Light and Electron Microscopic Studies on Tissue Mast Cells in the Tongue

By

TAKUO SASAKI

Department of Anatomy, School of Medicine, Keio University, Shinjuku, Tokyo 160, Japan

—Received for Publication, January 22, 1980—

Key Words: Mast cells, Tongue, Ageing, Cytochrome c

Summary. The present paper describes the distribution, development and histochemical characteristics of the tissue mast cells in mouse tongue. The following results were obtained. 1) In the tongue of newborn mice, only a small number of mast cells was apparent while in that of adult mice, a large number of mast cells could be found in loose connective tissue among the lingual muscle bundles. 2) All tissue mast cells found in the tongue of newborn mice, exhibited immature cell types which did not stain by the cytochrome c-leucopatent blue method, or indicate metachromasia with azure stains. 3) By electron microscopy, the immature mast cells were found to lack microvilli and contained a well-developed granular endoplasmic reticulum and a small number of immature granules in which small progranules and vacuoles could be seen.

Introduction

The distribution of tissue mast cells in the digestive tract of the rat has been studied previously by Mota et al.\(^\text{20}\) using toluidine blue. Apart from toluidine blue staining, a variety of indicators has been introduced for mast cells since the studies of Ehrlich\(^\text{7}\). Mitsui et al.\(^\text{14}\) recently devised a method called the cytochrome c adjective method for the demonstration of mucopolysaccharide, and succeeded in detecting sulphated mucopolysaccharides such as chondroitin sulphate or heparin in tissue sections.

In the present study, the tongue and masseter muscle of mice were mainly used to determine the distribution of mast cells in these organs and to elucidate the ageing process of mast cells by the cytochrome c adjective method, the Astra blue-safranin O method, and electron microscopy.

Materials and Methods

The materials used were mainly tongue tissues from 146 albino mice of the ICR strain. The number, age, and sex of the mice are shown in Table 1. The masseter and tibialis anterior muscles of the same mice, and tongue tissues from 12 humans of both sexes were also tested.

1. The tissues were dissected out and fixed for 24 hr in 10% formalin containing 1% cetylpyridinium chloride. The tissues were then rinsed in distilled water, dehydrated in a graded alcohol series, and embedded in paraffin.
Table 1. Age and number of the mice tested

<table>
<thead>
<tr>
<th>Age in weeks</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
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<tbody>
<tr>
<td>0</td>
<td>10</td>
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<td></td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
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<td>16</td>
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<td>1</td>
</tr>
<tr>
<td>56</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>58</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>59</td>
<td>146</td>
</tr>
</tbody>
</table>

? (sex was not checked)

2. Astra blue-safranin O method.

The formula for the stain is as follows:

- 0.5% aqueous solution of Astra blue ........................................... 50 ml
- 0.5% aqueous solution of safranin O ......................................... 1 ml
- Glacial acetic acid ................................................................. 20 ml
- Distilled water ................................................................. 29 ml

The final pH of the mixture is 2.3.

Deparaffinized sections were treated as follows:

1) Stain for 15 min at room temperature in the above mixture of stains.
2) Rinse in distilled water.
3) Counterstain with hematoxylin for 10 min.
4) Dehydrate in graded alcohol, clear in xylol and mount in Permount or balsam.

In this staining procedure, blue stained cells are thought to be young or immature, and red stained cells mature.

3. Cytochrome c-leucopatent blue method (cytochrome c adjective method).

Deparaffinized sections were treated as follows:

1) Immerse in a 0.1% aqueous solution of cytochrome c for 2 min. Cytochrome c isolated from horse heart was obtained from Sigma Co., U.S.A.
2) Wash in distilled water.
3) Stain with leucopatent blue-H$_2$O$_2$ solution for 1-2 min.
4) Wash in distilled water and counterstain with Kernechtrot solution for 2 min.
5) Dehydrate in graded alcohol, clear in xylol and mount in synthetic resin or balsam.

With this staining procedure, mast cell granules are usually stained blue and nuclei red. The intensity of the blue color parallels the maturity of the mast cell granules.

4. The P.A.S. reaction was performed according to the technique of McManus (Pearse, 1968). A dilute aqueous solution (0.02%) of azures (A and B) was used to observe the metachromasia of the mast cell granules.

