LOCALIZATION OF CATHEPSINS B AND D IN THE SYNOVIAL LINING CELLS OF THE NORMAL RAT TEMPOROMANDIBULAR JOINT BY IMMUNO-LIGHT AND -ELECTRON MICROSCOPY

TAMOTSU KİYOSHIMA, MIZUHO A. KIDO¹, TAKAYUKI TSUKUBA²,
HIDETAKA SAKAI, KENJI YAMAMOTO², AND TERUO TANAKA^{1,3}

*Department of Oral Pathology, First Department of Oral Anatomy¹ and Department of Pharmacology²,
Faculty of Dentistry, Kyushu University 61, Fukuoka 812*

Received for publication June 13, 1994 and in revised form October 11, 1994

The localization of cathepsins B and D in the synovial lining cells and distribution of the lining cells containing these cathepsins in the normal rat temporomandibular joint (TMJ) were examined immunocytochemically in thick (8–10 μ m) and semithin (1 μ m) cryosections with the avidin-biotin-peroxidase complex method and in ultrathin sections with the gold-labeled IgG method. In the thick sections, strong immunoreactivity for cathepsins B and D probably in the type A cells (not clear at this level), was observed simultaneously in the superficial layer of the synovial membrane. This reactivity was strong at the following portions of the synovial membrane located adjacent to the blood vessels: 1) at the anterior portion and 2) medial-posterior portion facing the upper joint cavity, and 3) at the anterior portion and 4) lateral portion facing the lower joint cavity. In semithin cryosections, strong immunoreactivity for cathepsins B and D was simultaneously found in the type A cells which have numerous pseudopodia and vacuoles, while weak granular immunoreaction products for both cathepsins were also observed in the type B cells. In control sections, no type A or B cells showed immunoreactivity for these cathepsins. In the ultrathin sections, numerous gold particles indicating cathepsins B and D were detected in the lysosomes and phagolysosomes of the type A cells facing the lateral intercellular spaces and joint cavity. In the phagolysosomes, these cathepsins were characteristically co-localized. On the other hand, in the type B cells, a few gold particles were localized only in the lysosomes.

Judging from these findings, it is suggested that type A cells predominantly contain cathepsins B and D, and that these cathepsins may be restricted within the cells (lysosomes) without extracellular release in the normal rat TMJ. It is also suggested that materials such as cell debris in the lateral intercellular spaces between the type A cells or in the joint cavity are endocytosed by phagosomes nearby, and are digested by proteolytic enzymes (cathepsins B and D) in the lysosomes. In addition, it is considered that this function in the type A cells is most conspicuous at the anterior portion of the synovial membrane of the normal rat TMJ.

Cathepsins B, D and L have generally been associated with destructive diseases, because these enzymes can degrade various connective tissue components such as collagen (15) and proteoglycan (16, 19). Besides its destructive ability, however, cathepsin D has the processing ability to activate procathepsins B and L (20), and cathepsin B also activates procollagenase (5). In particular, in arthritic knee joints, numerous investigators have reported that cathepsins

B, D and L are expressed in the synovium, specifically in synovial lining cells (6, 17, 21, 23).

This synovial membrane consists of a synovial lining layer and a sublining layer. The synovial lining layer faces the joint cavity lying on the sublining layer and is mainly formed by two types of cells, type A (macrophage-like) and type B (fibroblast-like) cells named by Barland *et al.* (2). However, it still remains unclear which types of cells contain cathepsins B, D and L. On the other hand, in the temporomandibular joint (TMJ), there have hitherto been few reports as to whether cathepsins are contained in the synovial lining

³ To whom correspondence and reprint requests should be addressed.

cells, except for our recent study (10) which showed that cathepsins B and D coexist in both types (A and B) of cells in the normal rat TMJ but are predominantly contained in the type A cells. However, this report only showed the localization of these cathepsins in the synovial lining cells in part of the synovium of the normal rat TMJ. Therefore, there is a need to demonstrate in detail the distribution of the cathepsin-containing lining cells in the whole synovium of the normal TMJ to better understand the pathophysiology of TMJ diseases.

