Immunoreactivity of Vacuolar H⁺-ATPase in Human Tissues —Using Polyclonal Rabbit Antibodies against V-ATPase Subunits

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We immunohistochemically examined tissue distribution and cellular localization of vacuolar H⁺-ATPase (V-ATPase), which is a proton-translocating ATPase known as an acidifier of cytoplasmic vesicles and vacuoles, using two polyclonal rabbit antibodies against A and B subunits of the V-ATPase purified from bovine chromaffin granules. The intense staining was found in epithelial cells of the renal tubules and the salivary ducts, pancreatic α cells, endocrine cells in colon, macrophages, osteoclasts, and chondrocytes in human tissues, and the following three localization patterns of V-ATPase were found in the examined cells: 1) cytoplasmic pattern with granular and diffuse types, 2)

polarized pattern, and 3) diffuse apical cytoplasm pattern. The granular type of cytoplasmic pattern was typically observed with high density in macrophages, α cells of the pancreatic islets and endocrine cells in colon. The diffuse type was often seen in most of ductal cells of exocrine glands, osteoclasts, nerve cells with moderate density, and in chondrocytes with high density, and the polarized pattern was represented by intercalated cells of the renal tubules with high density. The diffuse apical cytoplasm pattern was typically found along the brush border of proximal tubules from moderate to high density.

Key words: V-ATPase, Human tissues, Immunohistochemistry

I. Introduction

V-ATPase is a proton-translocating ATPase known as an acidifier of cytoplasmic vesicles and vacuoles. V-ATPase consists of multimeric complexes with cytoplasmic V1 and membrane-bound V0 domains. Cytoplasmic ATP is hydrolyzed in the V1 domain, forcing protons away from the cytoplasm through the V0 domain and across the membrane. The V1 domain, containing the ATP catalytic site, is composed of a hexamer of three subunit A (65–77 kDa) and three subunit B (55–60 kDa) proteins plus accessory subunit C (40–45 kDa), D (33–34 kDa) and E (26–33 kDa). The V0 domain, as a proton transporter, is composed of four subunits of 16 kDa, one subunit of 20 kDa and one subunit of 115 kDa. Multiple isoforms have so far been found for each of subunit A, B,

and E [5, 17, 26, 32, 33].

V-ATPase is highly conserved at the protein level throughout evolution from archaebacteria to mammals. V-ATPase mainly exists in the intracellular vesicles such as lysosomes, endosomes, secretory granules, and Golgi apparatus where it acidifies the intravesicular environment. This is necessary for keeping proteinases active to digest the intracellularly engulfed materials, and helping to transport the synthesized proteins to their destination. V-ATPase is also found on the plasma membrane of some cells such as the ruffled border of the osteoclasts and the plasma membrane of the renal tubular epithelial cells, where V-ATPase extrudes proton actively from blood to urine, to maintain the systemic acid-base balance [5, 17, 26, 32, 33]. Recently, the roles of V-ATPase have been noted in the apoptosis of cells, metastasis of some tumors, as well as in the infective pathways of virus [15, 25, 34]. These discoveries show a prospective feature in clinical application.

In humans, the V-ATPase protein has been found in

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brain [2], kidney [18], and osteoclasts [39]. Hille [19] reported mRNAs of subunits of A, C, D, and E existed in many organs including kidney, liver and pancreas using the probe developed from human osteoclastoma. Their distribution, however, in human tissues has not been studied systemically. Since there is a disagreement, for example, about whether the V-ATPase that exists in the plasma membrane of renal tubules is the same protein that exists in the ruffled border of osteoclasts [12], knowledge about the tissue distribution and cellular localization of the V-ATPase protein in human tissues is necessary to understand the V-ATPase function *in vivo*. In this study, we examined the expression of the subunit A of V-ATPase immunohistochemically using the antibody against that from bovine chromaffin granules. This subunit is known as the most preserved one among all of the 11 subunits in evolution. In addition, this is the most basic and functions in conjunction with subunit B as a catalytic unit [26]. Considering the variety of cell and tissue localization of subunit A, we also investigated the distribution of subunit B.

