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Immunocytochemical and enzymecytochemical studies on the intracellular transport mechanism of secretory immunoglobulin A and lactoferrin in human salivary glands

Tsuneaki Nakamura [1], **Hiroshi Nagura** [2], **Noriyuki Komatsu** [3], **and Keiichi Watanabe** [4]

[1] Applied Research Laboratories, Lion Corporation, Tajima 100, Odawara, 256
[2] Laboratories of Germfree Life Research, Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Shyowa-ku, Nagoya, 466
[3] Cell Biology Research Institute and [4] Department of Pathology, Tokai University School of Medicine, Bohseidai, Isehara, 259-11, Japan

Summary. The intracellular transport mechanism of secretory immunoglobulin A (sIgA) has been immunocytochemically defined in human submandibular glands. To examine the properties of the intracytoplasmic vesicles which contain IgA, the enzyme labeled antibody method for SC and IgA and Novikoff's method for acid phosphatase (ACPase) activity were employed on the same sections. The intracytoplasmic vesicles containing IgA and SC in the serous acinar cells were free of ACPase activity and were thus distinguishable from lysosomes. Neither IgA nor SC was localized in the secretory granules.

Lactoferrin was localized in the secretory granules and glandular lumen of the serous acinar cells, but not in the cytoplasmic vesicles, which were also free of ACPase activity. These findings suggests that the transport of sIgA was performed by intracytoplasmic vesicles and that lactoferrin is discharged from secretory granules into the lumen and finally makes a "rendezvous" with sIgA in the lumen of acinar cells.

Key words: Immunoglobulin A – Secretory component – Lactoferrin – Acid phosphatase – Immunocytochemistry

Secretory immunoglobulin A (sIgA), complex of dimeric IgA and secretory component (SC), plays an important role in the defense mechanisms against microbial antigens on mucosal surfaces (Brown 1978). The intracellular

Offprint requests to: T. Nakamura at the above address

transport mechanism of sIgA has been immunocytochemically defined in human submandibular glands in a previous study (Nakamura et al. 1982). It was suggested that dimeric IgA binds specifically to SC on the plasma membrane of serous acinar cells and ductal epithelial cells, is then endocytosed and transported by vesicles through the cytoplasm and discharged into the lumen at the apical surface as sIgA, a similar mechanism to that described by Brown (1978), Nagura et al. (1979) and Brandtzaeg (1981).

However, it is well known that lysosomes fuse to endocytic vesicles and digest endocytosed substances with lytic enzymes in the cytoplasm. This study aimed at understanding the specific properties of intracytoplasmic vesicles which contain IgA and distinguishing them from phago-lysosomes. In the present work immunocytochemical and enzymecytochemical methods were employed for localization of IgA, SC and acid phosphatase (ACPase), a lysosomal marker enzyme, on the same sections. Furthermore the localization of lactoferrin, a bacteriostatic iron-binding glycoprotein (Arnold et al. 1977; Arnold et al. 1980) was compared with sIgA by immunoelectronmicroscopical observation.

Materials and methods

Samples. The biopsy specimens of human submandibular glands were immediately fixed with periodate-lysine-4% paraformaldehyde (PLP) (McLeen and Nakane 1974) solutions for 6 hours at 4° C. The tissues were washed in increasing concentrations of sucrose in PBS and embedded in OCT compounds (Lab Tek products, Il, USA). The sections were sliced at 10 μm thick in a cryostat and dried in room air.

Antibodies. Rabbit anti-human IgA, anti-human SC and antihuman lactoferrin were purchased from Dakopatts (Kyowa Medics, Tokyo, Japan), and antibody specificy of these antisera was documented by electrophoresis and immunodiffusion in agarose gel as previously described (Nakamura et al. 1982). The Fab' fragment of the γ-globulin fractions of these antisera were labeled with horseradish peroxidase (HRP) after Wilson and Nakane (1978). For use in control experiments the Fab' fragments of nonimmune rabbit γ-globulin were also labeled with HRP.