The present study was designed to investigate the regional differences in distribution of synovial lining cells containing cathepsins B and D in the synovium of the normal rat TMJ by light microscopy, using an avidin-biotin-peroxidase complex method, and by electron microscopy, using a colloidal gold-labeled IgG method.

MATERIALS AND METHODS

Tissue preparation

Normal 7- to 8-week old Wistar rats (17 males) were perfused first with heparinized saline (pH 7.4) under sodium pentobarbital anesthesia (50 mg/kg, i.p.) and then with a fixative consisting of a mixture of 4% paraformaldehyde, 0.2% picric acid and 0.05% glutaraldehyde in 0.1 M phosphate buffer (PB) (pH 7.4) at 4°C. The bilateral TMJ were immediately removed and immersed in the same fixative for 2 hr at 4°C. After fixation, specimens were demineralized in 5% EDTA in PB containing 4% sucrose. The TMJ capsules were carefully dissected without the extra muscles and bones.

Immuno-light microscopy

The specimens (from 6 rats) were placed into 20% sucrose in PB overnight at 4°C for cryoprotection before being cut into thick sections (8–10 μ m) on a cryostat. Serial sections, 8–10 μ m in thickness, were cut in the sagittal or frontal plane with a Jung Frigocut 2800N (Leica instruments GmbH, Nussloch, Germany), then mounted on gelatin-coated glass slides and divided into three groups. Two adjacent sections were stained for cathepsins B and D, respectively, while the third sections were used as controls. Sections were stained immunohistochemically by the avidin-biotin-peroxidase complex (ABC) method with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) in a moist chamber. They were washed in PB and treated with 0.1% NaBH₄ in PB twice for 15 min to prevent background staining, followed by 0.3% H₂O₂ in PB for 1 hr at room temper-

ature to inhibit endogenous peroxidase activity. After rinsing in PB, they were preincubated with 3% normal goat serum in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 15 min at room temperature to block nonspecific sites. The serial sections were incubated overnight at 4°C with each specific antibody (anti-cathepsin B 10 μ g/ml and anti-cathepsin D 5 μ g/ml), diluted in a solution consisting of 1% bovine serum albumin (BSA), 0.1 M L-lysine-HCl and 0.02% NaN₃ in PBS. After washing, they were exposed to biotinylated goat anti-rabbit IgG, diluted 1 : 200 in PBS, for 1 hr at room temperature. The sections were washed and incubated with ABC reagent, diluted 1 : 100 in PBS, for 1 hr at room temperature. After a further rinse in PBS, they were reacted with DAB solution consisting of 0.02% 3,3'-diaminobenzidine (Dojin Laboratories, Kumamoto, Japan) in 0.05 M Tris-HCl buffer, pH 7.6, with 0.006% H₂O₂ for about 3 min. No immunostained sections (8–10 μ m thick) were counterstained. DAB reaction products in the immunostained sections were enhanced by a Kodak Wratten gelatin filter No. 44 and photographed with an OLYMPUS VANOX AH2.

For observation of cryo-semithin sections, immunohistochemical staining was performed as described previously by Kiyoshima *et al.* (10). Briefly, the synovium at the anterior portion of the TMJ (7 rats) was sectioned (1 μ m thick) and incubated with the antibodies to cathepsin B or D for 90 min using the ABC method, and reacted with DAB solution. Semithin sections were lightly counterstained with toluidine blue.

Control sections in the third group were treated in the same way with the following exceptions: replacement of the specific antibody by normal non-immune rabbit serum or primary antibody preabsorbed with cathepsin B or D, or omission of the incubation with the specific antibody.

