throughout all vascularized tissues. Their wide distribution has been observed in many human organs and tissues, such as the skin, foreskin, the alveolar wall of the lungs, the uterus, intestinal mucosa [5, 10, 14, 27, 34, 52] and also in periapical granulomas (Fonzi, 19). Ultrastructural and functional aspects of human mast cells have also been described in detail. Two types of mast cells are currently known. Both types share common morphological characteristics, such as a round or oval nucleus, a multitude of cytoplasmic granules, and few mitochondria; they do, however, differ in the ultrastructural aspect of their granular content [21, 31, 53, 54, 56], their anatomical localization, enzymatic content and response to secretagogue substances. Recently, an internationally accepted definition divided human mast cells into two subpopulations according to their enzymatic content [8, 9, 25]. Consequently, mast cells containing only tryptase in their granules are called McT, while mast cells containing both tryptase and chymase are called McTC. Immunoelectron microscopy studies have clearly indicated that McT (mast cells with only tryptase positive granules) are characterized by a granular content with concentrically arrayed electrondense laminae; in contrast McTC (tryptase and chymase positive granules) display a granular content of parallel fine electrondense bands.

Deeper interest in mast cell analysis has recently arisen due to the latest discoveries made by biochemical research. Modern techniques seem to confirm the possibility that, under appropriate stimuli, mast cells are able to secrete not only the well known preformed mediators (histamine, serotonin and heparin), but also a wide range of newly synthetized biochemically active molecules, such as prostaglandins [14, 15, 16, 17, 44] and cytokines [42, 45, 63, 65, 68, 69]. Because they contain cytokines, which were previously thought to be secreted only by macrophages, lymphocytes and endothelial cells, mast cells are presently considered to play a role in healthy and diseased tissue that is much more complex than was previously suspected [20, 21, 64].

However, despite the great number of studies on human mast cells, there are few studies concerning gingival mast cells. Mast cells in healthy gingival tissue were studied in the 1968 Weinstock paper from a morphostructural point of view [67]; recently, light microscopy studies have been performed with the aim of differentiating gingival mast cell subpopulations in rat and human gingiva [1, 28, 35, 70]. Surprisingly, international literature still lacks up-to-date studies on both morphostructural and functional characteristics of healthy human gingival mast cells.

In previous light microscopy report of ours on the cellular population of healthy gingival tissue [3, 6, 22, 24], we continuously described metachromatic cells in the connective tissue underlying epithelial layers. With the aim of assessing the presence of mast cells and characterizing their phenotypical differentiation, we examined in transmission electron microscope biopsies of healthy human gingival tissue. Considerations on their role in healthy gingival tissue as eventual cellular regulators of tissue homeostasis are hereby discussed.

MATERIAL AND METHODS

Thirty biopsies of healthy human gingival tissue were surgically removed with informed consent from patients, ranging in age from 18 to 35, and who were selected during daily dental practice for their good oral hygiene and «clinically healthy gingiva». Samples were immediately washed in idrosaline solution and fixed in Karnowsky's aldehyde fixative (1% formaldehyde, 3% glutaraldehyde in 0,1 M Cacodylate buffer, pH 7,4) without Calcium for 2 hours

at 4°C. Following rapid dehydration in ethanol and propilene oxide, samples were included in Araldite (Epon). 0,5 to 1 micron semi-thin sections were stained with toluidine blue and observed at light microscopy. Ultra-thin sections (500-600 nanometres) including sulcular epithelium and the underlying connective tissue were cut at microtome LKB ultratome IV, stained with lead citrate and uranyl acetate and examined using a Philips EM 201 transmission electron microscope. In each sample, a minimum of 10 mast cells were observed at T.E.M.