5. Electron microscopy.

Small pieces of mouse tongue, 1×1×1 mm, were fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4) for 1 hr at 4°C, washed in the same phosphate buffer for 1 hr, and further fixed in 1.0% phosphate-buffered osmium tetroxide (pH 7.4) for 1 hr. They were then washed in distilled water for 10 min, dehydrated in graded alcohol, and finally embedded in polyester or Quetol 812 after the method of
Kushida. This sections were cut with glass knives on a Porter-Blum MTII microtome, stained with uranyl acetate and lead citrate (Raynolds), and examined under an HU-11B or HU-12 electron microscope.

Furthermore, in order to observe the cytochrome c-benzidine-H₂O₂ reaction of the mast cell granules at the electron microscopic level, thin sections on a specimen grid were treated with the above-mentioned 0.1% cytochrome c solution for 15 min, washed in formate buffer (pH 3.0) for 30 min, and then stained with benzidine-H₂O₂ solution for 15 min. The benzidine-H₂O₂ solution consisted of 100 ml of a saturated aqueous solution of benzidine and 0.5 ml of 3% H₂O₂. These stained sections were then washed in distilled water, dried, and viewed under an electron microscope without additional staining with uranyl acetate or lead citrate.

Results

Many mast cells were found in the tongue tissue of the mouse. The number of mast cells was relatively small in the lamina propria, but large in the tela submucosa or loose connective tissue of the striated muscle. Mast cells could not be found within the stratified squamous epithelium, although they occasionally accumulated in the deep intermuscular connective tissue (Fig. 1). Mast cells were not recognized to aggregate around blood vessels or nerve bundles, in contrast to the data of Olsson who described clusters of mast cells in the vicinity of the endoneural blood vessels of rat sciatic nerve.

The mast cells were round, ellipsoid or spindly in shape. The nucleus was usually situated centrally in the cells, but was sometimes eccentric. The size of the cells was variable, ranging from 8.0 to 12.0 μm in diameter. The mast cell granules were stained deep blue by the cytochrome c-leucopatent blue method, and the granules appeared densely (Fig. 3) or sometimes coarsely arranged in the cytoplasm. The size of the granules was variable, ranging from 0.3 to 1.3 μm in diameter.

In electron micrographs (Fig. 4), mast cells stained by the cytochrome c-benzidine method revealed small electron dense deposits or patches which were diffusely arranged in the internal structure of the granules.

As regards location in the mouse tongue, the body of the tongue contained more numerous mast cells than the apex of the tongue, and the root of the tongue contained the smallest number of mast cells.

In the masseter muscle and anterior tibial muscle, mast cells were found to be very few in number. Especially in the masseter muscle, mast cells were found only in the fibrous sheath (epimysium) surrounding the whole muscle (Fig. 2). In thin cross sections of the anterior tibial muscle, only one or two mast cells were recognized either in the internal perimysium or in the epimysium. That mast cells are quite few in number in these striated muscles, represents a marked difference from the tongue which is mainly composed of striated muscles.

In the human tongue, relatively small numbers of mast cells could be recognized in the lamina propria and tela submucosa; they were rarely found in the stratified squamous epithelium and in the germinal center of the lingual tonsil. Mast cells were fewer in numbers in the intermuscular connective tissue as compared to the mouse tongue.

The mast cells in the tongue of newborn mice differed from those of adult mice. The mast cells of mice on the first day after birth, were very few in number.
They were round in shape, contained a small number of immature granules which remained unstained by the cytochrome c-leucopatent blue method, were stained very weakly by the P.A.S. reaction, stained blue with the Astra blue-safranin O method, and indicated no metachromasia with azure stains (Table 2).

By electron microscopy, the above-mentioned immature mast cells were found to lack typical microvilli around the cell body, but they contained a large amount of free ribosomes, granular endoplasmic reticulum and mitochondria, as well as a variety of immature granules, some of which were small or abnormally large in size, and aggregated progranules or vacuoles (Fig. 5). The presence of a large number of dense homogeneous granules in mast cells is indicative of cellular maturity (Fig. 3).

With age, the mast cells increased in number, and their cellular shape and arrangement in the tongue tissue gradually changed as shown in Table 3. Further, the immature granules changed as regards reactivity in the cytochrome c-leucopatent blue method, Astra blue-safranin O method and P.A.S. reaction (Table 2). In other words, the mast cell granules became the mature type at 3 weeks of age, and the mast cell population in the tongue attained a maximum after 35 weeks of age.

In the present study, no sexual differences were recognized in the distribution of mast cells or in the characters of the granules of mast cells from the mouse tongue throughout all stages examined after birth.