Immuno-electron microscopy

The anterior portion of the TMJ (4 rats) was fixed by the same procedure and fixative as described above. Specimens were cut into thick sections (200 μ m) with a Microslicer, then dehydrated through a graded ethanol series at low temperature, and embedded in LR gold resin (London Resin, Basingstoke, UK). The LR gold resin was polymerized by UV irradiation at about -30°C for 24 hr. Ultrathin sections of LR gold resin-embedded material were mounted on nickel grids coated with a Formvar membrane and immunostained. Briefly, the sections were treated with 3% normal goat serum in 0.1 M PBS containing 1% BSA, 0.1 M L-lysine-HCl

and 0.02% NaN_3 for 15 min, followed by incubation for 60 min with anti-rat cathepsin B or D IgG (anti-cathepsin B 80 $\mu\text{g}/\text{ml}$ and anti-cathepsin D 32 $\mu\text{g}/\text{ml}$). After washing in PBS, sections were incubated with the colloidal gold ($\phi 11 \text{ nm}$)-labeled IgG probe for 30 min. Colloidal gold-labeling of goat anti-rabbit antibody (IgG) was performed as described by Slot and Geuze (22). The sections were counterstained with uranyl acetate and examined with an electron microscope.

Antibodies

Antibodies to cathepsins B (25) and D (26) purified from rat spleen were raised in rabbits and purified by affinity chromatography using cathepsin B- or D-coupled Sepharose as described previously (24). The monospecificity of the antibodies was confirmed in the above previous studies (24, 25) and in other tissues such as osteoclasts (7).

RESULTS

The rat TMJ contains an intervening articular disc, which divides the joint cavity into upper and lower compartments, and the synovial membrane lines part of these compartments. The TMJ is depicted by schematic drawings in Fig. 1 (frontal view) and 2 (sagittal view).

Light microscopy

The medial to lateral portion of the TMJ was cut into 8–10 μm -thick serial cryosections in the sagittal plane.

In the sagittal sections of the medial portion (Fig.

3a, b) of the TMJ indicated by line 3 in Fig. 1, at the anterior peripheral portion of the disc facing the joint cavity, the superficial layer of this portion was positive for cathepsins B (not shown) and D (Fig. 3a). This cathepsin-positive layer probably consisted of type A cells containing cathepsins B and D (10) although it was difficult to identify cell type in these thick sections. There was no difference between the distribution patterns of the immunoreactivities of the two cathepsins. In this portion, the superficial layer facing the upper compartment (UC) was labeled positively for both cathepsins more extensively than that facing the lower compartment (LC). In the posterior peripheral portion of the disc (Fig. 3b), synovial folds protruded into the UC, and the superficial layer of the synovial folds was labeled for both cathepsins. However, this immunoreactive layer was discontinuous, because the synovial lining cells showed both positive and negative immunoreactivities. Large blood vessels were located closer to the cathepsin-positive layer. In the connective tissue under the lining layer, there were some cathepsin B- and D-positive cells, and these were probably macrophages (not clear in the thick sections). On the other hand, the peripheral portion facing the LC was either negative or negligibly stained (not shown).

In the sagittal sections (line 4 in Fig. 1), corresponding to a position slightly laterally over the central portion of the TMJ at the anterior peripheral portion of the disc (Fig. 4), the superficial layer facing the LC was continuously and strongly labeled for both cathepsins. However, in the superficial layer facing the UC, cathepsin-positive cells showed a dispersed distribution pattern. Thus, at this anterior portion, the

FIGS. 1, 2. Frontal (Fig. 1) and sagittal (Fig. 2) views of the center portion of rat TMJ. Each bold line indicates the cut plane of each section. Line numbers correspond to the respective figure number. Broken lines in the posterior side in Fig. 2 indicate medial cleft terminals. A: anterior, : actual disc, C: mandibular condyle, L: lateral, LC: lower compartment, M: medial, P: posterior, T: temporal bone, UC: upper compartment.

distribution of immunoreactivity was the inverse of that shown in Fig. 3. At the posterior portion, cathepsins-positive cells were sparse in the peripheral portion facing the UC and LC (not shown).

In the sagittal sections of the lateral portion (Fig. 5) (line 5 in Fig. 1), the immunoreactivity at the anterior peripheral portion of the disc facing the LC was stronger than that at the posterior peripheral portion. However, the anterior to posterior portion facing the UC was negative for cathepsins.