Immunocytochemistry and enzyme cytochemistry. The immunocytochemical procedure was based on the direct peroxidase-labeled antibody method as reported in the previous study (Nakamura et al. 1982) and enzyme cytochemistry was based on the Novikoff's method (Novikoff 1963). The sections were rinsed in 0.01M phosphate buffered saline pH 7.6 (PBS), immersed in 1% nonimmune rabbit serum for 10 min, and then incubated with HRP-labeled Fab' fragments of the antisera or non immune rabbit serum 4° C for over night. After fully washing in PBS, the sections were fixed in 0.5% glutaraldehyde in PBS for 10 min and washed in 0.1M Tris maleate buffer pH 7.4. They were treated with the Gomori-Novikoff's solution for 10, 20 or 30 min at room temperature, and washed sequentially with the Tris maleate buffer and 0.05M Tris HCl buffer, pH 7.6. And then they were incubated in 0.02% diaminobenzidine (DAB) for 30 min and continuously in DAB solution containing 10 mM hydrogen peroxide and 10 mM sodium azide to inactivate endogeneous peroxidase for 10 min. After osmification by 2% osmium tetraoxide, the sections were dehydrated in graded ethanol and embedding by Quetol 812. Ultrathin sections, either unstained or stained with lead citrate, were viewed using Hitachi H-600 electron microscopy.

Results

In serous type acinar cells, SC was localized on the basolateral and apical plasma membranes, in the glandular lumen, and in the cytoplasmic vesicles,

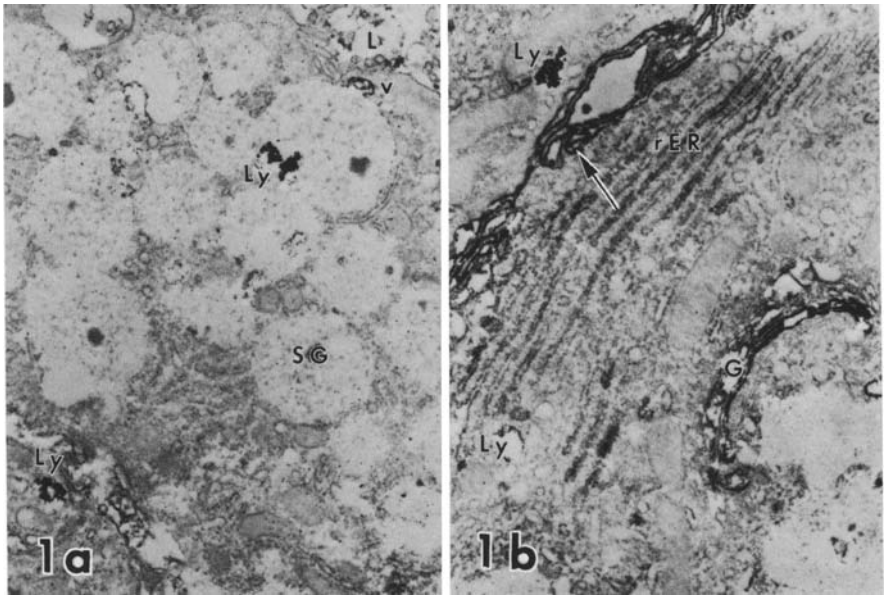

Fig. 1a The supranuclear and **b** basal regions of serous acinar cells, in which SC and ACPase activity are simultaneously stained. ACPase activity was present in the vacuoles of the lysosomes (*Ly*) which are free from SC. SC-containing cytoplasmic organelles; the cytoplasmic vesicles (*v*), rough endoplasmic reticulum (*rER*) and the Golgi complex (*G*), and basolateral plasma membranes (→) show no ACPase activity. **a** ×15,000, **b** ×15,000

but was not seen in secretory granules (Fig. 1). In addition, SC was also present in the protein-synthesizing organelles such as the perinuclear spaces and rough endoplasmic reticulum, and in the Golgi complex, suggesting that SC is synthesized in these cells (Fig. 1). IgA was localized on the basolateral and apical plasma membranes, in the glandular lumen, in the endocytic invaginations along the plasma membrane, in the cytoplasmic vesicles where SC was present, and in basement membranes and the interstitium (Fig. 2b). The vesicles containing SC or IgA in the apical cytoplasm occasionally opened into the lumen (Fig. 2a) or sometimes fused to secretory granules which existed beneath the luminal surfaces (Fig. 1a).

