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E-пошта: y.makeyeva@garvan.org.au

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ORCID IDs
Юлія Макеєва:
https://orcid.org/0000-0003-1576-0750
Стівен Т. Ліч:
https://orcid.org/0000-0001-9704-1666
Девід К. Рюґо:
https://orcid.org/0000-0002-5250-7503

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Створення концепції: Юлія Макеєва, Девід К. Рюґо;
Результати дослідження: Юлія Макеєва, Стівен Т. Ліч, Девід К. Рюґо;
Написання: Юлія Макеєва, Девід К. Рюґо;
Редагування та затвердження остаточного варіанту: Юлія Макеєва, Стівен Т. Ліч, Девід К. Рюґо.

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Мастоцити в середовищі: порівняльне дослідження у язику та в печінці

Юлія Макеєва[1,2], Стівен Т. Ліч[2] та Девід К. Рюґо[1,3,4]

[1]Інститут медичних досліджень Гарвана, Дарлінгхерст, Новий Південний Уельс, Австралія
[2]Дисципліна педіатрії, Школа клінічної медицини, Університет Нового Південного Уельсу, Новий Південний Уельс, Австралія
[3]Школа біомедичних наук, Університет Нового Південного Уельсу, Кенсінгтон, Новий Південний Уельс, Австралія
[4]Відділення отоларингології, хірургії голови, шиї та основи черепа, Лікарня Сент-Вінсент, Дарлінгхерст, Новий Південний Уельс, Австралія

Мастоцити відіграють різноманітну роль у здоров'ї та хворобах. Ці імунні клітини тканинно-специфічно мігрують до місць цільового розташування, де вони можуть попереджати або посилювати імунні відповіді шляхом екзоцитозу своїх біоактивних гранул. Екзоцитоз гранул може бути викликаний зовнішніми імунологічними та неімунологічними сигналами. Мастоцити є гетерогенними клітинами зі значною фенотиповою пластичністю та функціональною різноманітністю, залежно від місцевого середовища. Метою цього дослідження було дослідити відмінності у популяціях мастоцитів у язику та в печінці, щоб покращити розуміння того, як органно-специфічне місцеве середовище може впливати на фенотипову варіабельність та функції мастоцитів. Мастоцити досліджували за допомогою світлової та електронної мікроскопії у тканинах щурів, які перебували в ідентичних умовах утримання. Мастоцити сполучної тканини були ідентифіковані як у язику, так і в печінці та демонстрували фенотипові відмінності у секреторній активності та характеристиках гранул між двома органами. Отримані результати підтверджують що фенотипова варіабельність мастоцитів залежить від місцевого середовища розташування. Ця інформація може допомогти у розробці стратегій запобігання або лікування захворювань.

Ключові слова: імунна клітина, мастоцит, світлова мікроскопія, електронна мікроскопія, гістологія, фенотипова мінливість, імунотерапія.

Mast cells in the Milieu: A comparative study in the tongue and liver

Yuliya Makeyeva[1,2], Steven T. Leach[2] and David K. Ryugo[1,3,4]

[1]*Garvan Institute of Medical Research, Darlinghurst, NSW, Australia*
[2]*Discipline of Paediatrics, School of Clinical Medicine, UNSW, NSW, Australia*
[3]*School of Biomedical Sciences, UNSW, Kensington, NSW, Australia*
[4]*Department of Otolaryngology, Head, Neck and Skull Base Surgery, St. Vincent's Hospital, Darlinghurst, NSW, Australia*

Mast Cells (MCs) have diverse roles in health and disease. These immune cells migrate in a tissue-specific manner to target sites where they can alert or amplify immune responses through exocytosis of their bioactive granules. Granule exocytosis can be triggered by both external immunological and non-immunological signals. Depending on the local milieu, MCs are highly heterogeneous cells with significant phenotypic plasticity and functional diversity. The aim of this study was to investigate differences in MC populations in the tongue and liver to advance the understanding of how organ-specific location may affect MC phenotypic variance and functions. MCs were investigated using light and electron microscopy in tissues obtained from rats housed in identical conditions. Connective tissue MCs were identified in both the tongue and liver and showed phenotypic differences in secretory activity and granule characteristics. These findings indicate that phenotypic variance depends on tissue location. This knowledge may help develop strategies for preventing or treating diseases.

Keywords: Immune cell, mast cell, light microscopy, electron microscopy, histology, phenotypic variance, immunotherapy.

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For correspondence: Garvan Institute of Medical Research 384 Victoria Street Darlinghurst, NSW 2010, Australia
E-mail: y.makeyeva@garvan.org.au

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ORCID IDs
Yuliya Makeyeva:
https://orcid.org/0000-0003-1576-0750
Steven T. Leach:
https://orcid.org/0000-0001-9704-1666
David K. Ryugo:
https://orcid.org/0000-0002-5250-7503

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Conceptualization: Yuliya Makeyeva, David K. Ryugo;
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Introduction

Mast Cells (MCs) are mobile, long-lived granule-containing immune cells that act as both sensors and effectors of communication between the immune, vascular, and nervous systems [1,2]. They are derived from myeloid precursors, circulate in the blood as immature progenitors, and then migrate trans-endothelially into peripheral tissues to complete their maturation. Their development is influenced by growth factors, chemotactic agents, and the surrounding microenvironment, all of which cause MCs to be highly adaptable and functionally diverse [3-11]. MCs differ, not just across species, but also within the same organ of the same species [5,8,12-18]. MCs also differ in the magnitude and nature of their responses to different types of triggers. Whereas some stimuli cause a differential and selective release of mediators, others may cause a rapid and extensive degranulation of mediators [8,19-21].