The significance of V-ATPase in osteoclasts has supposedly been to acidify the extracellular lacunae between osteoclasts and bone surface, and to activate lysosomal enzymes such as cathepsins [9, 14], while carbonic anhydrase II (CA II) is known to be a producer of protons by CO₂ hydration [18]. In order to clarify whether these proteins are synthesized by the same cells, we further observed the mutual relationship among the localization of V-ATPase, carbonic anhydrase II (CA II) and cathepsin D using relevant antibodies.

II. Materials and Methods

Tissue samples

Human tissues, including lung, kidney, salivary gland, pancreas, esophagus, stomach, colon, adrenal gland, thyroid gland, brain, bone and lymph node were taken from 5 autopsy cases within 2.5 hr after death. The tissues of the above five cases used in this study showed no remarkable pathological changes. Normal tissues of kidney and salivary gland from surgical specimens were selected in order to understand the changes of immunostaining after death (Table 1).

Fixation and section preparation

All tissues taken from autopsy and surgery were prepared for paraffin sections. In order to confirm the results obtained from paraffin sections, cryosections were made from parts of tissues including kidney and salivary gland. For paraffin sections, tissues were fixed in 4% paraformaldehyde in phosphate-buffered saline (PFA-PBS) for 2 hr, embedded in paraffin and sectioned at a thickness of 2 μ m. They were stored at 4°C until staining. Bone samples were decalcified in EDTA for 48–72 hr, but otherwise they were processed as done in non-decalcifying tissues. For cryosections, tissues were fixed in paraformaldehyde-

Table 1. Five cases of autopsy and four surgical specimens

cases of autopsy				
No	Age	Sex	Postmortem time	Cause of death
1	72	M	1.0 hr	Acute myocardial infarction
2	34	F	1.0 hr	Hypertrophic cardiomyopathy
3	70	M	1.5 hr	Acute myocardial infarction
4	59	M	2.5 hr	Myocardial infarction
5	61	F	1.5 hr	Acute interstitial pneumonia

surgical specimens				
	No	Age	Sex	Clinical diagnosis
Kidney	1	73	M	Renal cell carcinoma
	2	74	F	Renal cell carcinoma
Salivary gland	1	69	M	Epithelioid granuloma
	2	61	M	Adenocarcinoma

lysine-periodate (PLP) for 5 hr at 4°C, immersed in gradient sucrose serials (10% for 6 hr, 15% for 6 hr and 20% for 12 hr) at 4°C, then frozen quickly in dry ice-acetone, sectioned into 4 μ m, dried for 30 min, and stored at -20°C until staining.

For Western blot analysis, tissues were frozen immediately after sampling, and stored at -70°C until use.

Fig. 1. Immunoblotting analysis of the cross reactivity of the antibody against the subunit A of V-ATPase in human salivary gland (lane C) and kidney (lane D). Subunit A (72 kDa) of V-ATPase purified from bovine chromaffin granules is also loaded (lane B). Purified V1 of V-ATPase from bovine chromaffin granules is electrophoresed and stained with Coomassie Brilliant Blue (lane A).