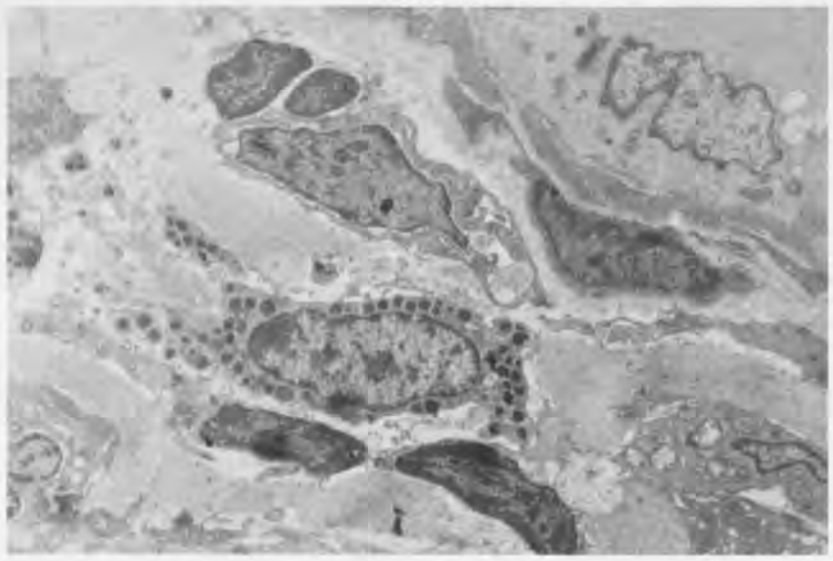


Fig. 1: Mast cell in healthy gingival tissue: collagen fibers, pericyte and endothelial cells, fibroblasts. A round nucleus and cytoplasmic granules are clearly visible (TEM 1650×).
Fig. 1: Tissu conjonctif gingival humain sain: mastocytes, fibres de collagène, cellules endothéliales, fibroblastes. Dans le mastocyte, le noyau arrondi et les granules cytoplasmiques apparaissent évidents (MET 1650×).

RESULTS

The T.E.M. examination demonstrated in all the specimens the presence of numerous mast cells in the gingival connective tissue. Mast cells, although ubiquitarious in the connective tissue within collagen fibers, appeared more abundantly near fibroblasts, plasma cells and macrophages, and surrounding blood vessels (Fig. 1, 2).

Observations under low magnification revealed the same ultrastructural aspects of mast cells observed in other human organs. Each mast cell possessed a round or oval single-lobed nucleus; the nuclear content was constituted by partially condensed chromatin. Cytoplasmic content showed rarely small and few mitochondria, with a typical morphological characteristics, oriented in a perinuclear fashion in the proximity of the Golgi Complex, which was usually well developed with flattened saccules and vesicles. Free ribosoms and non-membrane-bound lipid bodies were also present (Fig. 1, 2). Other cytoplasmic features included filaments and sometimes, electrondense particle granules (glicogen granules). The plasma membrane was characterized by the presence of many protrusions (1-1,5 millimicrons in length) projecting outwardly from the surface of the cell, sometimes parallel and adjacent to the cell surface (Fig. 1, 2, 5, 7, 8).

The cytoplasmic content was for the most part dominated by numerous, large, membrane-bound granules, varying in shape, density and internal compositon. Non-membrane bound granules were also present.

Cytoplasmic granules

An extreme variability in the shape and ultrastructural content of granules occupying most of the cytoplasm was observed. High resolution allowed us

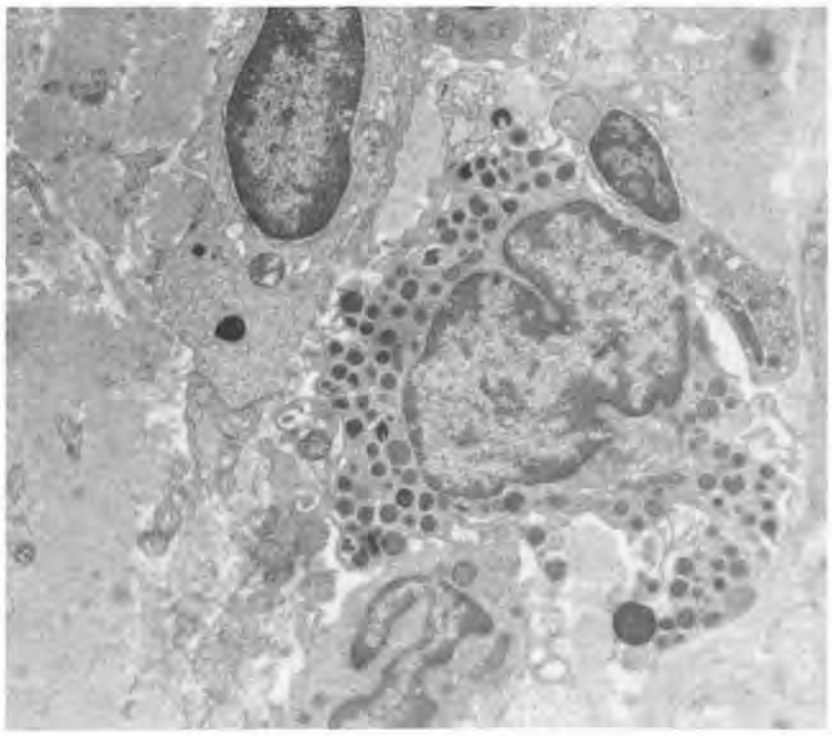


Fig. 2: Gingival mast cell, displaying a multitude of cytoplasmic granules, a single-lobed nucleus, and a well developed Golgi complex (TEM 1650×).
Fig. 2: Mastocyte gingival, avec des granules cytoplasmiques, un noyau bilobé et un complexe de Golgi (MET 1650×).

