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Immunoelectronmicroscopic study on the transport of secretory IgA in the lower respiratory tract and alveoli

Hajime Haimoto[1], Hiroshi Nagura[2], Munehisa Imaizumi[3], Keiichi Watanabe[4], and Soichi Iijima[1]

[1] Department of Pathology, [2] Laboratory of Germfree Life Research Institute for Disease Mechanism and Control, and [3] Department of Surgery, Nagoya University School of Medicine, 65 Tsuruma, Showa, Nagoya 466; [4] Department of Pathology Tokai University School of Medicine, Isehara, Japan

Summary. To define the immunocytochemical localization of secretory component (SC), IgA and J chain in human bronchioles and alveoli, a direct peroxidase-labeled antibody method was used. SC was found in non-ciliated cells of the bronchioles including respiratory bronchioles and type II alveolar epithelial cells, whereas SC was rarely present in ciliated cells and type I alveolar epithelial cells and was absent from goblet cells. In the positively reacting cells, SC was found in secretory protein synthetic organelles such as perinuclear spaces and endoplasmic reticulum, Golgi complexes, and on the external surfaces of the apical and basolateral plasma membranes. IgA and J chain were localized in the epithelial cells where SC was found. Ultrastructually IgA was present on the apical and basolateral plasma membranes, in pinocytic invaginations of the membranes, and in vesicles distributed through the cytoplasm, especially in the apical cytoplasm of the epithelial cells where SC was found. In addition, IgA and J chain were found to be associated with the endothelial cells of the capillaries, plasma cells and the surrounding interstitium. These observations suggest that SC is synthesized and secreted by epithelial cells, especially non-ciliated cells of the bronchioles including respiratory bronchioles and type II alveolar epithelial cells. They also suggest that secretory IgA (sIgA) is transported into alveolar spaces and the bronchiolar lumen through these cells by SC-mediated transport mechanism. This sIgA may play an important role in defense mechanisms of the lower respiratory tract and alveoli.

Key words: IgA-Secretory Component – J chain – Lung – Immunocytochemistry

The secretory form of IgA (sIgA) is a major immunoglobulin in bronchial secretions and other external body fluids (Tomasi et al. 1965; Tomasi and