Table 2. Localization of V-ATPase in systemic organs

Organ & Cell	Subunit A of V-ATPase	P-ATPase	Organ & Cell	Subunit A of V-ATPase	P-ATPase
Kidney			Pancreas		
glomerulus:			acinar cell	+	-
endothelial cell	-	-	duct:	+	-
mesangial cell	-	-	interlobular duct	+	-
tubule:			Langerhans' cell	++/++	-
proximal tubule*	++	-	Adrenal gland		
Henle's loop	+	-	cortical cell: zona glomerulosa	-	-
distal tubule**	++	-	zona fasciculata	-	-
collecting tubule**	++/-	-	zona reticularis	+	-
renal pelvic epithelium	+	-	medullary cell	+	-
Salivary gland			Thyroid gland		
acinar cell:			follicular cell	+	-
serous acinus	-	-	Brain		
mucous acinus	-	-	nerve cell	+	-
duct:			glia cell	-	-
intercalated duct	+	+	Lung		
striated duct**	++	+	bronchial epithelial cell	+	-
interlobular duct**	++	+	alveolar epithelium	-	-
Esophagus			alveolar macrophage	++	-
squamous epithelium	+	-	chondrocyte	++	-
esophageal gland: acinus	-	-	lymph node		
duct	+	-	dendritic cell	+	-
Stomach			lymphocyte	-	-
foveolar epithelium	+	-	Bone		
fundic gland: chief cell	-	++	osteoclast**	++	-
parietal cell	+	-	osteoblast	-	-
Colon			osteocyte	-	-
columnar cell	+	-	chondrocyte	+	-
goblet cell	-	-	Skin		
endocrine cell	++	-	squamous cell	+	-
Auerbach's myenteric plexus	+	-			
Smooth muscle cell	-	-			

* plasma staining, ** polarized staining
 -, +, and ++: negative, positive, and strongly positive.

Antibodies and the cross reactivity

Polyclonal antibodies against subunits A and B of V-ATPase were prepared as reported previously [27, 28]. The cross reactivity of the antibody against subunit A was confirmed by the immunoblotting analysis using fresh tissues from human kidney and salivary gland. In brief, frozen tissues were homogenized in SDS sample buffer (tissues/buffer: 1/20). The protein in the supernatant was separated by electrophoresis on SDS polyacrylamide gel (12% acrylamide), and electrophoretically transferred to Immobilon-P membranes (Millipore, USA). The antibody against the subunit A of V-ATPase was diluted to 1:1000 and reacted for 2 hr at room temperature. The strip was washed, reacted with the peroxidase-labeled second antibody diluted to 1:1000 for 1 hr at room temperature, washed and color was developed as per the manufacturer's instruction (Konica Immunostain HRP-100 Kit, Japan). The subunit-72 kDa of V-ATPase purified from bovine chromaffin granules [27] was used as a positive control.

An antibody against P-ATPase of hog stomach was provided by Dr. K. Omori (Kansai Medical University) [40]. Other antibodies used in this study included anti-insulin $\kappa 60$, anti-glucagon and anti-somatostatin monoclonal antibodies (DAKO, Denmark) for α , β and δ islet cells of pancreas; anti-CD68 (DAKO, Denmark) and anti-cytokeratin (COSMO Bio Co, LTD, Japan) monoclonal antibodies to identify V-ATPase-positive cells; anti-cathepsin D (Novocastra Laboratory, LTD, England) and anti-carbonic anhydrase II (CAII) antibodies (Rockland, USA) to observe the relationship between these two enzymes; and V-ATPase during the resorption of bone. Bound primary antibodies were detected with the goat anti-rabbit IgG secondary antibody (Dako, Japan).

Immunohistochemical staining

Paraffin sections were deparaffinized with xylene, treated with methanol containing 1% H_2O_2 for 30 min to block endogenous peroxidase activity, and rinsed in PBS. Sections were first exposed to the blocking solution (10%

Fig. 2. Immunocytochemical staining of the human kidney with the antibody against the subunit A of V-ATPase. **A)** All cells of proximal tubules (PT) are moderately positive on the apical plasma membrane (arrow). The intercalated cells (IC, arrowhead) of distal tubules (DT) are intensely stained on the apical plasma membrane and adjacent cytoplasm. All cells from the glomerulus are negative. **B)** The IC (arrowhead) in the inner medullary collecting tubules (IMCT) demonstrate similar staining patterns as those in DT, and the principal cells (PC, arrow) are weakly positive. **C)** Immunoreactivity turns negative when sections are reacted with normal serum instead of the primary antibody against V-ATPase. **A & C** $\times 50$, **B** $\times 100$.