Two major types of MCs have been identified in rodents: connective tissue MCs (CTMCs) and mucosal MCs (MMCs) [22-27]. These two MC types differ according to anatomical location, histological appearance, pharmacological properties, and granule content [19,28-30]. CTMCs are found in the connective tissues of skin, muscles, and the peritoneal cavity. They contain granules filled with glycosaminoglycan, proteases and lipid mediators, heparin and histamine (highly sulphated mucopolysaccharides) that have a strong affinity with Toluidine Blue (TB) and safranin stains. In contrast, MMCs are found in mucosal linings of the lungs and intestines. Their granules contain poorly sulphated mucopolysaccharides that react with Alcian blue stain but do not contain heparin [5,7,9,19,22,29,31-33]. MCs can also be identified by their granule protease. Rat MC protease (RMCP) I and II are highly specific granule markers. RMCP I is found in CTMCs, whereas RMCP II is found in the MMCs. Although biochemically similar, they are antigenically distinct [34-38]. Because MCs respond to a wide range of stimuli, they can benefit innate and adaptive immunity, host defence, homeostasis, immunoregulation, tissue integrity, and neuro- and angiogenesis [10, 39-41]. However, dysregulated activation of MCs contributes to allergic reactions, inflammation, autoimmune diseases, tumour metastasis, fibrotic diseases, and reproductive disorders [10,40,42-52].

The role of tissue milieu in determining MC phenotype and function has been actively investigated [8,53]. MCs are located in multiple body organs and tissues, including the lungs, heart, gastrointestinal tract, oral cavity, and liver [25,40,46,54-59]. In vascularized tissue, MCs are located near blood and lymphatic vessels, epithelia, smooth muscles and peripheral nerves [1,12,60-62]. The aim of the present study was to investigate phenotypic differences between CTMCs located in rat tongue and liver tissues to advance understanding of how organ-specific location may affect MC phenotypic variance and function.

Materials and Methods

Animals

Male Sprague Dawley rats (N=10) were used in accordance with the NHMRC Animal Experimentation Guidelines and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) and with the approval of the Animal Care and Experimentation Committee of the University of New South Wales (UNSW). Tissue samples from animals for this study were obtained through the tissue-sharing program in line with the principle of Reduction as per Code.

Tissue processing

Rats were given an overdose of intraperitoneal (i.p.) Lethabarb 100 mg/kg (pentobarbitone sodium, Vibrac Animal Health, Pty. Ltd., Australia), transcardially perfused with ice-cold 0.1 M phosphate-buffered saline (PBS), pH 7.2, followed by a solution of 4% paraformaldehyde (PFA) in 0.1 M PBS. Tongues (from apex to root) and liver sections (three pieces randomly cut) were dissected for histologic examination. Tissue was postfixed in 10% neutral buffered formalin (Sigma-Aldrich Corp.; Sydney, Australia) for 12 hr at 4 °C and embedded in paraffin using standard embedding procedures. Tongues were positioned longitudinally, and livers were positioned randomly. Sections were serially cut at 5 μm on a motorised microtome (Leica RM 2155; Leica Microsystems; Wetzlar, Germany) and mounted consecutively on electrostatic slides (Menzel-Glaser; Braunschweig, Germany).

Haematoxylin and Eosin staining

Haematoxylin and Eosin staining were performed to evaluate basic tissue structure and quality. Sections were dewaxed in Histo-Clear (National Diagnostic Products Pty Ltd., Australia) and rehydrated in a graded series of decreasing alcohol concentrations (100%, 70%, 50%, H2O). Sections were then stained in Harris Haematoxylin (Fronine Pty Ltd, Australia), differentiated in acid alcohol, and immersed in Scott's Blueing Solution (Fronine Pty Ltd, Australia). Then, they were dehydrated in 70% alcohol, counterstained with alcoholic eosin, and dehydrated in a series of increasing alcohol concentrations. Sections were cleared in Histo-Clear (National Diagnostic Products Pty Ltd., Australia) and cover-slipped using Aqua Mount (BDH Laboratories Supplies, England).

Toluidine Blue (TB)

TB staining was used to detect MCs in tongue and liver tissues. Paraffin sections were dewaxed, hydrated as previously described, and stained with TB (working solution of 1% TB in 70% ethanol, 5 ml and 1% sodium chloride in distilled water, 45 ml mixed well, pH around 2.3) for 60 secs. Sections were washed three times in distilled water and dehydrated quickly in 95% ethanol and then in 100% ethanol. Sections were cleared in xylene and coverslipped with DPX mounting medium. Slides were scanned (Aperio ScanScope AT; Leica Biosystems, Germany), viewed, and analyzed (Aperio ImageScope software). Image preparation (adjusting brightness and contrast only), assembly and analysis were performed in Adobe Photoshop 2020 (Adobe Systems; San Jose, CA, USA).