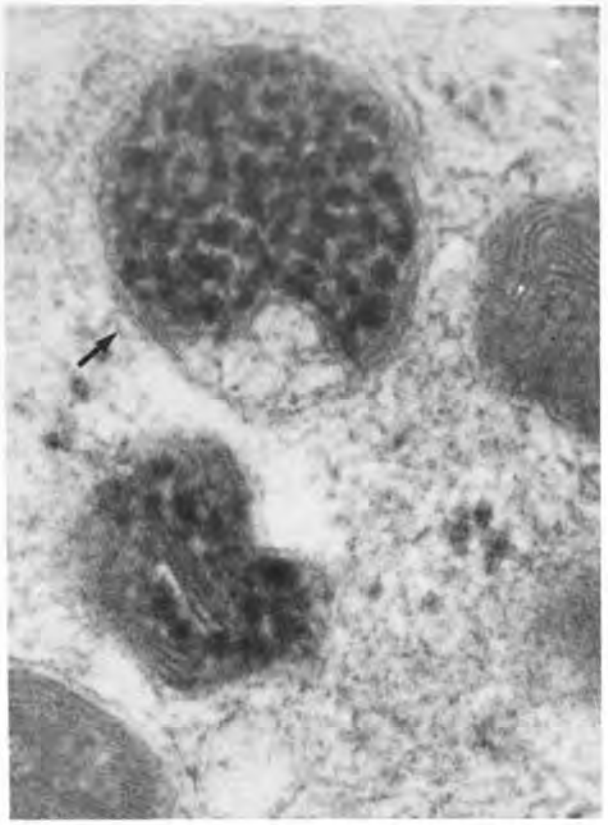


Fig. 3: Cytoplasmic granules showing a «particulate» content (closed arrow), constituted by electrodense particles with indistinct margins (TEM 20,000×).
Fig. 3: Granules cytoplasmiques mastocytaires caractérisés par un contenu «à particles» (flèche fermée), avec particules denses aux électrons, arrondies aux bords mal définis (MET 20,000×).

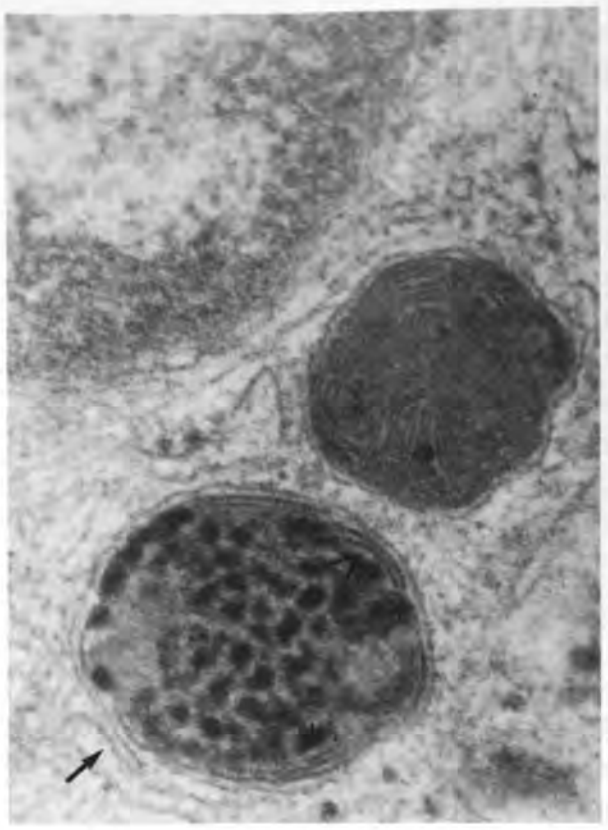


Fig. 4: Cytoplasmic granules showing round, electrondense particles filling out the granular content (closed arrow) and interlaced, thin, straight and curved bands maybe disentangled scrolls (open arrows) (TEM 20,000×).
Fig. 4: Granules cytoplasmiques dont le contenu est caractérisé exclusivement par des particles denses aux électrons (flèche fermée) et par de fines bandelettes entrelacées à la périphérie, souvent courbes peut-être des résidus de figures «en rouleaux» ouvertes (flèche ouverte) (MET 20,000×).

to distinguish three different ultrastructural aspects of granular content: «particulate», «grating» and «scroll».