Thus, in the sagittal sections, immunoreactivity for both cathepsins in the synovial lining layer was

basically stronger at the anterior peripheral than at the posterior peripheral portion of the disc.

The anterior to posterior portion of the TMJ was cut into the 8–10 μm -thick serial cryosections in the frontal plane.

In the frontal sections of the anterior portion (Fig. 6) (line 6 in Fig. 2), the peripheral portion of the disc was visible because numerous blood vessels, which are not found in the actual disc, were observed in this portion. The superficial layer facing the UC showed positive immunoreactivity in the center third of the disc. On the other hand, along the superficial layer

FIG. 3. Part of sagittal thick cryosections (8–10 μm thick) indicated by line 3 in Fig. 1. Panel a shows the anterior peripheral portion of the disc (D) immunostained with anti-cathepsin D antibody. The superficial layer (arrowhead) facing the upper compartment (UC) was labeled more strongly and extensively than that facing the lower compartment (LC). Panel b shows synovial folds protruding into the upper compartment (UC) at the posterior portion immunostained for cathepsin B. Both cathepsin B-positive (arrowheads) and -negative (open arrowheads) immunoreactive cells were found in the synovial lining layer of the folds. Blood vessels (V). Not counterstained.

FIG. 4. Part of a sagittal thick cryosection indicated by line 4 in Fig. 1, showing the anterior peripheral portion of the disc (D). The superficial layer facing the lower compartment (LC) was continuously and strongly labeled with the anti-cathepsin B (arrows), while the superficial layer facing the upper compartment (UC) was labeled poorly. Blood vessels (V). Not counterstained.

FIG. 5. Sagittal thick cryosection indicated by line 5 in Fig. 1. Cathepsin B immunoreactivity (arrowheads) at the anterior peripheral portion (A) of the disc (D) facing the lower compartment (LC) was stronger than that at the posterior portion (P) facing this compartment. The superficial layer facing the upper compartment (UC) was not labeled for cathepsin B. Condylar head (CH). Not counterstained.

FIG. 6. Frontal thick cryosection indicated by line 6 in Fig. 2. Numerous blood vessels (V) were located in this disc portion (not actual disc). Cathepsin D-positive superficial layer of the disc (D) facing the upper compartment (UC) is at the center third (arrows) of the disc. The immunopositive superficial layer (arrowheads) of the disc facing the lower compartment (LC) extended from the medial (M) to lateral terminals (L). Condylar head (CH). Not counterstained.

facing the LC, immunoreactivity was continuous from the medial to lateral terminal.

In the frontal sections cut through the thinnest part of the disc (line 7 in Fig. 2), at the medial peripheral portion, a few immunoreactive foci were observed at the intima facing the UC, while at the LC, some were found on the cleft terminal (not shown). On the other hand, in the lateral peripheral portion (Fig. 7), the cleft terminal facing the LC was immunopositive, while staining in the cleft terminal facing the UC was either negligible or negative.

In the frontal sections of the posterior portion (Fig. 8) (line 8 in Fig. 2), at the medial side, the cleft terminal facing the UC was lined by positive cells, while at the lateral side, some large and small synovial folds protruded into the UC and the surface of these folds was discontinuously lined by the positive cells. Characteristically, large blood vessels were located close to the UC.

In semithin sections (1 μ m thick sections) of the anterior peripheral portion (Fig. 9a-d) facing the UC, type A and B cells constituted the synovial lining layer. Type A cells formed the most superficial layer facing the joint cavity and possessed many short cytoplasmic processes such as pseudopodia and numerous vacuoles, thus resembling macrophages. On the other hand, type B cells were round in shape

FIG. 7. Part of a frontal thick cryosection indicated by line 7 in Fig. 2, showing the lateral peripheral portion of the disc (D). Cathepsin B immunoreactivity (arrowheads) was found from the peripheral portion of the disc to the condylar neck (N) facing the lower compartment (LC). On the other hand, no immunoreactivity was found along the cleft terminal facing the upper compartment (UC). Not counterstained.