Electron microscopy (EM)

Tongue and liver tissues were embedded in a gelatine-albumin mixture (25 mg gelatine and 1.5 g bovine serum albumin in 5 ml of warm water), hardened with a mixture of 10 drops of 37% formaldehyde and 400 µL of 5% glutaraldehyde. The hardened tissue block was then cut into 60 µm thick sections on a vibrating microtome (VT1200S; Leica Systems, Nussloch, Germany). Sections were washed 3 times (10 min each) in 0.1 M maleate buffer (pH 5), osmicated in 1% osmic acid in 0.1 M s-Collidine buffer (pH 7.4) for 15 mins, rinsed again in maleate buffer (6x; 10 min each), en bloc stained overnight in 1% uranyl acetate at 4 °C, dehydrated in ascending concentrations of alcohol, soaked in propylene oxide, infiltrated with PolyBed 812 (Polysciences, Inc.; PA, USA, and embedded in PolyBed 812 between two sheets of Aclar (Electron Microscopy Sciences; Hatfield, PA). Aclar sections were examined under a light microscope (LM), and selected regions of interest were photographed, cut out from the polymerized Polybed 812, and embedded in BEEM capsules (ProSciTech; QLD, Australia) for semithin and ultrathin serial sectioning (PowerTome X; Boeckler Instruments, Inc., Tucson, AZ). Semithin sections (250 nm) were collected on glass slides, stained with TB, and photographed under a LM. Serial ultrathin (75 nm) sections were collected on formvar-coated slotted grids, stained with uranyl acetate and lead citrate, and examined with an electron microscope.

Digital microscopy, image processing and photography

TB-stained slides were examined under LM using 2.5x (Plan-Neofluar, 2.5x/0.075), 10x (Achroplan, 10x/0.25), 20x (Plan-Neofluar, 20x/0.50), 40x (Plan-Neofluar, 40x/0.75), 63x (Plan-Apochromat, 63x/1.4 oil), and 100x (Olympus Plan, 100x/1.25 oil) objectives and then photographed with a ProgRes C5 CCD 5.0 camera (Jenoptik; Jena, Germany). Regions of interest were followed through each stage of processing under LM. Guided by LM images, serial ultrathin sections were examined using EM at 2000x, 5000x, and 15000x magnification (H-7650; Hitachi, Tokyo, Japan).

Quantitative analyses

For all quantification methods, tissues were obtained from rats (N=4) of the same age (four weeks) that were individually housed under identical conditions: conventional open cages, controlled temperature (18 °C) with 12-hr light/12-hr dark cycles, and fed ad libitum a Standard Laboratory Diet (Gordon's Speciality Stockfeeds, NSW, Australia). Rats were monitored for signs of stress/distress and weighed weekly. During the seven-week experiment, all rats appeared healthy and exhibited normal behaviour and weight gain. At the start of the experiment, selected rats had similar weights (104-114 g). The weight of each rat increased linearly over 7 weeks with <10% differences among the subjects (446 - 482 g).

Size and variability of MCs

TB-stained tongue and liver slides were scanned using Aperio ImageScope (Leica Biosystems Imaging Inc., CA, USA). Counting was performed manually and blindly on randomly selected scanned slides under a 40x objective lens. All MCs from the tip to the root in the tongue (total: 2,286 MCs) and MCs around portal triads in the liver (total: 611 MCs) were analyzed. To determine MC size and variability, the circumference of the external violet contour (silhouette area) of MCs with visible nuclei was outlined. This area (size) was recorded in μm2 (FIJI software), statistically analyzed, and data were compared between the tongue and liver.

Statistical analyses

Data were analyzed using mean, standard deviation (SD), standard error of the Mean (SEM), Min, Max, median (Me), 25th percentile, and 75th percentile. Three statistical tests were applied: the Mann-Whitney U test for two independent samples when data were not normally distributed; the Student's paired t-test for one-sample population studies; and the t-test for two-sample population studies. Variations in MC sizes were determined using a coefficient of variation (CV), a statistical measure of probability dispersion around the mean value. CV was expressed as percentages (%). MCs were considered highly variable in size (high dispersion around the mean) when CV was greater than 25%. Variability was considered low (low dispersion around the mean) when CV was less than 10%. Data are reported as Mean ± SD. Statistical analysis with $p < 0.05$ (two-tailed) was considered significant and marked by an asterisk (*).

Results

Identification of MCs

Light (LM) and electron microscopy (EM) were used to examine MCs in tongue and liver tissues. MCs identified under LM were then examined using EM (Fig. 1). In LM, MCs were identified by their distinctive content of darkly stained cytoplasmic granules (Fig. 1, inset). EM confirmed these same cells to be MCs by the presence of (a) membrane-bound, heterogeneous granules; (b) unilobular nuclei; (c) numerous projections and folds on cell surfaces; (d) cell organelles; and (e) signs of exocytosis. EM further showed that intracytoplasmic granules varied in size, shape and opacity with lucid, well-defined contours and were surrounded by perigranular membranes. Granules located at the margin of a cell appeared to be in contact with the plasma membrane (Fig. 1).

The unilobular nucleus was usually located centrally and exhibited clumps of partially condensed chromatin. Many elongated, filiform projections emerged across the cell surface, except when MCs were tightly adjoined by neighbouring cells. Cell organelles were squeezed between granules (Fig. 1).