normal goat serum) for 30 min at room temperature to reduce nonspecific background staining. Sections were then incubated with each primary antibody for 12 hr at 4°C. Polyclonal antibodies against subunits A and B of V-ATPase were used at a dilution of 1 : 1000 and 1 : 800 respectively. After washing with PBS (3 times, 5 min each), sections were incubated for 30 min with the biotinylated goat anti-rabbit IgG antibody diluted to 1 : 50, rinsed in PBS, and then incubated for 60 min with avidin-biotin-peroxidase complex (ABC). After washing in PBS, they were reacted with 3'-3' diaminobenzidine tetrahydrochloride (DAB) for 30 min, rinsed in PBS and counterstained with methyl green.

For cryosections, the anti-subunit A antibody was used at the dilution of 1 : 20000.

Control

Normal serum as the substitute for the primary anti-

bodies was adopted as negative controls. Staining of P-ATPase serves as a comparative control, and the antibody against P-ATPase was used with a dilution of 1 : 1000.

Evaluation of staining results

The results of V-ATPase staining were graded as – (negative), + (positive) and ++ (strongly positive).

III. Results

The immunoblotting test with the antibody against V-ATPase A subunit demonstrated a clear band at the same position of the marker 72 kDa in kidney and salivary gland (Fig. 1).

The results of the V-ATPase immunostaining are summarized in Table 2. Various kinds of cells in human tissues were positive for V-ATPase. The distribution and staining pattern of subunits A and B were substantially the

Fig. 3. Striated ducts (arrow) of submandibular gland show strong immunoreactivity for V-ATPase. The reactivity is diffuse in the cytoplasm with a tendency of concentrating to the lumen. The intercalated duct (arrowhead) is weakly stained. A) Paraffin section, and B) cryostat section. C) P-ATPase is present in the salivary ducts. A $\times 50$, B & C $\times 100$.

Fig. 4. Immunoreactivity for V-ATPase in epithelial cells of various ducts and glands. A) Ducts of esophageal gland, B) pancreatic ducts, C) bronchial epithelial cells, D) colonic gland, and E) gastric pits (E2) and glands (E3), (E1 and E4: the negative controls). All of these epithelial cells are positively stained with a cytoplasmic pattern. A–D $\times 100$, E1–E4 $\times 200$.

Fig. 4

same.

1) Epithelial cells of the urinary system including tubules and pelvis (Fig. 2A, B), ducts of salivary gland (Fig. 3A, B), ducts of esophageal gland (Fig. 4A), pancreatic ducts (Fig. 4B), bronchial wall (Fig. 4C), and gastrointestinal tract, such as colon (Fig. 4D) and stomach (Fig. 4E):

Most of these cells were diffusely stained in the cytoplasm to a moderate degree, and epithelial cells of renal tubules and salivary ducts were strongly stained, especially on their apical cytoplasm.

In kidney, V-ATPase was expressed in more variable patterns than in any other tissues. V-ATPase was concentrated in the apical part of intercalated cells (IC) of the distal tubules (DT), and the cortical and inner medullary collecting tubules (CCT and IMCT). It was also localized along the luminal surface of the proximal tubules (PT) with a strong to moderate degree. Furthermore it was diffuse in the cytoplasm with lower density in the rest of epithelial cells including principle cells (PC) of DT and CT. Parts of the collecting tubules were negative for V-ATPase. The glomerular cells composed of epithelial, endothelial and mesangial cells were almost negatively stained (Fig. 2A).

In salivary gland, epithelial cells of the striated and interlobular ducts were strongly stained in their apical cytoplasm. The intercalated ducts were moderately stained, while the serous and mucous acini were not stained (Fig. 3A). In the cryosections, a more clear tendency of the antigen concentrating to the apical plasma membrane was noticed (Fig. 3B)

In the stomach, epithelial cells of gastric pits were diffusely stained in their cytoplasm (Fig. 4E2). The parietal cells of gastric gland were weakly stained, while the chief cells were negative (Fig. 4E3).