Offprint requests to: H. Haimoto at the above address

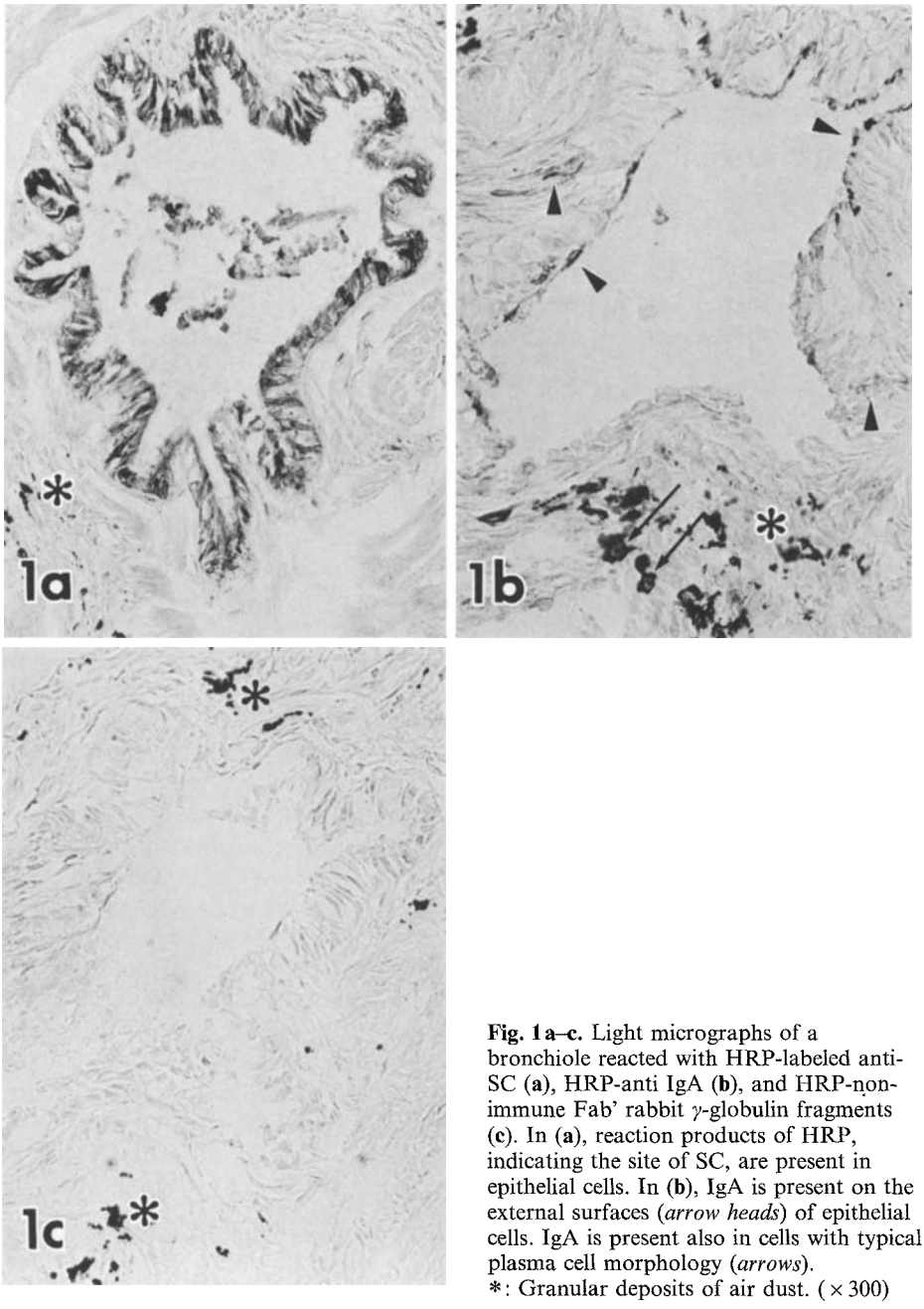

Fig. 1 a–c. Light micrographs of a bronchiole reacted with HRP-labeled anti-SC (**a**), HRP-anti IgA (**b**), and HRP-non-immune Fab' rabbit γ-globulin fragments (**c**). In (**a**), reaction products of HRP, indicating the site of SC, are present in epithelial cells. In (**b**), IgA is present on the external surfaces (*arrow heads*) of epithelial cells. IgA is present also in cells with typical plasma cell morphology (*arrows*). *: Granular deposits of air dust. ($\times 300$)