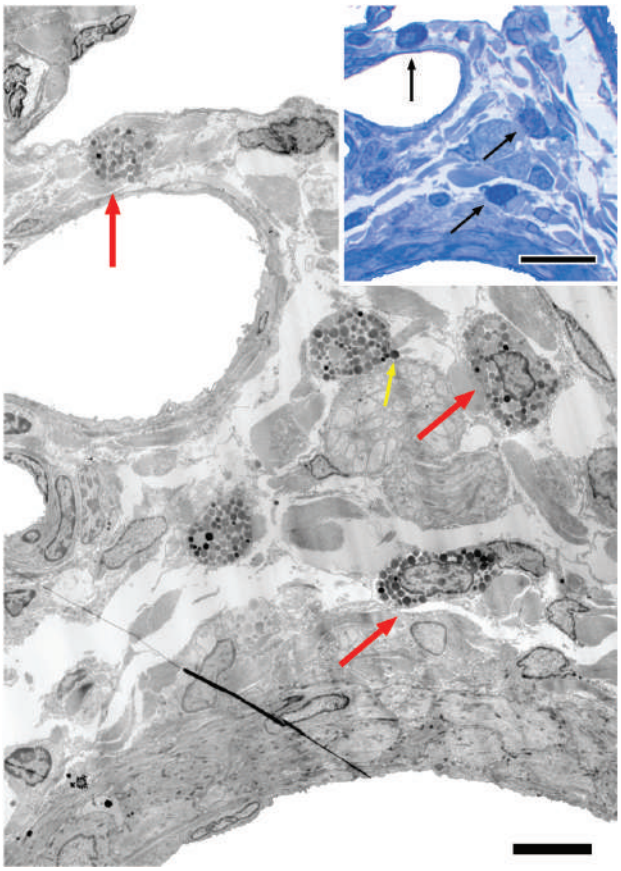

Figure 1. Identification of MCs in rat liver. Under LM, intensely blue-TB+MCs (black arrows) are seen around the portal triad (upper right inset). EM shows these cells to be mononuclear (red arrows), located in the connective tissue of vessels, and filled with granules. Individual granules are internally homogeneous but can vary in size and opacity. Secretory activity (exocytosis) is evident as a single electron-dense granule (yellow arrow) appears to be released from MC to extracellular space. Abbreviations: EM, electron microscopy; LM, light microscopy; MCs, mast cells; TB, toluidine blue; TB+, positive staining. Scale bars: inset = 20 μm; electron micrograph = 10 μm

EM revealed resting (inactivated), as well as degranulating MCs. In resting MCs, cell membranes were well-defined and intact with typical filiform projections; mature, well-defined, homogeneous granules were evenly distributed across cells (Fig. 2A). In degranulating MCs, two types of degranulation were observed. In some cases, single granules exited from an MC without disturbing cell integrity (Fig. 1, yellow arrow). In cases of profound degranulation, many extracellular granules were dispersed around the MC (Fig. 2B).

Characterization of MCs in tongue

LM and EM confirmed that MCs in the tongue and liver were connective tissue (CT) MCs, which is consistent with published reports [12,15,21,63-71]. LM showed that MCs were distributed across the entire dorsal surface in all TB-stained tongue tissue samples (Fig. 3A). MCs, individually or in groups, were located between muscle fibres, in the connective tissue, and around nerve bundles. Denser accumulations were observed around blood vessels and in the region of the lamina propria adjacent to the stratified squamous epithelium (SSE) but not in the SSE itself (Fig. 3A). MCs were not observed in taste buds. LM at higher magnification showed that MCs varied greatly in size and shape, being round, oval, elongated or spindle-shaped. They contained roundish, pale blue nuclei with blotches of what appeared to be clumped chromatin (Fig. 3B). Each MC contained numerous intracytoplasmic metachromatic granules. Some MCs with a lower density of granules exhibited extracellular granules, suggesting they were involved in ongoing degranulation (Fig. 3B). Quantitative analysis showed that the size of MCs in the tongue was 121.8 ± 58.6 μm2 and that cell size was highly variable: CV = 48.1% (Fig. 7).

EM showed that MCs were located between collagen fibres, fibroblasts, and small blood vessels. Irrespective of shape, MCs had a well-defined cell membrane with projections and folds. Mitochondria, Golgi vesicles, endoplasmic reticulum, centriole, and lipid bodies were occasionally seen in the MC cytoplasm. In resting MCs, granules were evenly distributed within the cell cytoplasm.