2) Some endocrine cells including pancreatic islet cells (Fig. 5B), endocrine cells in the colon (Fig. 6A), follicular

cells of the thyroid gland (Fig. 6B1), most zona reticularis cells of adrenal cortex and some of the adrenal medullary cells (Fig. 6C1):

These cells showed a diffuse cytoplasmic staining in a moderate degree except endocrine cells in the colon and one type of pancreatic islet cells. Both of them were strongly stained with a granular pattern. The intensely stained pancreatic islet cells were found mostly in the peripheral region of islets. In serial sections, further followed by mirror sections for V-ATPase and glucagon (Fig. 5A, B), these V-ATPase-positive cells corresponded to α cells.

3) Cells of a macrophage lineage including alveolar macrophages (Fig. 7A), osteoclasts (Fig. 8A) and dendritic cells of lymph nodes (data not shown):

Alveolar macrophages, which were CD68-positive and cytokeratin-negative were intensely stained for V-ATPase in their cytoplasm in a granular pattern (Fig. 7A-C).

Osteoclasts were diffusely stained in the cytoplasm (Fig. 8B), and some of them presented a concentration in the ruffled border membrane (Fig. 8D, arrowhead). In order to prove that cathepsin D and carbonic anhydrase II exist in osteoclasts where V-ATPase is localized, these three proteins were stained in serial sections (Fig. 8A-C). The results showed that three of them existed in the same osteoclasts. Dendritic cells were moderately stained in a diffuse cytoplasmic pattern (data not shown).

4) Chondrocytes from bronchioles and joints:

They were intensely stained with a diffuse cytoplasmic pattern (Fig. 9A).

5) The other cells:

Moderate staining was noted in squamous epithelial cells of skin (Fig. 9B), nerve cells of Auerbach's myenteric plexus in colon (Fig. 9C), and nerve cells of brain (Fig. 9D). All of these cells showed a diffuse cytoplasmic pattern.

Fig. 5. V-ATPase and glucagon immunostaining of islet cells of pancreas by mirror sections: B) V-ATPase-positive cells in islets correspond to A) the glucagon secreting α cells (arrowhead). C) the negative control. $\times 100$.

Fig. 6. V-ATPase in the other endocrine cells: **A**) endocrine cells in colon, **B1**) thyroid follicular cells (**B2**: the negative control), and **C1**) cells of zona reticularis of adrenal cortex (arrow) and parts of the cells of medulla (arrowhead) (**C2**: the negative control). V-ATPase distributes diffusely in the cytoplasm of these cells with different density. **A** $\times 200$, **B** $\times 100$, **C** $\times 25$.

Fig. 7

Fig. 8

All examined tissues demonstrated negative staining after replacing the primary antibodies with normal serum (Figs. 2C, 4E1 4E4, 5C, 6B2, 6C2).

The results from surgical specimens of kidney and salivary gland were substantially the same as those from autopsy. This assured that the results mainly obtained from the observation on autopsy specimens were reliable.

Contrasted to V-ATPase, the expression of P-ATPase was found only in parietal cells of the stomach and epithelial cells of the salivary duct (Fig. 3C). All other tissues demonstrated negative immunoreactivity for P-ATPase in their parenchymal cells.

IV. Discussion

In this study, the cross reactivity of rabbit antibody against the A subunit of V-ATPase from bovine chromaffin granules in selected human tissues including kidney and salivary gland was confirmed by the immunoblotting. A reactive band was identified at the position of 72 kDa, suggesting that a similar molecular structure to that in bovine chromaffin granules exists in human tissues. This result proves that the immunohistochemical stainings were reliable.

According to the localization pattern of V-ATPase in

Fig. 9. The immunoreactivity in other cells: **A)** chondrocytes, **B)** squamous cells of skin, **C)** nerve cells from Auerbach's myenteric plexus of colon and **D)** nerve cells from brain. Besides chondrocytes which are stained strongly, the other three kinds of cells are stained in a weak to moderate degree. **A, C & D** $\times 200$, **B** $\times 100$.