Particulate content was characterized by the assembling of small particles which were more or less electrondense, as well as fine filaments in a homogeneous less dense matrix. Particles sometimes filled out the granular content, in other cases they left a less electrondense area (Fig. 3,4). At high magnifications, the content seemed composed by round particles, which

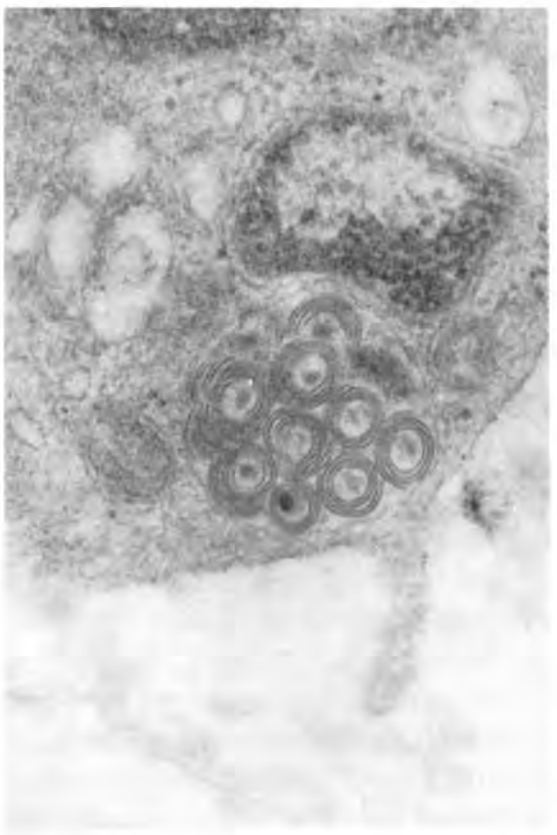


Fig. 5: «Scroll pattern»: granules cut in transversal section showed aggregates of two to four electrondense laminae concentrically coiled together, often including in their center an amorphous electrondense core (TEM 70,000×).
Fig. 5: Le contenu granulaire caractérisé par des groupes de deux ou plus «laminae» denses aux électrons, disposées, en section transversale, concentriquement, assumant ainsi un aspect «en rouleaux». Souvent à l'intérieur des laminae «en rouleaux» on peut remarquer un noyau de matériel amorphe dense aux électrons (MET 70,000×).

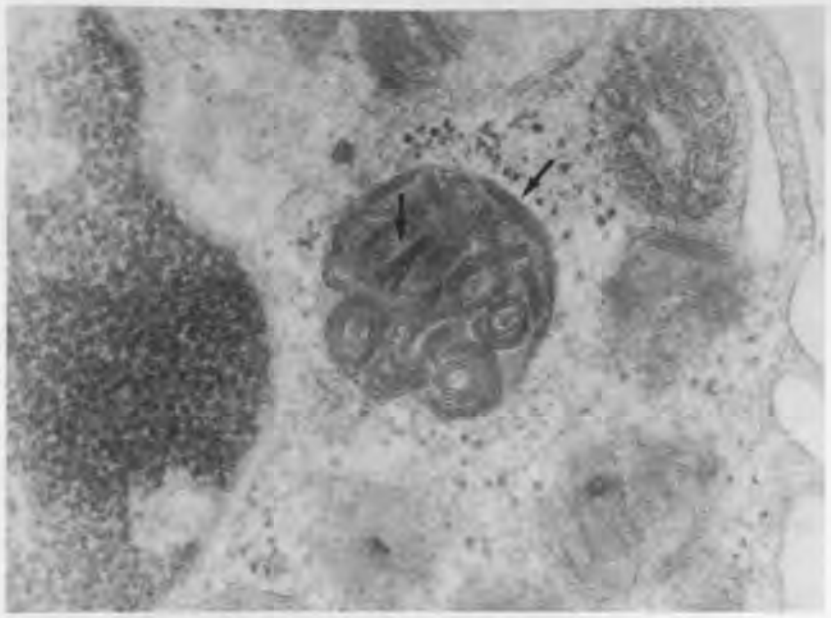


Fig. 6: Non membrane-bound granule with visible transversal and longitudinal sections of the scroll pattern. The constituting laminae are parallel and straight when the section is longitudinal (arrows) (TEM 70,000×).
Fig. 6: Granules non délimités par une membrane avec des sections longitudinales et transversales des figures à l'aspect «en rouleaux». Les sections longitudinale sont caractérisés par laminae droites (flèche) (MET 70,000×).

were usually distinctly separated, while others showed indistinct margins and resemble thread pearls. Particulate content seemed organized by coarse, electrondense, interlaced strands measuring 50 nanometres in width. The structure of these strands consisted of a multitude of dense particles embedded in a less dense matrix. Thin electrondense lines forming «whorls» or «scroll-like» figures, cut in different sections, were observed at the periphery of the granule (Fig. 4).

