A Histochemical and Immunohistochemical Study of Mucous Cyst—With Special Reference to Mucoid Substance and Characteristics of Inflammation—

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Abstract
Mucous cysts are relatively common lesions in the oromaxillofacial region. A mucous cyst is composed of a mucoid substance and foamy mononuclear macrophages (mucinophages), with inflammatory cell infiltration and the overgrowth of blood vessels and fibrous tissue forming a mucoid granuloma. Although the mucoid substance characteristics and the inflammatory characteristics are thought to be important factors, details of the histopathogenesis in relation to the mucinophages remain unclear. Therefore, the present study was conducted to investigate the pathogenesis of mucous cysts with reference to the histochemical and immunohistochemical characteristics of thirteen cases and a review of the literature. Lectin histochemistry and immunohistochemistry showed that mucinophages gave a strong reaction to concanavalin A and lysozyme, and a weak or negative reaction to wheat germ agglutinin. In contrast, the mucoid substance, salivary ducts and acinar cells were strongly to weakly reactive to wheat germ agglutinin and weakly or negatively reactive to concanavalin A. The reactivity to the other lectins and salivary markers in mucous cysts was similar with those in the salivary gland tissues. These results suggested that mucoid degeneration resulted from phagocytosis by the mucinophages and that the mucoid substance in mucous cysts was derived from the saliva. The cell rate of CD68-positive mucinophages was higher in mucous cysts than in control tissues. The cell rates of panT-positive T-cells and panB-positive B-cells were lower in mucous cysts than in control tissues. In addition, the count of mast cells showing metachromasia with toluidine blue at pH 2.5 in mucous cysts was equal to that in control tissues. For immunoglobulin–producing plasma cells, the IgE positivity rate was higher in mucous cysts than in control tissues, whereas the IgA positivity rate was lower in mucous cysts than in control tissues. These results suggested that the accumulation of mucinophages, which had phagocytic and antigen-presenting abilities, was mainly associated with the pathogenesis of the mucous cyst, in addition to a local T-cell–mediated cellular immune mechanism or to an acute allergic immune response by IgE–producing cells and mast cells.

Keywords: mucous cyst, mucoid substance, mucinophage, inflammatory characteristics

Introduction
Mucous cyst, a non–neoplastic condition affecting the salivary glands, consists of mucoid granulation tissue with cyst formation. The lesion is histopathologically subclassified into two subtypes: the extravasation type and the retention type. The extravasation type, which is more common, lacks an inner luminal epithelial lining; conversely, the retention type has an epithelial lining (1).

Although the pathogenesis is associated with abnormal salivary secretion and chronic inflammation (1), the details are still unclear. Therefore, to clarify the pathogenesis of the mucous cyst and to understand its behavior, it is important to study the
morphological and inflammatory characteristics.

Histochemical and immunohistochemical studies have been performed using lectins (2, 3), inflammatory markers, and immunological factors (4–8) to characterize the pathogenesis and morphological nature of various lesions.

The purpose of the present study was to investigate, using histochemical and immunohistochemical methodology, the morphological characteristics of mucous cysts with special attention to mucoid substances and inflammatory changes.

**Materials and Methods**

Thirteen cases of mucous cysts, of which 7 were the extravasation type and 6 were the retention type, diagnosed at the Department of Pathology, Nihon University School of Dentistry at Matsudo during 1971–2003, were used for the present study. The patients, seven males and six females, ranged in age from 6 to 63 years (average 26.8 years). The most commonly affected site was the lower lip (10 cases); the sites of the remaining three cases were the buccal mucosa (1 case), tongue (1 case), and a nonspecific site. Consideration was given to patient privacy, diagnosis, and the management and prognosis of the lesions (ethics committee recognition number EC03–038). The resected specimens were immediately fixed in 10% neutral formalin, and then made into paraffin–embedded blocks, which were sectioned at a thickness of 4 μm for histopathological and immunohistochemical observation. The sections were stained with hematoxylin–eosin (HE), periodic acid–Schiff reaction (PAS), and toluidine blue, pH 2.5 (TB–2.5).