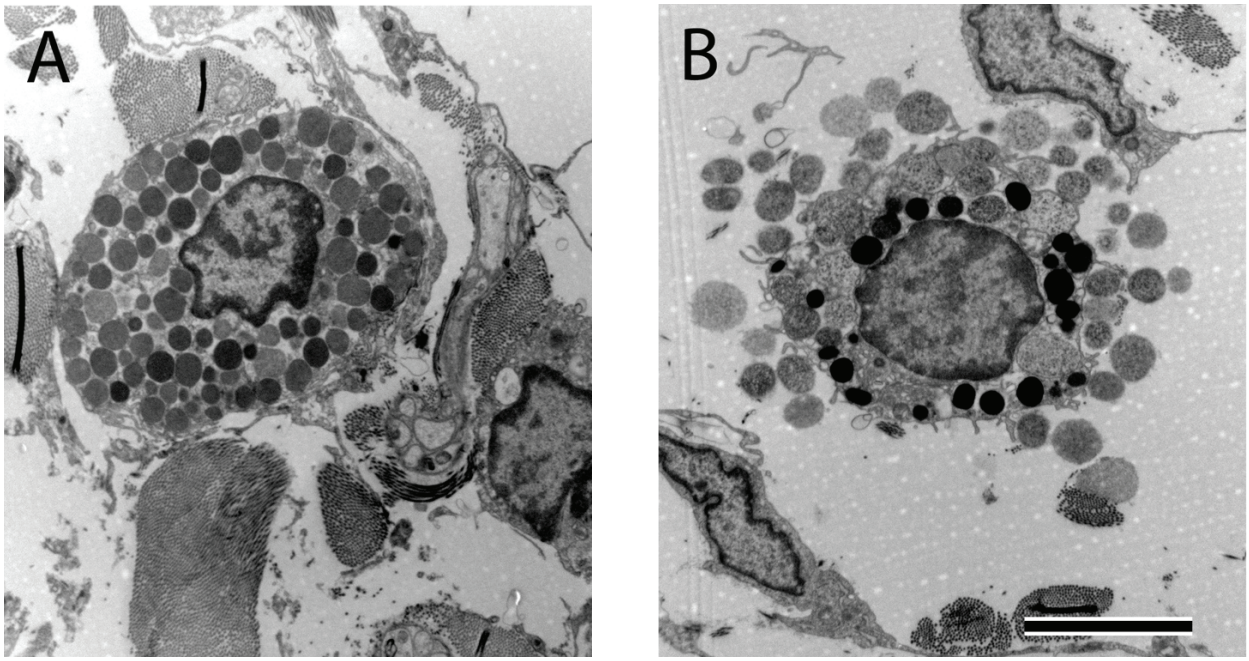

Figure 2. Representative electron micrographs of MCs in the liver. EM shows (A) resting (inactive) and (B) degranulating MCs. (A) In resting cell, well-defined granules of variable size and opacity are evenly distributed in the cell. The smallest granules represent recent "buds" released from the Golgi apparatus. The cell membrane is intact and has numerous distinct folds and projections. The unilobular nucleus exhibits clumps of partially condensed chromatin. (B) In degranulating cell, signs of membrane disruption and exocytosis are seen. Many altered electron-lucent granules are seen outside the cell. Abbreviations: EM, electron microscopy; MCs, mast cells. Scale bar = 10 μm

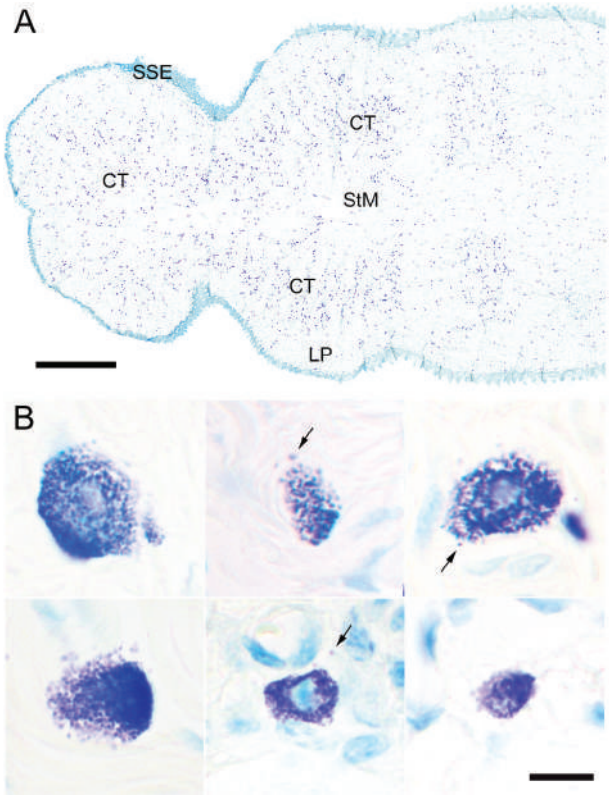

Figure 3. Representative photomicrographs show a TB-stained tongue. (A) Under LM, TB+MCs (CTMCs) are seen as small dark blue-violet flecks widely distributed across the dorsal surface. MCs are dispersed between the lamina propria (LP) and the deeper-lying core of striated muscle (StM). No MCs are seen in SSE (pale blue outline marking the tongue's surface). (B) At higher magnification, MCs of different sizes and shapes are seen in the connective tissue (CT), around small blood vessels, and between StM. MCs are characterized by the presence of numerous intracellular metachromatic granules (dark blue/violet). Some granules are seen in extracellular space near MCs (arrows). Abbreviations: CT, connective tissue; CTMCs, connective tissue MCs; LM, light microscopy; LP, lamina propria; MCs, mast cells; TB, Toluidine Blue; TB+, positive staining; SSE, stratified squamous epithelium; StM, striated muscle. Scale bar = 2 mm for A, and 4 μm for B

They exhibited variable size and shape. Most of the granules appeared spherical, but some were irregular. MCs had granules with different opacities. The majority of granules, however, were homogeneously opaque (that is, electron-dense and black). In degranulating MCs, granules were enlarged and somewhat translucent, with small electron-dense particles suspended inside them. These pale granules were sometimes clustered and found between intact electron-dense granules (Fig. 4).