Scroll-like configuration was the most frequently observed configuration, characterized by compact coiled electrondense laminae, concentrically arrayed inside the granule. Granular content was exclusively constituted by scroll configuration (Fig. 5, 6), often displaying condensed material in the center of the scroll configurations (Fig. 5) or scroll and particulate configurations. When cut along their long axis, the scroll patterns seemed composed of parallel and closely packed dense lines separated by electron lucent spaces with constant periodicity (Fig. 6). Nuclei of electrondense material, of different radiopacity, sometimes resembling particulate content, were present within granules characterized by the presence of dense amorphous material and residues of coiled and open laminae (Fig. 7).

Grating configuration: other mast cells (Fig. 8) showed both scroll configuration and an homogeneous granular content when observed at low magnification. High resolution observation allowed to distinguish a complex ultrastructural granular organization, with scrolls and fine electrondense filaments parallely arrayed, separated by electron-

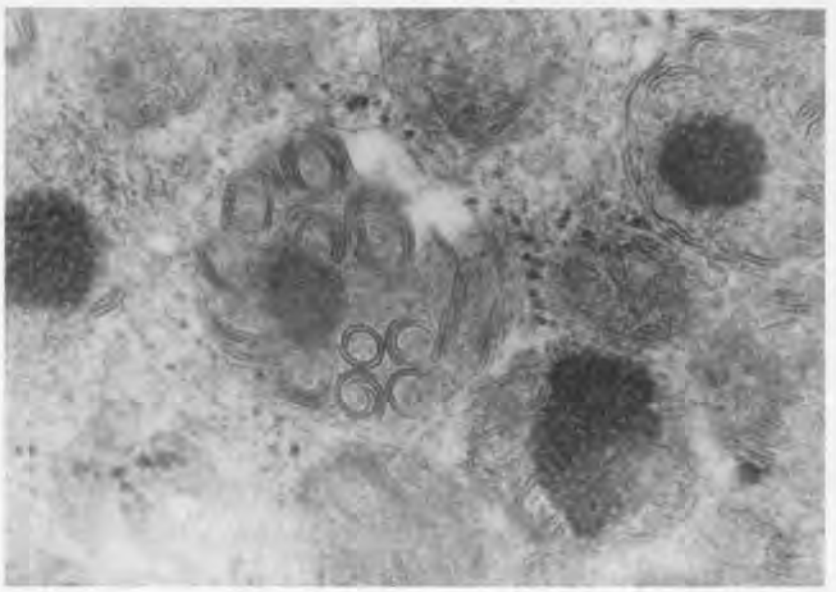


Fig. 7: Non membrane-bound granule, displaying «scroll pattern» laminae, concentrically arrayed, embedded in a less dense matrix, containing an eccentrical electron dense nucleus (TEM 70,000×).
Fig. 7: Granules non délimités par une membrane laminae «en rouleaux» contenant un noyau dense aux électrons (MET 70,000×).