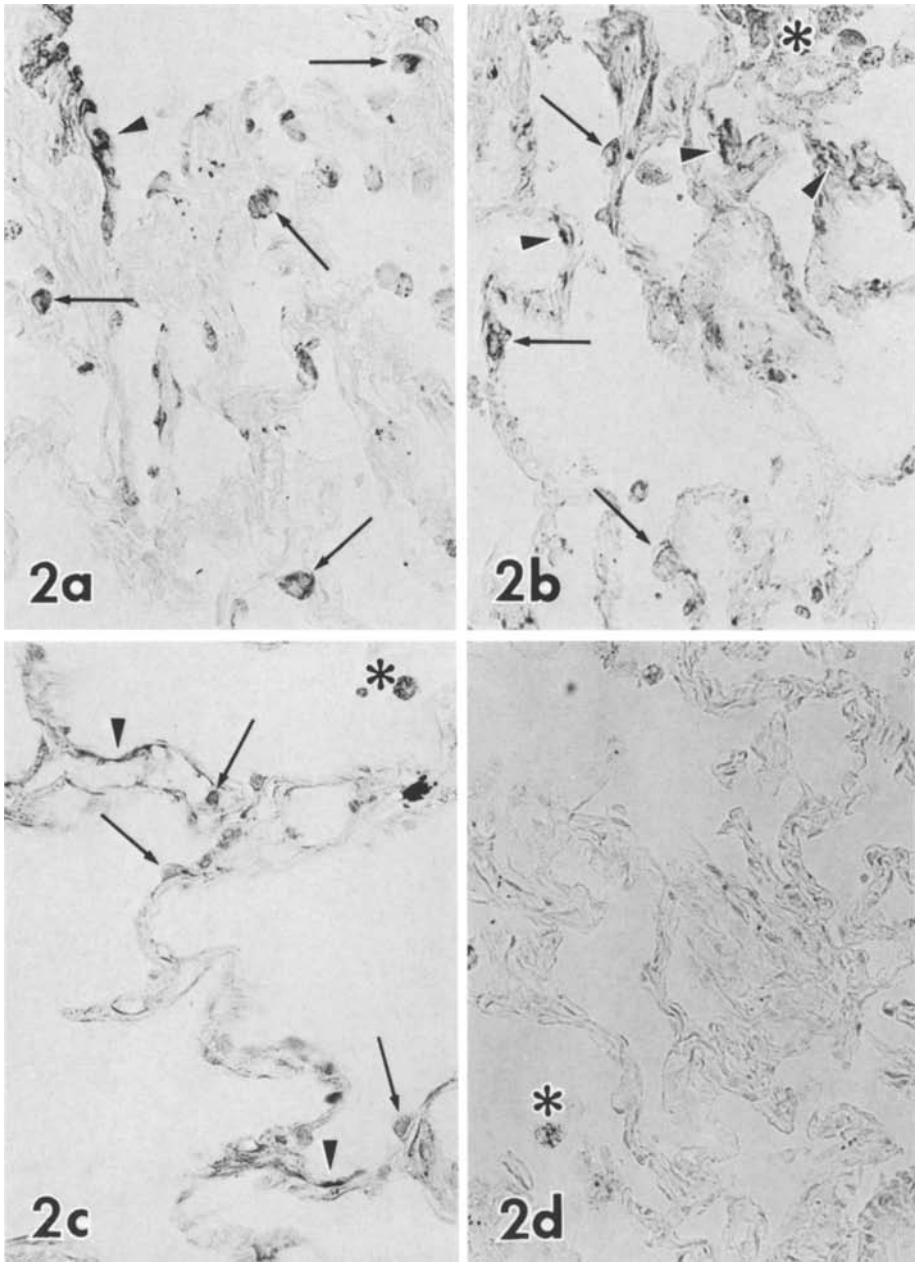

Fig. 2a–d. Light micrographs of a respiratory bronchiole and alveoli reacted with HRP-anti-SC (**a**), HRP-anti-IgA (**b**), HRP-anti-J chain (**c**) and HRP-non-immune Fab' rabbit γ-globulin fragments (**d**). In (**a**), SC is present in the epithelial cells of the respiratory bronchiole (arrow head) and type II alveolar epithelial cells (*arrows*). In (**b**) and (**c**), IgA and J chain are present also in type II alveolar epithelial cells (arrows) and in the capillary lumen of the alveolar septa (arrow heads). Several alveolar macrophages (*) are observed. ($\times 300$)