**Discussion**

Combs (1966) described a detailed process for granule formation in tissue mast cells of the tongue and lymph nodes involving elaborate participation of the Golgi apparatus and granular endoplasmic reticulum. The animals used by him were 35 mm crown-rump embryos and newborn rats.

Mitsui et al. reported the presence of tissue mast cells and eosinophil leukocytes in mouse tongue, and examined the structures of these cells at the electron microscopic level.

The cytochrome c-leucopatent blue method was devised by Mitsui et al. to demonstrate acid mucopolysaccharide based on the experimental finding that

<table>
<thead>
<tr>
<th>Cytochrome c-leucopatent blue method</th>
<th>Soon after birth</th>
<th>1 week after birth</th>
<th>3 weeks after birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>blue cell</td>
<td>negative*</td>
<td>positive</td>
<td>strongly positive</td>
</tr>
<tr>
<td>red cell</td>
<td></td>
<td>positive</td>
<td>++</td>
</tr>
<tr>
<td>mixing of blue and red cells</td>
<td></td>
<td>positive</td>
<td>+</td>
</tr>
<tr>
<td>Azure A and B</td>
<td>orthochromasia</td>
<td>metachromasia</td>
<td>metachromasia</td>
</tr>
<tr>
<td>P.A.S. reaction</td>
<td>weak</td>
<td>strong</td>
<td>strong</td>
</tr>
</tbody>
</table>

* (becomes positive on the 6th day after birth), – (absent), + (few in number), ++ (many)
Table 3. Number and shape of mast cells in the tongue tissue of mice.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of mast cell</th>
<th>Shape of mast cell</th>
<th>Arrangement of mast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Round</td>
<td>Spindly</td>
</tr>
<tr>
<td>24 hr after birth</td>
<td>±</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>1 week</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>2 weeks</td>
<td>++</td>
<td>++</td>
<td>±</td>
</tr>
<tr>
<td>3 &quot;</td>
<td>++</td>
<td>+ ±</td>
<td>+</td>
</tr>
<tr>
<td>5 &quot;</td>
<td>++</td>
<td>+ ±</td>
<td>+ ±</td>
</tr>
<tr>
<td>12 &quot;</td>
<td>++</td>
<td>+</td>
<td>+ ±</td>
</tr>
<tr>
<td>35 &quot;</td>
<td>+ ++</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>56 &quot;</td>
<td>+ ++</td>
<td>+</td>
<td>+ +</td>
</tr>
</tbody>
</table>

- (absent), ± (quite few in number), + (few), + ± (moderate), ++ (many), +++ (quite many)

cytochrome c intensely oxidized leucopatent blue-H$_2$O$_2$ solution and altered it to the original deep blue. It was clarified by Mitsui et al.\(^1\)\(^3\) that this method provided a technique for the histochemical localization of sulphated mucopolysaccharides such as heparin based on linkage formation between sulphate groups of heparin and amino groups of cytochrome c. The method is useful for the detection of mast cells which are defined as connective tissue cells containing characteristic granules. It was found by Chiu and Lagunoff\(^3\) that an N-sulphated mucopolysaccharide (heparin) was present in the mast cells of all animal species examined except the carp.

In the present study, the granules in the mast cells of the mouse tongue showed remarkable differences between newborn and adult mice. That is to say, the mast cell granules of newborn mice did not stain by the cytochrome c-leucopatent blue method or indicate metachromasia with azure stains, but stained blue by the Astra blue-safranin O method. These findings indicate that the tongue mast cells from newborn mice contained only immature granules which probably possessed fewer sulphate groups in a free state than mature granules.

In electron micrographs, the immature granules of mast cells exhibited certain differences from mature ones. In immature mast cells, the plasma membrane was not specialized and typical microvilli were not developed in the periphery. Further, the granules contained aggregated progranules and vacuoles as pointed out previously by Combs\(^4\) and Fujita\(^8\). In the mouse tongue, large numbers of mast cells were found in the body, smaller numbers were present in the apex, and the smallest number was seen in the root. It is of interest to note that the mast cells of the mouse tongue generally existed in loose connective tissue, and that neutrophil leukocytes could not be detected in the fibrous connective tissue of the mouse tongue although a very small number of eosinophil leukocytes might be detected together with the tissue mast cells.