Fig. 7. Immunocytochemical staining of macrophages from pulmonary alveoli in serial sections with antibodies against **A)** subunit A of V-ATPase, **B)** CD68, and **C)** cytokeratin. Cells showing a prominent granular staining with the antibody to subunit A of V-ATPase are CD68-positive macrophages, but not cytokeratin-positive type I pneumocytes (arrow). $\times 100$.

Fig. 8. Immunocytochemical staining of osteoclasts shows mainly diffuse cytoplasmic pattern (**A**) and partly diffuse apical cytoplasmic pattern (**D**, arrowhead). Similar diffuse cytoplasmic staining in the same osteoclasts is observed in serial sections using antibodies against **A)** subunit A of V-ATPase, **B)** carbonic anhydrase II (CAII), and **C)** cathepsin D. $\times 200$.

cells, it can be divided into two categories: (1) plasma membrane type in which it functions in proton secretion and subsequently in transport of salt and water, and (2) intracellular membrane type in which it is involved in acidifying the intravesicular compartment of organelles [5, 17, 26, 32, 33]. The former is limited to parts of cells like epithelial cells of the renal tubules, osteoclasts, macrophages, and so on [4, 12, 18, 31, 42, 43], while the latter is thought to have an ubiquitous existence. Corresponding to the above classification, in this study, we observed three staining patterns, that is, 1) diffuse cytoplasmic pattern, which was the most common in positively stained cells, 2) diffuse apical cytoplasm pattern, which was typically observed in the proximal tubules of kidney, and 3) polarized cytoplasmic pattern, which was found typically in the intercalated cells from distal and collecting tubules of kidney and salivary gland. V-ATPase in the latter two patterns may reside in both cytoplasmic vesicles or vacuoles and plasma membrane. It is not yet clear whether the enzyme in different membrane compartments has the same structure. The antibodies we used could identify the V-ATPase localized on plasma membrane, as well as those that existed in the intracellular membrane. This finding suggests that V-ATPase from different membrane compartments in most human cells has immunologically a similar structure as reported by Khadouri *et al.* [22] and Bastani *et al.* [1].

V-ATPase has been intensively studied in renal tubules. Consistent with previous reports [1, 7, 22], we found strong staining in the tubules, though the density and location were variable from tubules to tubules along the urinary stream. In terms of the staining density of V-ATPase of renal tubules, we could assess the following turn: intercalated cells (IC) from distal tubules (DT), cortical collecting tubules (CCT) and inner medullary collecting tubules (IMCT) > cells of proximal tubules (PT) > principle cells (PC) of DT, and CT, and cells of Henle's loop. This distribution tendency corresponds well with the physiological function of H^+ transport, that is, the main part of H^+ secretion in kidney is at CT and then PT, and V-ATPase participates in nearly 1/3 of H^+ secretion at PT and almost all at CT, while Na^+-H^+ exchanger participates in the rest [13]. V-ATPase was all found to be located in the apical cytoplasm of the epithelial cells of PT, DT and CT. This localization pattern suggests that H^+ is pumped out from the intracellular region to the tubular lumen, but not into the blood, as proved in the previous experimental study [13]. Although Brown *et al.* [6, 7] reported that there was a basolateral gradient in intensity of the enzyme expression in some intercalated cells in cortical CT, we found only the apical staining in DT and CT. Such a diversity may be due to the difference between species and/or antibodies employed, because Brown *et al.* made their observation in rabbit kidney and used antibodies against the enzyme from bovine kidney microsome.

Both isozymes of V-ATPase were present in human salivary ducts. They were found to be localized densely in

cytoplasm near the apical plasma membrane. This finding is consistent with observation by Just and Walz [20] in cockroach and observation by Maddrell and O'Donnell [24] in insect. Such a high expression suggests that V-ATPase is actively functioning in salivary duct cells. According to the functions of V-ATPase, it can be generally divided into two categories: one is directly regulating the pH inside organelles, intracellular spaces, and outside of cells by extruding protons across the membrane, and the other is indirectly driving the other ion movements, or regulating further salt transport and water movement by producing an electrical potential difference across the plasma membrane in which they reside. The possible functions of V-ATPase in salivary gland came only from insects, in which a relationship with the secretion of K^+ is proved. The flux of KCl sets up a local osmotic gradient, and enables water to flow into the lumen as pH remains near neutral [23, 24].