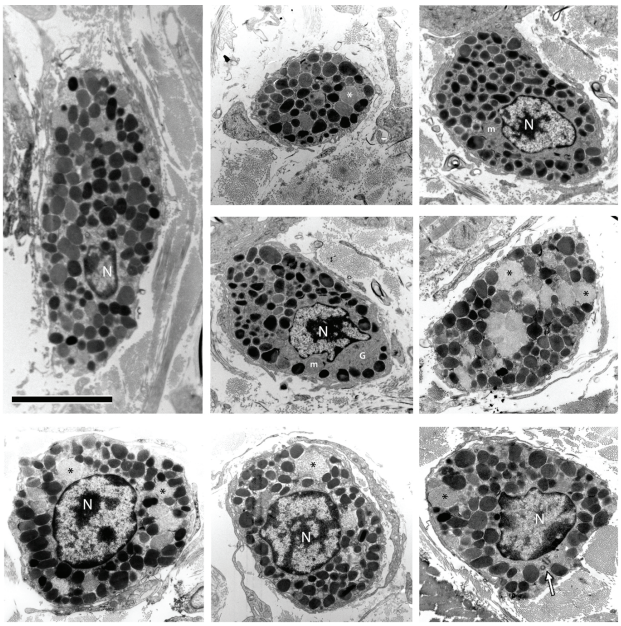

Figure 4. Representative electron micrographs of MCs in the tongue. EM shows that MCs are densely filled with intracellular granules that vary in size, shape, and opacity. Individual granules are internally homogeneous and mostly opaque. The nucleus (N) has a dense inner lining of chromatin of variable thickness along its envelope. Mitochondria (m), part of the centriole (arrow), and the Golgi apparatus (G) are seen in some MCs. Lipid bodies are undoubtedly present but difficult to distinguish from electron-dense granules. In degranulating MCs, enlarged translucent granules (*), sometimes clustered together, are seen between opaque electron-dense granules inside the cytoplasm. Abbreviations: EM, electron microscopy; G, Golgi apparatus; MCs, mast cells; m, mitochondria; N, nucleus. Scale bar = 5 μm

Characterization of MCs in liver

LM showed that MCs in the liver were not observed amongst hepatocytes of the parenchyma. In TB-stained sections, MCs were observed in the connective tissue around the portal vein, branches of the hepatic artery, and the bile duct (Fig. 5A). MCs of the portal triad exhibited a pale nucleus and cytoplasm packed with granules (Fig. 5B). MC degranulation was inferred by the presence of metachromatic granules scattered outside but in proximity to the cell body (Fig. 5B). MCs of the liver were variable in shape, being predominantly round or oval. The mean size of MCs in the liver was 50.4 ± 17.8 μm2, and significantly smaller than MCs of the tongue (*$p < 0.001$). MCs of the liver, like those of the tongue, were highly variable in size: CV = 35.3% but were less variable in size than MCs of the tongue (Fig. 7).

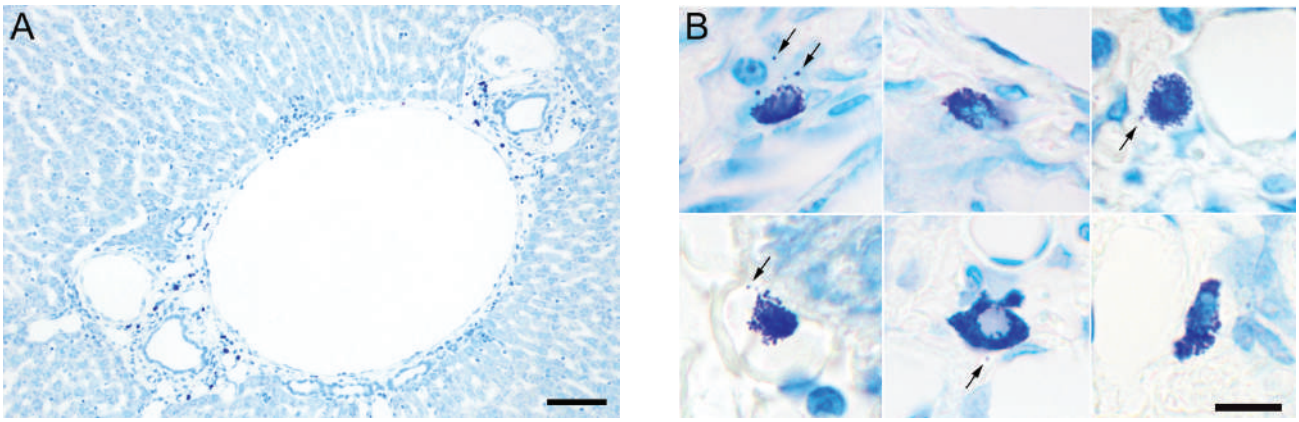

Figure 5. Representative photomicrographs show TB-stained liver tissue. (A) Under LM, TB+MCs (CTMCs) are seen as prominent, small dark blue dots distributed around the portal triad. No MCs are seen among hepatocytes (pale blue stained cells). (B) At higher magnification, round or oval MCs are seen in connective tissue around vessels. Metachromatic granules (dark blue/violet) are seen within the cytoplasm. Released granules (arrows) are seen in the extracellular space near secreting MCs. Pale blue nuclei are visible in some MCs. Abbreviations: CT, connective tissue; CTMCs, connective tissue MCs; LM, light microscopy; MCs, mast cells; TB, Toluidine Blue; TB+, positive staining. Scale bar = 100 μm for A, and 10 μm for B

EM showed round and oval MCs located between collagen fibres. Typically, they had well-defined cell membranes with projections and folds. Some MCs were tightly encircled by compact tissues so that membrane projections were not present. The somata of MCs were filled with granules of variable opacity and density (Fig. 6). Most MC granules in the liver were of varying shades of grey, indicating less electron-dense content (Fig. 6) than those of the tongue (Fig. 4). In addition, MCs of the liver exhibited only a few electron-dense black granules within the cytoplasm (Fig. 6). Most MCs exhibited features of secretory activity (Fig. 6).