and had a large nucleus, narrow cytoplasm and occasionally a few long cytoplasmic processes. The type B cells were mostly located beneath the type A cells but were occasionally located facing the joint cavity. In the synovial lining layer, immunoreactivity for cathepsins B and D was markedly stronger in the type A than in type B cells (Fig. 9a, b). Type A cells simultaneously showed strong immunoreactivity for both cathepsins B and D in their vacuoles and granules. On the other hand, the type B cells showed weak granular cathepsin immunoreactivity. In the controls described in the materials and methods section, no immunoreactivity for either cathepsin B or D was observed in either type A or B cells (Fig. 9c, d).

At the posterior peripheral portion (not shown) facing the UC, cathepsin-positive cells showed a dispersed distribution. However, in the connective tissue under the synovial lining layer, numerous cathepsin-positive cells which seemed to correspond to

FIG. 8. Part of a frontal thick cryosection indicated by line 8 in Fig. 2. On the lateral side (L), the surface of some synovial folds (arrows) was discontinuously positive for cathepsin D, while on the medial side (M), the cleft terminal (arrowheads) facing the upper compartment (UC) showed continuous positive immunoreactivity. Blood vessels (V). Not counterstained.

macrophages were found (not clear at this level). Also, many blood vessels were located close to the joint cavity.

Electron microscopy

In ultrathin sections (Figs. 10–12), the type A cells (Fig. 10) were seen to have numerous cytoplasmic processes, mitochondria and both granular and vacuolar structures of various sizes which were partially or completely filled with an electron-dense material. There were wide intercellular spaces between type A cells. Gold particles, indicating cathepsins B and D, were heterogeneously distributed in most granular and vacuolar structures, while some of these structures were negative for cathepsins. Numerous gold particles were occasionally found in the granular and vacuolar structures close to the nucleus or close to the joint cavity and lateral intercellular spaces. The vacuolar structures close to the joint cavity and intercellular spaces seemed to correspond to phagolysosomes. It is likely that cathep-

sin-negative phagosomes fused with cathepsin-positive lysosomes to form these structures. Lastly, gold particles were scarcely found in other organelles in type A cells or in intercellular spaces. In the adjacent ultrathin sections stained with anti-cathepsin B (Fig. 12a) or D (Fig. 12b), gold particles indicating cathepsin B were detected in the cathepsin D-positive lysosomes in type A cells. Thus, cathepsins B and D were co-localized in the same lysosomes of type A cells. On the other hand, type B cells (Fig. 11) were found in the deeper layer and had well-developed rough endoplasmic reticulum, in addition to a few granules and small vacuoles. A few gold particles, indicating cathepsins B and D, were detected only in some lysosomes, which seemed to correspond to the cathepsin-positive granular structures seen in the type B cells in the semithin sections. However, the vacuoles were negative for both cathepsins.

DISCUSSION

It has been demonstrated in the TMJ (10, 13, 18) as well as in other joints (2, 8, 9), that the lining cells of the synovial membrane mainly consist of two types of A and B cells. However, which types of synovial lining cells of the various joints except the TMJ contain cathepsins B and D have not hitherto been described precisely. Our previous study (10) demonstrated for the first time that large amounts of cathepsins B and D coexisted in the type A cells in the synovial lining of the TMJ, as well as small amounts in the type B cells. In the present study, the detailed localization of these cathepsins in the type A and B cells was reported, and we demonstrated that there are regional differences in distribution patterns of cathepsin-containing synovial lining cells in the normal TMJ.