ACPase activity was identified in lysosomes ar larger and more electron-dense granules when compared with the reaction product of HRP labeled with anti-SC or anti-IgA sera, but was not seen in the secretory granules (Fig. 1 and 2). The lysosomes did not fuse to secretory granules even when they were adjacent to each other. The vesicles containing SC of IgA were also free from the ACPase activity and definitely distinguishable from lysosomes.

Lactoferrin was present in secretory granules and occasionally in glandular lumens in serous acinar cells (Fig. 3). The staining intensity of lactoferrin varied considerably between different secretory granules and was sometimes associated with rough endoplasmic reticulum.

Control sections for IgA, lactoferrin and SC were uniformely negative.

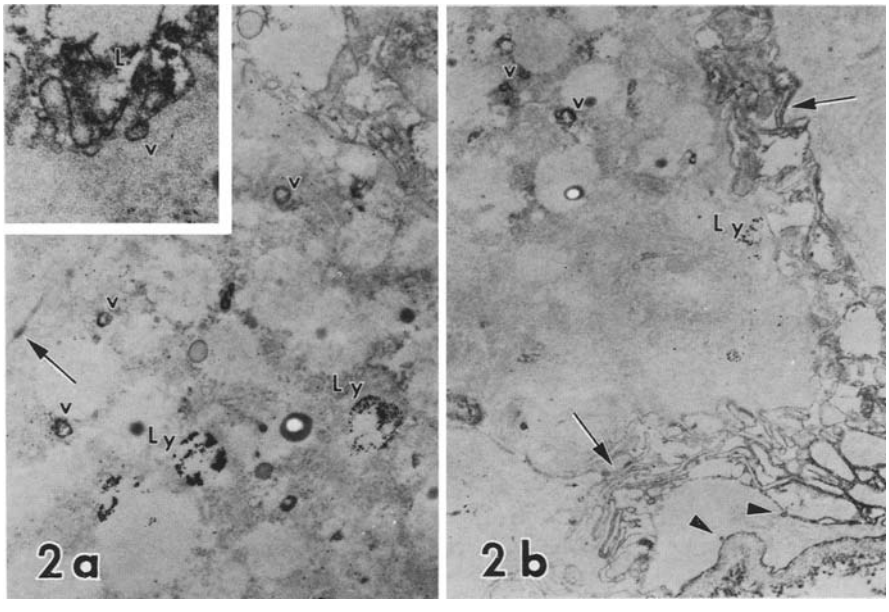

Fig. 2a The supranuclear and **b** basal regions of serous acinar cells, in which IgA and ACPase activity are simultaneously stained. The lysosomes (*Ly*) showing ACPase activity are distinguishable from the cytoplasmic vesicles (*v*) containing IgA. IgA is present on the apical and basolateral plasma membrane (→), endocytic vesicles (►) and in the glandular lumen (*L*), but not in the secretory granules (*SG*). The vesicles containing IgA (v) are sometimes opening to the lumen (*insert*). **a** ×16,000, **b** ×13,000