V-ATPase has been found in small synaptic vesicles (SSV) and large dense core vesicles (LDCV) of the neuron [8, 38]. The transmembrane proton electrochemical gradient formed by V-ATPase plays a vital role in the movement of neurotransmitters or peptides into the above secretory organelles. Microvesicles (MV) and endocrine granules are the structures in endocrine cells that are similar to SSV and LDCV of the neuron [8]. Recently, V-ATPase was found to exist in MV of the axons of posterior pituitary [30], and some endocrine cells such as pinealocytes [10], β cells of pancreas islets [29] and thyroid parafollicular cells [41]. These V-ATPases play a role in the uptake of norepinephrine, L-glutamate, γ -aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT). Thus it is not surprising that V-ATPase is present in nerve cells and many endocrine cells. Among these cells, the heavily stained cells in pancreatic islets were identified through examination of serial sections, and were found to be compatible with α cells secreting glucagon. However, it is not clear whether V-ATPase is related to the release of the glucagon or not.

It is known that mechanical loading of cartilaginous tissue generates an increase in the concentration of cations in the extracellular matrix. This is accompanied by a decrease of the extracellular pH, leading to a drop in the intracellular pH, namely acidification [11, 35]. Since cartilaginous tissues are exposed to continuous compressing stress [37], causing variation in pH, the extrusion of H^+ by H^+ transporters is active, and the transporters themselves should be well developed. We think this is the reason for highly concentrated V-ATPase are found in chondrocytes. Our findings are also consistent with those of Dascalu *et al.* [11], who proved by chemical analysis that 25% of H^+ transportation comes from V-ATPase as compared with other H^+ transporters.

Cells of a macrophage lineage include macrophages, osteoclasts, and dendritic cells. The immunoreactivity for V-ATPase in these cells was intense or moderate. Although the presence of V-ATPase in lysosomes of mac-

rophages has been well documented [16], its localization in the plasma membrane still lacks direct evidence. In this study, strong staining with a granular pattern observed in the cytoplasm is the non-polarized pattern. Thus we could not make a judgment whether there was a plasma membrane type of V-ATPase localization in macrophages because it was difficult to distinguish the plasma membrane type from the strongly stained cytoplasmic type at the light microscopic level.

Osteoclasts are multinucleated cells that are responsible for resorption of bone under the condition of acidification of the extracellular lacunae formed between osteoclasts and bone surface, resulting in demineralization, activating proteinases, and further absorption of bone [9, 14]. Proton pump and carbonic anhydrase [21] are thought to be important in the acidification of extracellular lacunae and activation of lysosomal enzymes such as cathepsins. Since these data were based on separate observations, the question as to whether lysosome enzymes and V-ATPase are present in the same osteoclasts should be answered. Thus we observed the localization of CAII, cathepsin D and V-ATPase in serial sections of the same tissue. V-ATPase was found in a diffuse cytoplasmic pattern, sometimes in a diffuse apical cytoplasmic one, and the dense distribution of CA II and cathepsin D in the same osteoclasts was proved. This suggests a intimate relationship among the three enzymes. The variable functional status of osteoclasts may be a reason for the different distribution of V-ATPase in osteoclasts, because it is known that osteoclasts have two types, such as resting ones and activated ones, and the polarized type may be represented as the activated one [3, 36].

Contrasted to V-ATPase, P-ATPase is only expressed in the parietal cells in stomach and epithelial cells in salivary ducts. The localization of P-ATPase in the base of the tubular cells of kidney and epithelial cells of rabbit distal colonic gland was reported previously [40, 44], but it was not found in this study.

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VI. References

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