The labeled streptavidin–biotin method in a commercial kit (DakoCytomation, Glostrup, Denmark) was used for lectin histochemistry and immunohistochemistry. The sections were deparaffinized, dehydrated, and treated with 0.1% trypsin for lectin histochemistry to enhance any specific tissue staining. After a thorough washing, the sections were covered with seven types of lectin, each with a specific sugar content, as follows: concanavalin A (Con A; D-mannose and D-glucose), wheat germ agglutinin (WGA; N-acetyl-D-galactosamine and N-acetyl neuraminic acid), *Dolichos biflorus* agglutinin (DBA; N-acetyl-D-glucosamine), soybean agglutinin (SBA; N-acetyl-D-galactosamine), *Ricinus communis* agglutinin I (RCA-I; D-galactose), peanut agglutinin (PNA; D-galactose and N-acetyl-D-galactosamine), and *Ulex europaeus* agglutinin I (UEA-I; L-fucose) (Vector Laboratories, Burlingame, CA). The concentration of each of these lectins was 25 μg/ml and the incubation time at room temperature was 60 min.

For the immunohistochemical study, the sections were deparaffinized and dehydrated. The antibodies used were anti-lysozyme (LZ) (polyclonal, DakoCytomation), anti-amylase (AM) (polyclonal, Sigma), anti-lactoferrin (LF) (polyclonal, DakoCytomation), anti–secretory component (SC) (polyclonal, DakoCytomation), anti–CD68 (CD68) (monoclonal, DakoCytomation), anti–HLA–DR (HLA–DR) (monoclonal, DakoCytomation), anti–CD20 (panB) (monoclonal, DakoCytomation), anti–CD45RO (panT) (monoclonal, DakoCytomation), anti–IgG (IgG) (polyclonal, DakoCytomation), anti–IgE (IgE) (monoclonal, DakoCytomation), anti–IgA (IgA) (polyclonal, DakoCytomation), and anti–IgM (IgM) (polyclonal, DakoCytomation). All of the antibodies were incubated with the tissue sections for 60 min at room temperature. Peroxidase activity was visualized using diaminobenzidine.

Positive control tests for all antibodies were performed on salivary gland tissue showing mild inflammatory change and lymph node tissue. For negative control testing, the primary antibodies were omitted from the procedures. Finally, the immunohistochemical sections were counterstained with Mayer’s hematoxylin.

The average cell positivity rates (percentages) for CD68, panB, panT, IgG, IgE, IgA, IgM, and metachromasia with TB–2.5 were determined for each of the 13 cases at ×600 original magnification in three areas of the lesion and in the control tissues.

**Results**

**Histopathology**

The lesion was characterized by an amorphous
Fig. 1. Mucous cyst formation encapsulated with fibrous tissue and adjacent to the salivary gland. (HE, original magnification ×10)

Fig. 2. High power view of Fig. 1. Mucous cyst consists of amorphous mucoid substance, mucinophage with foamy cytoplasm, over-growth of vessels and fibrous tissue. (HE, original magnification ×200)

Fig. 3. Retension type of mucous cyst reveals epithelial lining. (HE, original magnification ×50)

Fig. 4. PAS-positivity shows in mucoid substance and existing duct cells. (original magnification ×200)

Fig. 5. Mast cells show metachromasia with TB-2.5 (arrows) (original magnification ×150)
mucoid substance, mucinophage accumulation, lymphocyte or neutrophil infiltration, overgrowth of blood vessels, and fibroblasts and fibrous connective tissue; these components formed a cystic mass, usually adjacent to the salivary gland (Figs. 1, 2). An epithelial lining was observed in the retention-type mucous cysts (Fig. 3). The mucoid substance and mucinophages in the mucous cyst, and the acinar and duct cells in the salivary gland, were positive for PAS staining (Fig. 4) [DDI] but negative for staining with toluidine TB-2.5. In addition, some of the inflammatory cells showed metachromasia with TB-2.5 stain (Fig. 5).

**Lectin histochemistry**

The mucinophages showed strong positive reactivity for Con A (Fig. 6), weak reactivity for WGA, RCA-I, and PNA, and negative to weak reactivity for DBA, SBA, and UEA-I. The mucoid substance showed strong to weak reactivity for WGA (Fig. 7), weak reactivity for RCA-I, weak to negative reactivity for PNA, weak to negative reactivity for Con A, and negative reactivity for DBA, SBA, and UEA-I. The salivary acinar cells adjacent to the lesion exhibited strong to weak reactivity for WGA, weak to negative reactivity for RCA-I and PNA, and negative reactivity for Con A, DBA, SBA, and UEA-I. The duct cells adjacent to the lesion showed strong to weak reactivity for WGA, weak to negative reactivity for RCA-I, weak to negative reactivity for Con A and PNA, and negative reactivity for DBA, SBA, and UEA-I. There were no differences in lectin histochemical characteristics between the extravasation type and the retention type of cysts. The results are summarized in Table 1.