No extracellular cathepsin D has been observed in noninflamed synovium (21), whereas in rheumatoid synovium, the extracellular release of cathepsins B and D has been demonstrated (17), using the same method in both the studies. Thus, in the inflamed synovium, cathepsins B and D may be released to degrade various connective tissue components such as proteoglycan (16, 19) and collagen (15). In the present study using normal TMJ, large amounts of cathepsins B and D were observed intracellularly in the lysosomes of type A cells, while these cathepsins were scarcely released extracellularly; although a very low level of extracellular gold particles was detected, this was thought to be nonspecific because the same level was also found in control sections. It is possible that, in the noninflamed synovial lining cells, cathepsins B and D would be retained in the lysosomes. However, with

FIG. 9. Light micrographs of semithin (1 μ m) cryosections of the synovial membrane of the anterior peripheral portion of the disc immunostained with anti-cathepsin B (a) or D (b), with nonimmunized rabbit serum (c), or without primary antibody (d) as controls. Type A cells (arrows) had numerous vacuoles and cytoplasmic processes facing the joint cavity and showed strong immunoreactivity for cathepsins B and D. Type B cells (arrowheads) had a large nucleus and little cytoplasm, and showed weak granular immunoreactivity. In controls (c, d), no immunoreactivity was found in type A (arrows) or B (arrowheads) cells. Capillaries (C). All sections were counterstained with toluidine blue.

the development of inflammation, type A cells along with neutrophils and macrophages may release these proteases extracellularly, participating in the degradation of the joint tissue.

Strong immunoreactivity for cathepsins was found in the type A cells of the synovial membrane, as well as in macrophages and Kpfer cells in which large amounts of cathepsins B and D are contained (4, 11, 27, 28). In the present study, gold particles indicating cathepsins B and D were found in the amorphous vacuoles close to the lateral intercellular spaces between type A cells and facing the joint cavity. The heterogeneous localization of gold particles within these structures suggests that they may be digestive vacuoles (phagolysosomes) formed by fusion of cathep-

sin-containing lysosomes with cathepsin-negative phagosomes. Furthermore, cathepsins B and D were characteristically co-localized in these lysosomes. From these findings, it is certain that type A cells, as well as macrophages and Kpfer cells, have digestive capability. In addition, in a tracer experiment reported by Kubo (13) using horseradish peroxidase, type A cells were shown to endocytose foreign materials. Various materials are transuded from the fenestrated capillaries close to the synovial lining cells and pass into the joint cavity through the intercellular spaces (14). Therefore, these findings suggest that the materials are endocytosed by phagosomes from intercellular spaces on their way to the joint cavity and/or from the cavity, and that they are digested by

FIGS. 10, 11. Electron micrographs of type A and B cells at the anterior portion of the TMJ immunostained with anti-cathepsins B and D, respectively. Fig. 10; type A cells contained numerous vacuoles, vesicles, cytoplasmic processes and mitochondria (M). Gold particles indicating cathepsin B immunoreactivity were distributed heterogeneously in the amorphous vacuoles (AV) (probably phagolysosomes) formed by fusion of phagosomes with lysosomes. These vacuoles were located close to the joint cavity (JC) and lateral intercellular spaces (IS). Granules and vacuoles taken to be lysosomes (L) close to the nucleus also contained gold particles. Fig. 11; type B cells had well-developed rough ER, a few granules and small vacuoles (V). Gold particles indicating cathepsin D immunoreactivity were restricted within granules (lysosomes) (arrows). Counterstained with uranyl acetate.

FIG. 12. Electron micrographs of adjacent ultrathin sections of part of a type A cell immunostained with anti-cathepsin B (a) or D (b), respectively. Amorphous vacuoles indicated by arrows in panel a were labeled with gold particles indicating cathepsin B immunoreactivity. The same amorphous vacuoles (arrows in panel b) were also labeled with gold particles indicating cathepsin D immunoreactivity. Intercellular spaces (IS), mitochondria (M). Counterstained with uranyl acetate.

proteolytic enzymes such as cathepsins B and D in the lysosomes. Thus, type A cells may control the transport of materials from the blood vessels into the joint cavity and may also be involved in the removal of materials such as fibrous fragments and debris from the synovial fluid by their endocytotic and digestive ability.