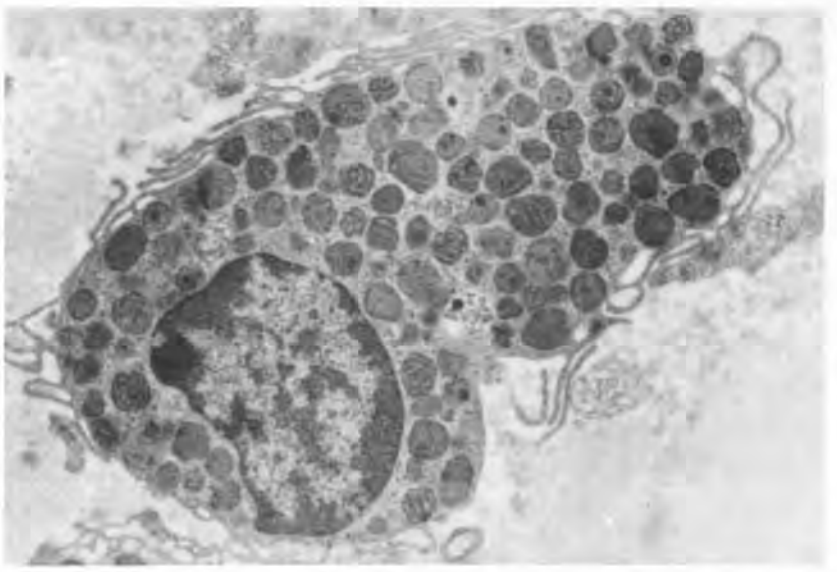
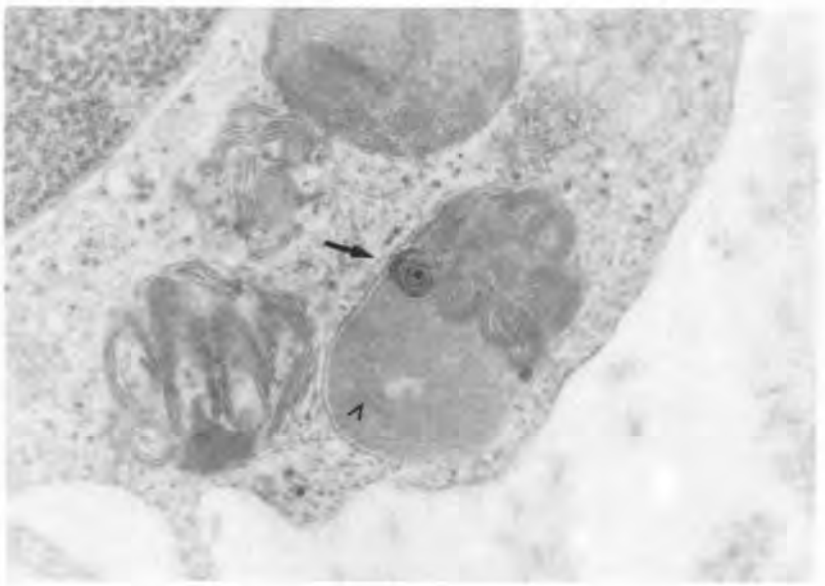


Fig. 8: Gingival mast cell showing few mitochondria, rounded nucleus, protrusions and cytoplasmic granules with granular content characterized by «whorls»-like figures and less electron dense, indistinct material (TEM 10,000×).
Fig. 8: Gingival mastocyte à un faible grossissement. Le noyau arrondi, les protrusions de la membrane cytoplasmique et un contenu granulaire caractérisé par des «figures en vortex» et par un matériel peu dense aux électrons, sont évidents (MET 10,000×).

transparent spaces, constituting a net (Fig. 9-10). Often the «grating» configuration showed one or more electrondense nuclei, deliminated by a narrow electrontransparent a narrow circular transparent area (Fig. 11).



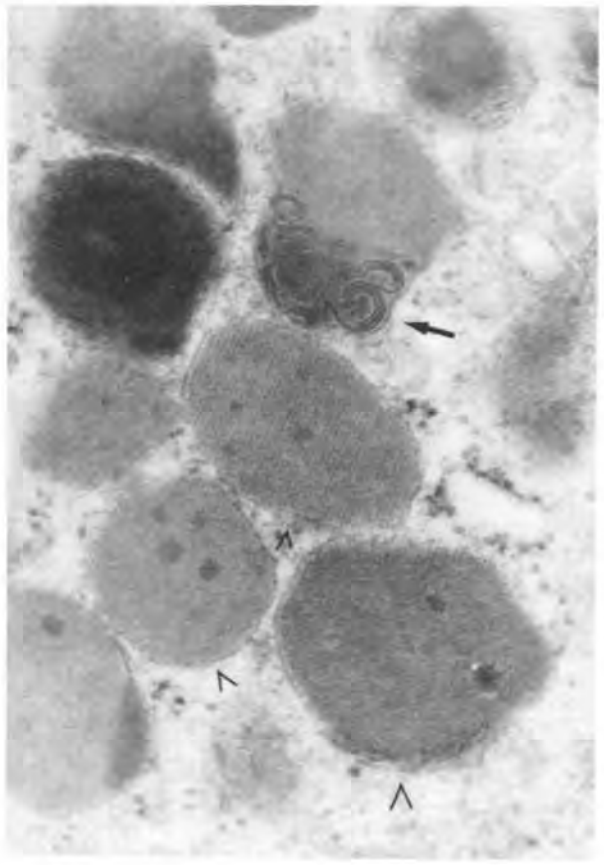


Fig. 9-10: At high resolution, granular content of the granules showed mixed «scrolls-like figures» (closed arrows) and thin, parallel bands, regularly ordered (open arrows) (TEM 45,000×).
Fig. 9-10: A un plus fort grossissement, le contenu granulaire présente des images mixtes «en rouleaux» (flèche fermée) et de fines bandelettes peu denses aux électrons disposées parallèlement (flèche ouverte) (MET 45,000×).