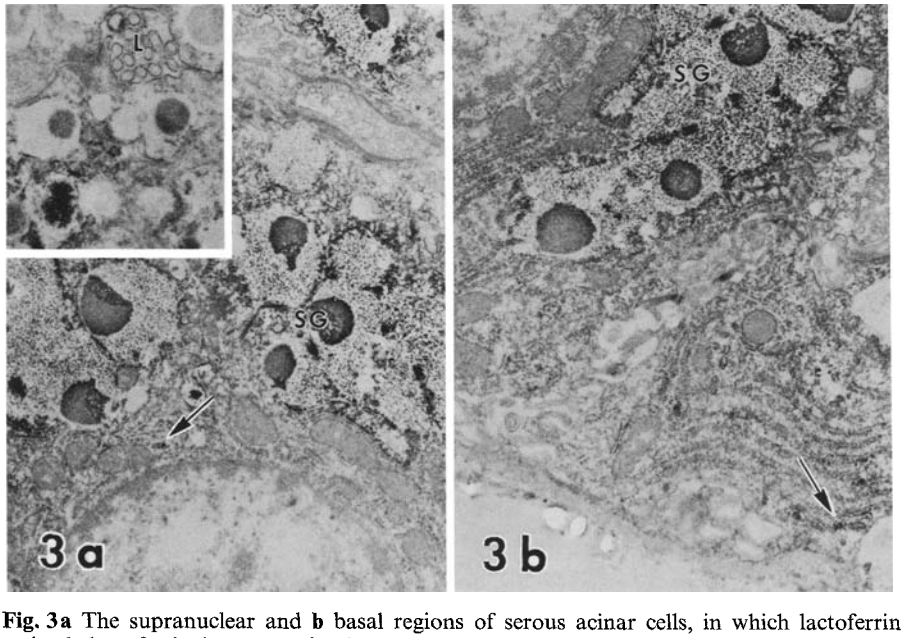

Fig. 3a The supranuclear and **b** basal regions of serous acinar cells, in which lactoferrin is stained. lactoferrin is present in the secretory granules (*SG*) and the glandular lumen (*L*), and sometimes associated with rough endoplasmic reticulums (→). **a** ×12,000, **b** ×8,000

Discussion

sIgA is secreted from salivary glands into the oral cavity. We reported in previous study that the SC-mediated transcellular transport of IgA occurs through intracytoplasmic vesicles in serous acinar cells (Nakamura et al. 1982). In present study, simultaneous staining by immunocytochemical and enzymecytochemical methods proved that lysosomes, acid phosphatase positive organelles, and cytoplasmic vesicles cotaining SC and IgA or secretory granules did not fuse with each other. It is suggested, following this and our previous studies on salivary glands (Nakamura et al. 1982) and digestive organs (Nagura et al. 1979; Nagura et al. 1981) that IgA, after being formed on the basolateral surface of the cells is transported as SC-IgA complex (sIgA) in cytoplasmic vesicles to the lumen without intracellular digestion. Such a transcellular vesicular transport mechanism has already been shown in the FC recepter-mediated transport of IgG through the epithelium of neonatal intestine (Nagura et al. 1978).

Lactoferrin, iron-binding glycoprotein, is presented in various human mucosal secretions (Masson and Hermans 1966; Friedman et al. 1983) and known to have bacteriostatic effects on some micro-organisms (Arnold et al. 1977; Arnold et al. 1980); Arnold et al. 1982; Stephen et al. 1980). Tourville et al. (1969) Caselitz et al. (1981), Korsrud and Brandtzaeg (1982) and Moro et al. (1984) localized lactoferrin in serous acinar cells of human salivary glands by immunofluorescene or immunoperoxidase staining. Our present observations showed that lactoferrin was present in secretory granules, which were also free from acid phosphatase activity, and in the glandular lumen, but not in the cytoplasmic vesicles where SC and sIgA were identified. These findings suggest that lactoferrin is transported by a different intracytoplasmic pathway from sIgA, discharged and finally co-exists with sIgA in the lumen of acinus.

We demonstrated that lactoferrin binds to sIgA in the secretions, and speculated that sIgA-lactoferrin complexes play a protective role against microbial infections on mucous membranes (Watanabe et al. 1984). Our ultrastructural observations provide evidence that a possible binding site of sIgA and lactoferrin exists on the external surfaces of mucous membranes, for example, on the acinar lumenal aspect of salivary glands.

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