**Immunohistochemistry of salivary secretion marker**

The mucinophages showed strong positive reactivity for LZ (Fig. 8), weak reactivity for SC, weak to negative reactivity for LF, and negative reactivity for AM. The mucoid substance showed weak reactivity for LZ and SC, weak to negative reactivity for AM, and weak to negative reactivity for LF. The acinar cells showed weak reactivity for LZ and SC, and weak to negative reactivity for AM and LF. The duct cells showed weak reactivity for SC, weak to negative reactivity for LZ and LF, and negative reactivity for AM. The results are shown in Table 2. There were no differences in immunohisto-

### Table 1. Results of lectin histochemistry

<table>
<thead>
<tr>
<th>Mucinophage</th>
<th>Mucoid substance</th>
<th>Acinar cell</th>
<th>Duct cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con A</td>
<td>++</td>
<td>-/+</td>
<td>-</td>
</tr>
<tr>
<td>WGA</td>
<td>+</td>
<td>++/+</td>
<td>++/+</td>
</tr>
<tr>
<td>DBA</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SBA</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RCA-I</td>
<td>+</td>
<td>+</td>
<td>-/+</td>
</tr>
<tr>
<td>PNA</td>
<td>+</td>
<td>+/-</td>
<td>-/+</td>
</tr>
<tr>
<td>UEA-I</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

++: strong reactivity  +: weak reactivity  -: negative

### Table 2. Results of immunohistochemistry using salivary secretion markers

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Mucinophage</th>
<th>Mucoid substance</th>
<th>Acinar cell</th>
<th>Duct cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>LZ</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-/+</td>
</tr>
<tr>
<td>AM</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>LF</td>
<td>-/+</td>
<td>-/+</td>
<td>+/-</td>
<td>-/+</td>
</tr>
<tr>
<td>SC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

++: strong reactivity  +: weak reactivity  -: negative
Fig. 6. Positive reactivity for Con A in the mucinophages is stronger than that in the mucoid substance. (original magnification ×200)

Fig. 7. Positive reactivity for WGA in the mucoid substance is stronger than in the mucinophages. (original magnification ×100)

Fig. 8. Positive immunoreactivity for LZ is observed in the mucinophages and mucoid substance. (original magnification ×200)

Fig. 9. Positive immunoreactivity is for CD68 (a) and HLA-DR (b) are indicated in the mucinophages. (original magnification ×150)

Fig. 10. PanT-positive lymphocytes (arrows) (original magnification ×100)

Fig. 11. IgE-positive plasma cells (arrowheads). Membranously positive immunoreactivity in the mast cells. (arrows) (original magnification ×100)
chemical characteristics between the extravasation type and the retention type of cysts.

**Distribution of inflammatory cells**

The mucinophages showed strong positive reactivity for CD68 and HLA-DR (Fig. 9a, b). Both panT-positive (Fig. 10) and panB-positive lymphocytes were present around the area occupied by mucinophages. The plasma cells were also reactive for IgG, IgE (Fig. 11), IgA, or IgM. Membranous positive immunoreactivity for IgE was observed in some of the inflammatory cells (Fig. 11).

The average proportion of cells positive for CD68 was 74.3% in mucous cysts and 28.3% in control tissues. The panT positivity rate was 16.3% in mucous cysts and 54.9% in control tissues. The proportion of mast cells showing metachromasia with TB-2.5 was 6.8% in mucous cysts and 6.9% in control tissues. The panB positivity rate was 2.6% in mucous cysts and 9.9% in control tissues. These results are summarized in Table 3.

Table 4 shows a comparison of the proportions of immunoglobulin-producing plasma cells in the mucous cysts. The IgG positivity rate was 36.8% in mucous cysts and 34.6% in control tissues. The IgE positivity rate was 25.5% in mucous cysts and 4.9% in control tissues. The IgA positivity rate was 22.6% in mucous cysts and 49.4% in control tissues. The IgM positivity rate was 15.1% in mucous cysts and 11.1% in control tissues. There were no differences in the proportions of inflammatory cells between the extravasation type and the retention type of cysts.

**Discussion**

Mucous cysts are thought to be caused by secretion disorders following injury, infection, or trauma-related complications (1). In addition, using an experimental model of mucous cysts, Ichikawa (9) demonstrated that the secretion disorder was the result of exudation caused by rupture of the salivary ducts, rather than by retention of saliva. An inflammatory response occurred at the rupture site, causing a mucoid granuloma and cyst formation. This etiology resulted in the extravasation type of cyst (1).