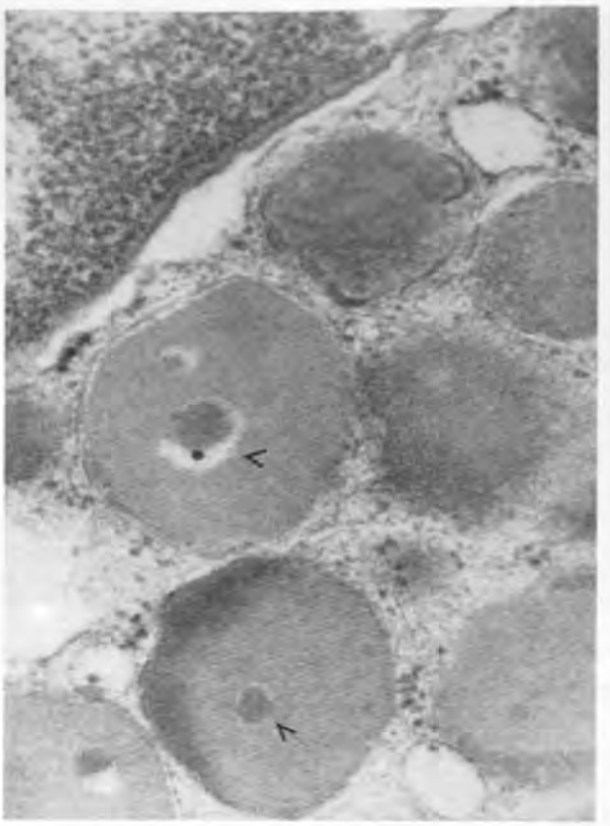


Fig. 11: The majority of the membrane-bound granules of the second mast cell subpopulation showed parallel, regularly spaced, slightly electrondense bands, with characteristical small, more electrodense, central nuclei. Nuclei were always posed in a transparent area (open arrows) (TEM 70,000×).
Fig. 11: La plupart des granules observés dans la seconde sous-population mastocytaire présentent presque constamment à l'intérieur du grillage formé par les bandes parallèles, de petits noyaux plus denses aux électrons, souvent entourés d'une auréole transparente (MET 70,000×).

Mast cells always displayed a granular content organized into two models: «scroll-particulate pattern» (Fig. 3, 4) and «grating-scroll pattern» (Fig. 8). However, no mast cell, among the observed samples, showed the contemporary presence of granules with «particulate» and «grating» configurations.

DISCUSSION

The present study clearly shows that mast cells, with two different morphostructural aspects, are constantly present (resident or migratory) cells of gingival tissue, usually close to blood vessels. The detailed observation at T.E.M. allowed us to distinguish two morphostructural different mast cell subpopulations. The first mast cell population showed a prevalence of cytoplasmic granules with

«particulate» and «scroll» figures, common beeing the observation of the two configurations inside the same cell. Electrondense nuclei embedded in a homogeneous matrix are common, though not constant. The second subpopulation was characterized by prevalence of granules with «grating» aspects. Scroll-like figures and granules with intermediate aspects between «scroll» and «grating» were also present, while it was never possible to observe, in our specimens, mast cells granules containing both «particulate» and «grating» aspects.

In human organs and tissues, mast cells are subdivided by most of Authors according to their ultrastructural aspect and enzymatic content. Only recently however, immunoelectron microscopy studies allowed to associate definitively different enzymatic content and ultrastructural configurations (Craig *et al.*, 1988, 1989). Therefore, mast cells with a prevailing «particulate-scroll aspect» of their granules contain only tryptase (McT), while the granular aspect «grating-lattice» is related to mast cells containing tryptase and chymase. The two subpopulations differ for their anatomical localization, in fact they are not equally distributed in human body. In the lung, McT represent more than 90% of total mast cell population, while in skin, foreskin and intestinal mucosa McTC seem to prevail. The reasons for the contemporary presence of the two subpopulations are not yet clear, and many attempts of correlating morphostructural aspects, biological properties and function are currently in progress. Beyond their ultrastructural aspect and anatomical localization, the two subpopulations degranulate when stimulated by different secretagogue substances. McT degranulate when stimulated by anti-IgE; McTC are instead stimulated by 48/80, substance P, C 5a, A 23187, Ca ionophore. Moreover, the two types may eventually secrete a great variety of cytokines, such as interleukins (IL-1, IL-2, IL-3, IL-4), Tumor Necrosis Factor (TNF-alfa), Nerve Growth Factor (NGF) and Interferon gamma (IFN-gamma) [2, 20, 39, 48, 49, 57, 65, 69).