The cytoplasm of degranulating cells had reduced granule density and contained granules that showed signs of swelling and alteration. They were either translucent with small electron-dense particles evenly distributed within the granule or seemingly empty, amorphous granules. These altered granules were often clustered inside the cytoplasm and juxtaposed with intact electron-dense black granules. Signs of MC secretory activity were often observed in either the form of single granules being released from the cell or granules already located outside the cell (Figs. 1 and 6, arrows). In the liver, some MCs showed profound degranulation, with most of their granules located extracellularly (Fig. 2B). Cell organelles were rarely seen, but lipid bodies were observed.

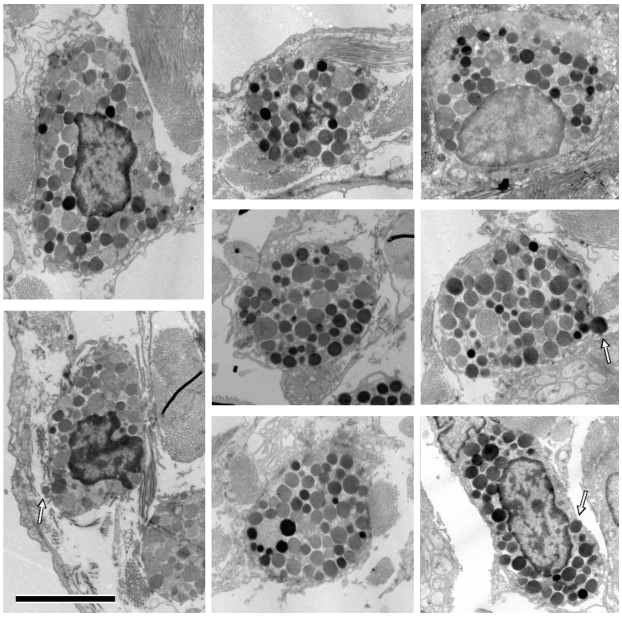

Figure 6. Representative electron micrographs of MCs in the liver. EM shows that MCs are located amongst collagen fibres. Granules of variable size and opacity, most of the medium density, are seen intracellularly. Unilobular nuclei have variable locations within MCs. In degranulating MCs, enlarged translucent granules, sometimes clustered together, are seen inside the cytoplasm. Single granules being released from the cell (arrows), or granules located outside the cell are evident. Abbreviations: EM, electron microscopy; MCs, mast cells. Scale bar = 5 μm

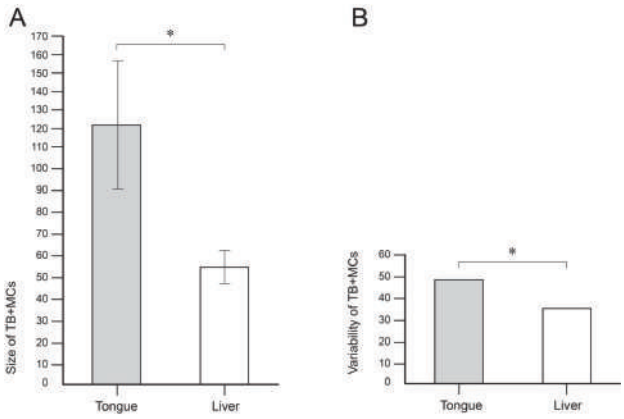

Figure 7. Size and variability of MCs. (A) This graph shows the *size difference* between MCs in the tongue (grey: 121.8 ± 58.6 μm2) and liver (white: 50.4 ± 17.8 μm2). On average, the liver's MCs are 2.4 times smaller than those in the tongue. (B) This graph shows *the variations* in the size of MCs in the tongue (grey: 48.1%) and liver (white: 35.3%). MCs in the liver vary in size by 1.4 times less than MCs in the tongue. Significance for A and B: *p < 0.001. Abbreviations: MCs, mast cells; TB, Toluidine Blue

Discussion

The study was conducted on tongue and liver tissues from rats housed under identical conditions to ensure that MC characteristics were consistent across all experimental animals. This study showed that CTMCs in the tongue and liver exhibited some phenotypic differences, such as cell distribution, size, secretory activity, and granule characteristics. Additionally, using metachromatic staining alone was inadequate for high-resolution MC analysis, and EM is the gold standard for detecting all MC populations and phenotypes.

TB staining was used to identify MCs of rats as it is the most frequently used metachromatic stain for MC detection [72,73]. TB is a basic hydrophilic thiazine metachromatic dye that distinctly stains MC granules. MC granules are composed of biologically active mediators that bind TB. Thus, TB staining detects both non-degranulated MC (compact, granular) and degranulated MC (lysis, externally located granules) [70,72,74-76].

TB-stained MCs were present in the tongue and liver tissues of rats, and they shared similar characteristics in both tissues, such as location near vascular vessels and the presence of intracellular granules, consistent with the findings of related studies [25,77,78]. However, light microscopic and quantitative analyses showed some differences between MCs of the tongue and liver, including cell distribution, secretory activity, size, and variability. In the tongue, MCs were evenly distributed across the dorsal surface and were located between muscles and connective tissues, with denser distribution around blood vessels and the lamina propria. In the liver, MCs were only observed in the connective tissue around the portal triad. These observations are consistent with the results of related studies [25,78]. MCs in the tongue and those in the liver also varied in shape and size. In the tongue, MCs were highly variable in shape (spindle, elongated and round/oval), whereas MCs in the liver were primarily round/oval in shape. These differences are consistent with published data showing that most elongated MCs were found in the interstitium, whereas most round MCs were found in proximity to blood vessels [73].