As shown by the above results, cathepsin-positive type A cells were not located throughout the surface of the synovium, but were instead localized in limited areas such as the anterior portion of the TMJ. These findings suggest the following: 1) In the synovium at the anterior portion of the TMJ, type A cells participate most extensively in control of synovial fluid and removal of materials from the joint cavities (UC and LC). 2) At the medial-posterior portion of the TMJ, the cells have control and removal function only in the UC side, and at the lateral portion they function only in the LC side. 3) In particular, in the synovium at the posterior portion of the TMJ, the lining cells hardly contribute to the removal function, because cathepsin-positive type A cells were very few or absent. Rather, in this portion, numerous macrophages were detected in the sublining layer and therefore

macrophages rather than type A cells may contribute to removal of the materials in the cavities.

In this study, type B cells were shown to contain very small amounts of cathepsins B and D only in their lysosomes. This type are known as fibroblast-like cells which are associated with protein synthesis such as collagen (12). Newly synthesized procollagen is degraded prior to secretion from collagen-producing cells such as fibroblasts (3). Cathepsins B and L may participate in the intracellular proteolysis in the processing pathway of newly synthesized procollagen (1). Therefore, the small amount of cathepsins observed in type B cells may be used for intracellular proteolysis required for the production of collagen.

The regional distribution of cathepsin-containing synovial type A cells and the detailed localization of these cathepsins within type A and B cells described in this study in the normal TMJ may be useful reference points for future histopathological investigations of the progression of arthritis in the TMJ.