Recent acquisitions on cytokines, as biomolecular paracrine regulators and indicators of biological processes, widen problems related to the presence of the two mast cell subpopulations in tissues and suggest their active role in the homeostasis regulation.

Among all the cytokines, preliminar results of an immunological investigation conducted at our Institute on healthy gingival tissue cultures seem to indicate for gingival mast cells the possibility to secrete TNF-alfa [3, 6, 23].

TNF-alfa is a multifunctional citokine with effects on inflammation, lipidic and proteic metabolism, ematopoiesis, angiogenesis and tumour cells [2, 7, 46, 63]. TNF-alfa was thought to be released by only macrophages and monocytes until recently, when Walsh *et al.*, with the help of immunohistochemistry, demonstrated mast cells to be the principal source of TNF-alfa in normal human skin [64]. Secreted in a variety of pathophysiological situations including septic shock, TNF-alfa has one of its main targets in the vascular endothelium [7, 13, 29, 30], which has TNF-alfa receptors and, as it has been demonstrated since 1985, can express a handful of cell surface molecules capable of supporting leukocyte adhesion. One of the three cell surface glycoproteins, generally named selections, has been known to be expressed by cytokine (TNF-alfa)-activated endothelial cells: Endothelial Leukocytes Adhesion Molecule-1 (ELAM-1) [4, 36, 40, 45, 56]. Studies have clearly indicated that TNF-alfa exposed cultured endothelial tissues synthetize and express ELAM-1, while other selections (ICAM, VCAM) are mainly expressed in diseased tissues [14, 43].

ELAM-1 is one of the mightest factors capable of promoting adhesion of leukocytes and polimorphonucleats (PMNs), mediated by interactions with PMNs and lymphocytes receptors, to the vascular wall [18, 29, 40, 60]. As well known, in gingival tissue, adhesion is the first step for lymphocytes and PMNs for subsequent diapedesis and chemotaxis through connective tissue [37, 38, 47, 51, 55, 58, 59, 61, 62, 66]. Today, TNF-alfa induced expression of ELAM-1 on the venular endothelial cells of many tissues has been virtually proved in every connective tissue.

Actually, both TNF-alfa and ELAM-1 are recognized to be normally expressed in human healthy gingiva and upregulated with the development of inflammation [11, 12, 26]. The observed location of gingival mast cells in the proximity of blood vessels may support their challenge of secreting proinflammatory mediators in the immediate vicinity of post-capillary venular endothelium. Beyond the «histamine deposit» role given to mast cells, the function of «gatekeepers» or «sentry cells» seem therefore more than possible for mast cells in the healthy gingival tissue, particularly for their content in protease and histamine which maight facilitate cell migration through degradation of basement membrane and forming interendothelial gaps [32, 33, 41]. If preliminary results of our immunological study will

confirm the possibility that gingival mast cells may control through TNF-alfa secretion PMNs migration through the healthy tissue, mast cells may become the most important regulators of the tissue cellular homeostasis, thus regulating cellular flow through connective tissue.

To test TNF-alfa mediated mast cell induction of ELAM-1 in healthy gingival tissue we are actually trying to induce ELAM-1 expression by means of stimulation of gingival mast cell with mast cell active secretagogue substances (i.e.: Substance P and Immunocomplexes).

If preliminary results will be confirmed, mast cells would acquire an important role in the homeostasis of healthy gingival tissue, being not anymore the deposits of histamine, but also the «sentry cells» of tissues, thus regulating the cellular flow through the connective tissue, of PMNs. PMNs are the powerful defense cells which first are activated, in the case of bacterial aggression, to discharge their granular content. The continuous migration of PMNs towards the gingival sulcus might consequently upregulated in the gingiva, by TNF-alfa. The expression of ELAM-1 is considered a necessary but one of the biological mechanisms involved in controlling early PMNs trafic into inflamed areas. However, the possibility that also other citokines, such as IL-1, IFN-gamma, would be involved in the expression of ELAM-1 is a question already posed.

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