Quantitative analysis showed that MCs in the tongue and liver varied in size, possibly due to differences in secretory activity. MCs in the liver were 2.4 times smaller than those of the tongue. Although MCs in the tongue and liver were highly variable in size, those in the liver were 1.4 times less variable than those in the tongue. Unlike MCs in the tongue, many liver MCs exhibited a reduced density of intracellular granules that had an affinity with TB, and an abundance of extracellularly located metachromatic granules. These findings suggest higher secretory activity and exocytosis of TB-stained granules in liver MCs than in the tongue. These observations are consistent with previous research showing that the size, shape and function of MCs vary according to tissue residency and microenvironment [8,20,78,79]. Because TB staining does not detect granules inside MCs after degranulation, some fully degranulated MCs may not be detected [80-82]. This LM limitation in the resolution of MCs may lead to confusion regarding the numbers and size of all MC populations present in tissues.

EM showed that tongue and liver MCs exhibit unilobular nuclei containing clumps of partially condensed chromatin and heterogeneous

intracellular granules. These MCs contain numerous projections and folds on cell surfaces. Ultrastructural investigation revealed differences in granule opacity and density between the MCs of the tongue and liver. In the tongue, densely opaque granules dominated. However, in the liver, the majority of granules were of varying shades of grey and distinctly less electron-dense. Enlarged and altered translucent granules, often clustered together, were observed in the cytoplasm of MCs in both tongue and liver tissues. However, the secretory activity of liver MCs was inferred by the presence of single extracellular granules away from the MC somata or by profound degranulation, characterized by impaired cell membrane integrity and extracellular granules.

MC populations can undergo significant changes in numbers, phenotype, content of stored mediators and function due to the local microenvironment and immunologic and/or inflammatory responses [83]. Intracellular factors (such as strain, MCs source) and extracellular factors (such as tissue type and parasite presence) influenced the granule phenotype in mice [84,85]. Additionally, electromagnetic radiation has been shown to alter MC numbers, morphology, and/or granule mediator content in rats and humans [86].

The finding that CTMCs in the tongue and liver exhibited some phenotypic differences may be due to differences in anatomical location and functional demands. Furthermore, different local stimuli can trigger MCs, and their exocytosis may initiate different cascades of reactions to specific triggers. Moreover, we speculate that MCs of the liver and tongue have different granule contents. EM revealed a dominance of homogeneously opaque granules with electron-dense content (black granules) in the tongue. In contrast, in the liver, the majority of granules were of varying shades of grey and held less electron-dense content, with only a few electron-dense black granules seen within the cytoplasm. These findings are consistent with previous reports. MC populations in the rat liver have some characteristics of immature CTMC and display a mucosal cell-like phenotype [25,87-89]. Subepidermal CTMCs exhibit some common features with MMCs, such as low histamine content and poor functional responsiveness to a trigger [90].

The dominance of grey granules may also be explained by a higher rate of exocytosis of electron-dense black granules in the liver. Electron-dense granules are composed of proteoglycan matrix, heparin in particular [83], which have an affinity with TB. Thus, the presence of fewer electron-dense black granules against the dominance of grey granules in the liver cytoplasm is consistent with the finding that liver TB-stained MCs are significantly smaller than those of the tongue. The unique role of liver MCs in the function of the gastrointestinal system is of interest [11,89,91-95]. Liver MCs are located around the portal area, including the portal triad and portal tracts. The portal triad comprises the portal vein, which brings nutrient-rich blood from the intestine via the mesentery, branches of the hepatic artery and the bile duct. Liver MCs can interact with and influence hepatocytes, cholangiocytes, portal fibroblasts, Kupffer cells, vascular endothelial cells, pit cells, hepatic stellate cells, and sympathetic and parasympathetic nerve fibres. Therefore, MCs play a significant role in liver functions and hepatic disorders, such as liver fibrosis, hepatitis, steatosis, liver cancers, alcoholic liver injury, allograft rejection, and liver aging [91-93,96,97].

Recent evidence suggests that the hepatobiliary system is involved in IgE-mediated gastrointestinal hypersensitivity, gut mucosal homeostasis, and inflammatory bowel diseases, including ulcerative colitis and Crohn's disease [95]. The interaction between gut inflammation and MCs has been investigated, and it's thought that MCs (via their release of histamine) may be a key player in IgE-mediated intestinal damage [94]. Liver MCs can also contribute to inflammatory mediators, such as histamine and TNFα, previously detected in the bile of rats [89].

MCs have the potential to interact with and affect other cells, providing a pathway for participation in many pathological conditions. Therefore, MCs are a highly attractive therapeutic target for treatment. Reducing MC numbers, modulating MC activation and phenotype, and altering secreted MC mediators and their downstream effects have been suggested in MC-directed immunotherapy in clinics. The challenge is to resolve the broad heterogeneity and high plasticity of MCs, which depends

on their localization and milieu, to devise accurate markers for granule content and key signalling pathways involved in selective degranulation, and to identify precise pharmacological treatments for each pathophysiological condition (stage) of disease [11,58,98,99]. A better understanding of CTMC phenotyping will contribute to the development of successful strategies for preventing and/or treating diseases, including anaphylaxis, mastocytosis, and certain leukemias and autoimmune disorders.

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