REFERENCES

1. Asokan, R., Kesava Reddy, G. and Dhar, S. C.: Studies on the intracellular degradation of newly synthesized collagen in 3-methylcholanthrene induced fibrosarcoma cells. *Life Sci.* 51; 1065-1071, 1992.
2. Barland, P., Novikoff, A. B. and Hamerman, D.: Electron microscopy of the human synovial membrane. *J. Cell Biol.* 14; 207-220, 1962.
3. Bienkowski, R. S.: Intracellular degradation of newly synthesized secretory protein. *Biochem. J.* 214; 1-10, 1983.
4. Dingle, J. T., Poole, A. R., Lazarus, G. S. and Barrett, A. J.: Immunoinhibition of intracellular protein digestion in macrophages. *J. Exp. Med.* 137; 1124-1141, 1973.
5. Eeckhout, Y. and Vaes, G.: Further studies on the activation of procollagenase, the latent precursor of bone collagenase: effects of lysosomal cathepsin B, plasmin and kallikrein, and spontaneous activation. *Biochem. J.* 166; 21-31, 1977.
6. Etherington, D. J., Taylor, M. A. J. and Henderson, B.: Elevation of cathepsin L levels in the synovial lining of rabbits with antigen-induced arthritis. *Br. J. Exp. Path.* 69; 281-289, 1988.
7. Goto, T., Kiyoshima, T., Moroi, R., Tsukuba, T., Nishimura, Y., Himeno, M., Yamamoto, K. and Tanaka, T.: Localization of cathepsins B, D, and L in the rat osteoclast by immuno-light and -electron microscopy. *Histochemistry* 101; 33-40, 1994.
8. Graaebak, P. M.: Ultrastructural evidence for two distinct types of synoviocytes in rat synovial membrane. *J. Ultrastruct. Res.* 78; 321-339, 1982.
9. Graaebak, P. M.: Characteristics of the two types of synoviocytes in rat synovial membrane: an ultrastructural study. *Lab. Invest.* 50; 690-702, 1984.
10. Kiyoshima, T., Tsukuba, T., Kido, M. A., Tashiro, H., Yamamoto, K. and Tanaka, T.: Immunohistochemical localization of cathepsins B and D in the synovial lining cells of the normal rat temporomandibular joint. *Archs. Oral Biol.* 38; 357-359, 1993.
11. Knook, D. L.: The role of lysosomal enzymes in protein degradation in different types of rat liver cells. *Acta Biol. Med. Germ.* 36; 1747-1752, 1977.
12. Krane, S. M., Dayer, J. -M., Simon, L. S. and Byrne, M. S.: Mononuclear cell-conditioned medium containing mononuclear cell factor (MCF), homologous with interleukin 1, stimulates collagen and fibronectin synthesis by adherent rheumatoid synovial cells: Effects of prostaglandin E₂ and indomethacin. *Collagen Rel. Res.* 5; 99-117, 1985.
13. Kubo, Y.: The uptake of horseradish peroxidase in monkey temporomandibular joint synovium after occlusal alteration. *J. Dent. Res.* 66; 1049-1054, 1987.
14. Levick, J. R.: Synovial fluid; Determinants of volume turnover and material concentration. In "Articular Cartilage and Osteoarthritis", ed. by K. E. Kuettner, R. Schleyerbach, J. G. Peyron and V. C. Hascall, Raven Press Ltds, New York, 1992, pp. 529-541.
15. Maciewicz, R. A. and Wotton, S. F.: Degradation of cartilage matrix components by the cysteine proteinases, cathepsins B and L. *Biomed. Biochim. Acta* 50; 561-564, 1991.
16. Morrison, R. I. G., Barrett, A. J., Dingle, J. T. and Prior, D.: Cathepsins B1 and D: action on human cartilage proteoglycans. *Biochim. Biophys. Acta* 302; 411-419, 1973.
17. Mort, J. S., Recklies, A. D. and Poole, A. R.: Extracellular presence of the lysosomal proteinase cathepsin B in rheumatoid synovium and its activity at neutral pH. *Arthritis Rheum.* 27; 509-515, 1984.
18. Murnane, T. W. and Feagans, W. M.: Fine structure of synovial lining cells in the squamosal-mandibular joint of the rat. *J. Dent. Res.* 49; 1068-1073, 1970.
19. Nguyen, Q., Mort, J. S. and Roughley, P. J.: Cartilage proteoglycan aggregate is degraded more extensively by cathepsin L than by cathepsin B. *Biochem. J.* 266; 569-573, 1990.
20. Nishimura, Y., Kawabata, T., Yano, S. and Kato, K.: Intracellular processing and activation of lysosomal cathepsins. *Acta Histochem. Cytochem.* 23; 53-64, 1990.
21. Poole, A. R., Hembry, R. M., Dingle, J. T., Pinder, I., Ring, E. F. J. and Cosh, J.: Secretion and localization of cathepsin D in synovial tissues removed from rheumatoid and traumatized joints. *Arthritis Rheum.* 19; 1295-1307, 1976.
22. Slot, J. W. and Geuze, H.: A new method of preparing gold probes for multiple-labeling cytochemistry. *Eur. J. Cell Biol.* 38; 87-93, 1985.
23. Trabandt, A., Gay, R. E., Fassbender, H. G. and Gay, S.: Cathepsin B in synovial cells at the site of joint destruction in rheumatoid arthritis. *Arthritis Rheum.* 34; 1444-1451, 1991.
24. Yamamoto, K., Kamata, O., Katsuda, N. and Kato, K.: Immunochemical difference between cathepsin D and cathepsin E-like enzyme from rat spleen. *J. Biochem.* 87; 511-516, 1980.
25. Yamamoto, K., Kamata, O., Takeda, M. and Kato, Y.: Characterization of rat spleen cathepsin B and different effects of anti-inflammatory drugs on its activity. *Jpn. J. Oral Biol.* 25; 834-838, 1983.
26. Yamamoto, K., Katsuda, N., Himeno, M. and Kato, K.: Cathepsin D of rat spleen. *Eur. J. Biochem.* 95; 459-467, 1979.
27. Yokota, S., Tsuji, H. and Kato, K.: Localization of cathepsin D in rat liver: immunocytochemical study using post-embedding immuno-enzyme and protein A-gold techniques. *Histochemistry* 82; 141-148, 1985.
28. Whitaker, J. N. and Rhodes, R. H.: The distribution of cathepsin D in rat tissues determined by immunocytochemistry. *Am. J. Anat.* 166; 417-428, 1983.