Several reports\(^5\) on the skin and heart, including human materials, have described a decrease in mast cells with advancing age. Wada\(^6\) emphasized that mast cells decreased in number in the palmar skin of the mouse with age. Simpson and Hayashi\(^4\) found mast cells to be more
numerous in the dermis of old male mice of the C57 Black strain and less numerous in old female mice compared to young individuals of the same sex. Marx et al.\textsuperscript{11} found an increase with age in the mast cells of rat liver, lung, kidney, and thymus. It is said that at the end of the embryonal life of the rat, a considerable increase occurs in the number of mast cells in the skin, in all the loose connective tissue and organ capsules\textsuperscript{7}. Webb\textsuperscript{25} stated that a maximum is attained from the 10th to 13th day after birth, whereupon a short decrease in number occurs in rats. Subsequently, Webb\textsuperscript{26} found a constant concentration of mast cells in rats. On the other hand, in the myocardium of the rat, Constantinides and Rutherdale\textsuperscript{5} and Wexler\textsuperscript{27} detected a decrease in number of mast cells with increasing age.

The present study clarified that the mast cells in the tongue were very few in number in newborn mice, that the number of mast cells increased with age attaining a maximum at about 35 weeks of age, and that the number never decreased even in old mice (60 weeks of age). Also, no sexual differences were detected in the number of mast cells in the mouse tongue.

Based on all the above data, it can be said that the ageing process of a population of mast cells in animals may vary according to the species, sex and region of the body. Further, it is worthy of note that the number of basophil leukocytes in the peripheral blood is broadly in inverse proportion to that of tissue mast cells in the tongue, as already mentioned by Mitsui et al\textsuperscript{18}. For example, animals which possess very few basophil leukocytes in the blood, display numerous tissue mast cells in the tongue, whereas those which possess relatively large numbers of basophil leukocytes in the blood, have very few or no tissue mast cells in the tongue (Table 4).

The author wishes to express his sincere thanks to Professor T. Mitsui for his kind help and criticism during the course of the present study.

References

8) Fujita, T.: Mast cells. In basic derma-

Table 4. Relationship of appearances between basophil leukocytes in the blood and tissue mast cells in the tongue.\textsuperscript{16}

<table>
<thead>
<tr>
<th>Animal</th>
<th>% of basophil leukocytes in the blood</th>
<th>Number of mast cells in the tongue</th>
</tr>
</thead>
<tbody>
<tr>
<td>man</td>
<td>0.5</td>
<td>few</td>
</tr>
<tr>
<td>hamster</td>
<td>0 (Ref. 28)</td>
<td>many</td>
</tr>
<tr>
<td>rat</td>
<td>0.1</td>
<td>many</td>
</tr>
<tr>
<td>mouse</td>
<td>0.07</td>
<td>many (Ref. 21)</td>
</tr>
<tr>
<td>dog</td>
<td>0.1 (Ref. 23)</td>
<td>absent</td>
</tr>
<tr>
<td>guinea pig</td>
<td>0.36</td>
<td>absent</td>
</tr>
<tr>
<td>rabbit</td>
<td>3.9</td>
<td>absent</td>
</tr>
<tr>
<td>tortoise</td>
<td>33.0 (Ref. 17)</td>
<td>absent</td>
</tr>
</tbody>
</table>
Tissue Mast Cells in the Tongue


Explanation of Figures

Plate I

Fig. 1. Tongue tissue of an adult mouse. Note that mast cells lie deeply in the loose connective tissue between the lingual muscle bundles. The cytochrome c-leucopatent blue method was employed. E: stratified squamous epithelium. ×200

Fig. 2. Epimysium of the masseter muscle of an adult mouse. Several spindly-shaped mast cells are recognized in the loose connective tissue. The cytochrome c-leucopatent blue method was employed. ×260
Plate 1

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Plate II

Fig. 3. Electron micrograph of a mature mast cell from the tongue of an adult mouse. The large number of dense homogeneous granules and numerous microvilli are indicative of cellular maturity. F: fibroblast, M: muscle fiber. ×13,500
Plate III

Fig. 4. Electron micrograph of a mature mast cell from the tongue of an adult mouse. This section was treated with both cytochrome c and benzidine-\( \text{H}_2\text{O}_2 \) solutions to stain for acid mucopolysaccharide. The reaction products are apparent as dense materials localized in the granules. \( \times 13,500 \)
Plate III

T. Sasaki
Plate IV

Fig. 5. Electron micrograph of an immature mast cell from the tongue of a mouse on the first day after birth. The cytochrome c adjective method was not employed. The prominent nucleus exhibits a small indentation, and the relatively sparse cytoplasm contains mitochondria (m), rough-surfaced (or granular) endoplasmic reticulum and immature granules (arrows). The plasma membrane is not specialized and typical long microvilli are not recognized. ×22,400