Mucoid matrices are biochemically and/or histochernically classified into several kinds of polysaccharides: glycogen, epithelial or mesenchymal acidic mucopolysaccharide, neutral mucopolysaccharide, and glycolipid (10). The present study used PAS [DD2] and TB-2.5 staining to reveal the mucoid substance in the components of the mucous cyst. The mucoid substance was stained with PAS, but did not show metachromasia with TB-2.5 staining. Reactivity to PAS staining indicates that the mucoid substance consists of glycogen or neutral mucopolysaccharides, whereas metachromasia with TB-2.5 staining means that the component consists of chondroitin sulfate. Therefore, the present results indicated that the mucoid substance of the mucous cyst was composed of glycogen and/or neutral mucopolysaccharides, rather than a mesenchyme-specific mucoid matrix (10, 11), and also suggested that the mucoid

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**Table 3. Distribution of inflammatory cells in mucous cyst**

<table>
<thead>
<tr>
<th></th>
<th>CD68-positive mucinophage</th>
<th>Pan T-positive T-cell</th>
<th>Mast cell showed metachromasia with TB-2.5</th>
<th>Pan B-positive B-cell</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucous cyst</td>
<td>74.3</td>
<td>16.3</td>
<td>6.8</td>
<td>2.6</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>28.3</td>
<td>54.9</td>
<td>6.9</td>
<td>9.9</td>
<td>100</td>
</tr>
</tbody>
</table>

Number : cell rate
Unit : %
substance was derived from salivary secretions (1, 2, 12).

Lectin histochemistry revealed a difference in the distribution pattern between mucinophages and the mucoid substance or the salivary gland components. The mucinophages showed strong reactivity for Con A, whereas the mucoid substance and salivary gland component revealed slight or no reactivity. Wheat germ agglutinin reactivity of the mucoid substance and salivary gland components was stronger than that of the mcinophages. In addition, mcinophages were positive for DBA, SBA, and UEA-[DD3]I. Reactivity for DBA and UEA-I in human obstructive submandibular lesions is more marked than that in normal glands, suggesting a specific effect on secretion (2). Therefore, the difference in the distribution pattern observed in the present study suggested a degenerative change in the mucoid substance of the cyst, indicating the additional binding of D-mannose, D-glucose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, and L-fucose, and the reduction of N-acetyl neuraminic acid.

Immunohistochemistry showed that the LZ reactivity of mcinophages was relatively stronger than that in the mucoid substance and salivary gland components. Lysozyme, which is a kind of digestive enzyme, is present in salivary gland cells. Therefore, the distribution of LZ in the mucous cyst suggests an association with degeneration of the mucoid substance.

In the inflammatory component of the cysts we examined, CD68- and HLA-DR-positive mucinophages were higher in mucous cysts than those in the control tissues. CD68 and HLA-DR are expressed in macrophage-lineage monocytes (4-6). The macrophage is an antigen-presenting cell that plays a role in phagocytosis. The accumulated mcinophages observed in this study appeared to be macrophage-lineage cells because they were associated with degenerated mucoid substance and showed positivity for CD 68 and HLA-DR. Saliva does not induce an inflammatory change when it passes through the salivary ducts. However, when saliva leaks outside of the parenchyma of the salivary gland, a localized self-defense reaction as if to a foreign body occurs, resulting in an accumulation of macrophages (1). Arteriosclerosis is also a common lesion caused by accumulation of macrophages in response to injury of the arterial endothelium and to hypercholesterolemia, causing atheroma formation (13). A similar phenomenon may occur in the pathogenesis of mucous cysts, as mcinophages accumulate due to retention of the mucoid substance.

The second most predominant type of inflammatory cell observed in the present study was the T cell, although the cell rate was lower in mucous cysts than in control tissues. T cells possess receptors for the superficial antigen-presenting sites of macrophages, and also induce the production of immunoglobulin by B cells and/or plasmacytes (14). From these findings, although the most important pathogenetic factor of mucous cyst formation was the accumulation of mcinophages as a foreign body reaction to the emission of saliva, a T-cell-mediated immune reaction as chronic inflammation was also associated with the pathogenesis.

In addition, the present findings indicated that IgE-positive plasma cells were more numerous than IgA-positive cells. Previous reports of salivary gland diseases in the subepithelial region have described the appearance of IgE-positive cells and mast cells (8, 15). Given that mast cells were also observed in the present study, an acute allergic immune response might also play an important role in the pathogenesis of mucous cysts. [DD4]

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References