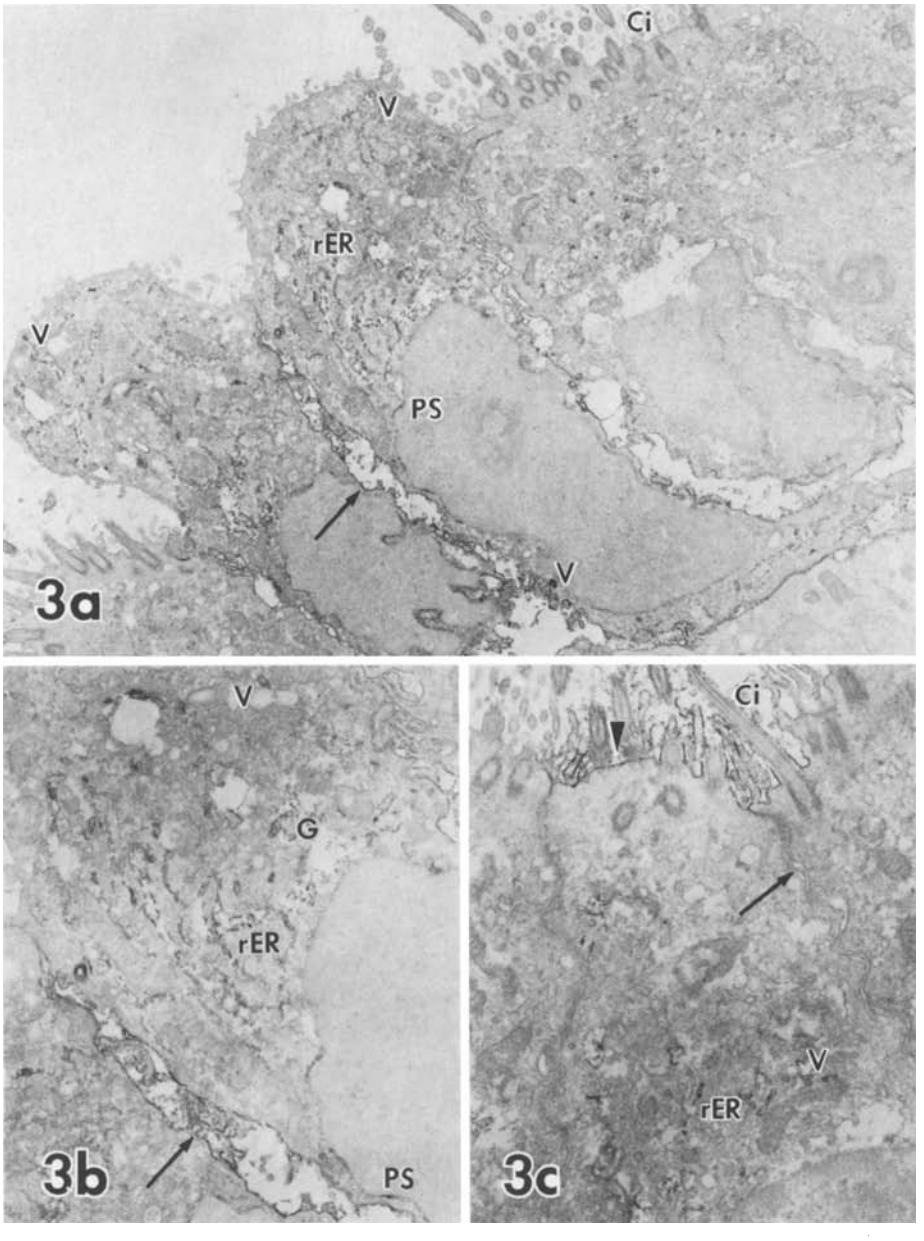

Fig. 3a–c. Electron micrographs of epithelial cells of a bronchiole reacted with HRP-anti-SC. In (**a**) and (**b**), there is a sharp contrast in the SC-staining of non-ciliated cells and ciliated cells (*Ci*). SC is present in the perinuclear spaces (*PS*), rough endoplasmic reticulum (rER), Golgi complexes (*G*), and cytoplasmic vesicles (*V*). *SC* is present also along the lateral plasma membrane (arrow). In (**c**), SC is present much less frequently in the rough endoplasmic reticulum (*rER*) and cytoplasmic vesicles (*V*) of ciliated cells than of non-ciliated cells. Occasionally, SC is localized along the apical plasma membrane (*arrow head*), but not along the lateral plasma membrane (*arrow*). (**a**: × 7,000, **b**: × 11,000, **c**: × 11,000)

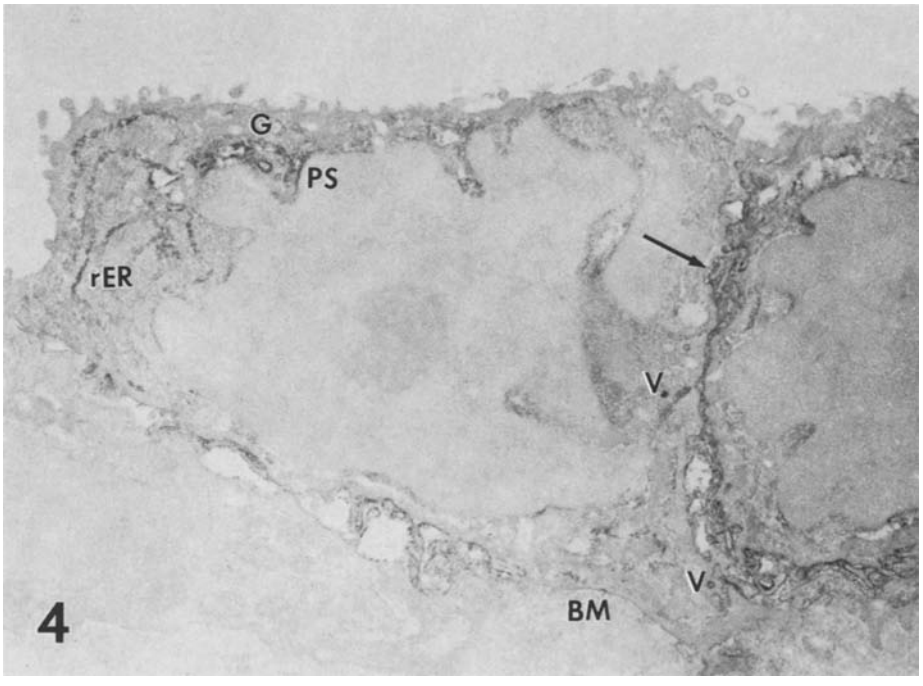

Fig. 4. Electron micrograph of the epithelial cell in a respiratory bronchiole reacted with HRP-anti-SC. *SC* is present in the perinuclear spaces (PS), rough endoplasmic reticulum (*rER*), Golgi complexes (*G*) and cytoplasmic vesicles (*V*). In addition, *SC* is present prominently along the lateral plasma membrane (*arrow*). *BM*: Basement membrane. (× 11,000)

Grey 1972), and consists of two molecules of IgA linked by a peptide J chain and a glycoprotein, secretory component (SC) (Koshland 1975; Brandtzaeg 1976a). The events in the assembly and transfer of sIgA into external fluids such as jejunal juice and bile have been defined by immunocytochemical methods (Brandtzaeg 1974; Brown et al. 1976; Nagura et al. 1979a; Nagura et al. 1981; Nagura et al. 1983). In the intestine, IgA and J chain molecules are synthesized in plasma cells in the lamina propria, whereas SC is synthesized in epithelial cells and expressed on the basolateral surfaces of the cells (Brandtzaeg 1974; Brown et al. 1976; Nagura et al. 1979a). The J chain-linked IgA dimer binds specifically to the SC on the epithelial surfaces and is internalized by endocytosis, and the vesicles containing SC-IgA complexes are transferred to the apices of the cells and released as sIgA into the luminal surfaces (Crago et al. 1978; Nagura et al. 1979b; Brown et al. 1976).

In the lung, little is known about the transcellular transport mechanism of sIgA although sIgA has been reported to be a major immunoglobulin component of the lung surfactant and bronchial secretion (Paciga et al. 1980; Neuhouse et al. 1976). Recntly it was shown that IgA dimer can be translocated into the lumen by human bronchial glandular epithelium (Goodman et al. 1981), but the question of whether the epithelial cells of

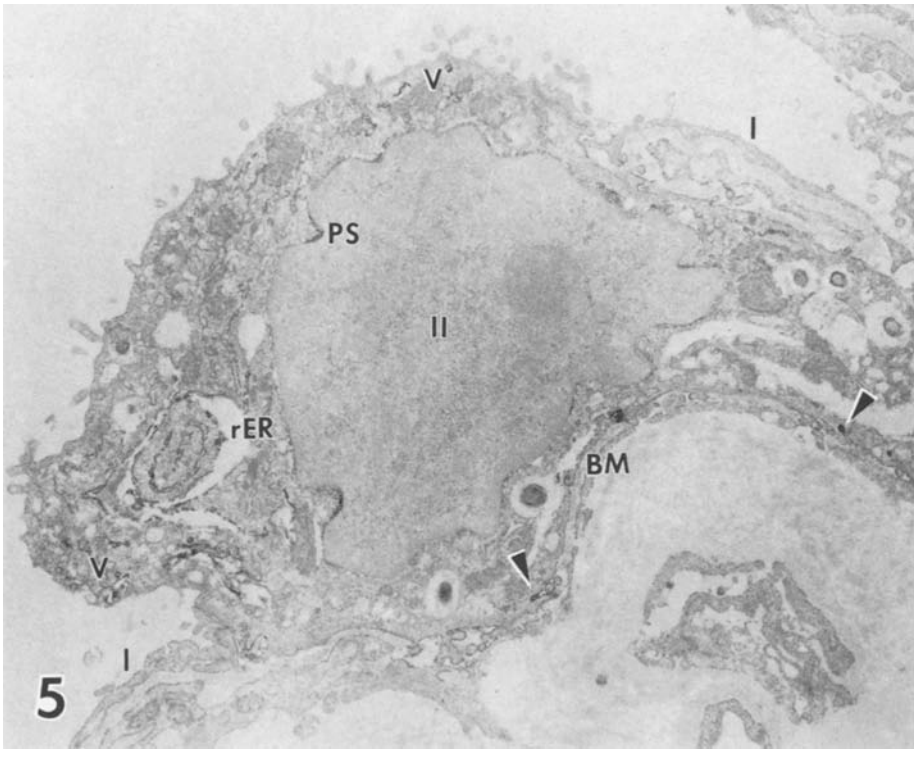

Fig. 5. Electron micrograph of an alveolus, reacted with HRP-anti-SC. *SC* is present in the perinuclear spaces (*PS*), rough endoplasmic reticulum (*rER*) and cytoplasmic vesicles (*V*) of a type *II* alveolar epithelial cell (*II*). SC is also present on the basal plasma membrane and in endocytic like vesicles (*arrow heads*) just inside the plasma membranes. *BM*: Basement membrane, *I*: Type *I* alveolar epithelial cell. (× 10,000)

the lower respiratory tract and alveoli participate in the secretion of sIgA has not been answered. Therefore, we have employed peroxidase-labeled antibody immunoelectronmicroscopy to localize SC, IgA and J chain in the lower respiratory tract and alveoli of the human lung.

Materials and methods

Patients and tissue specimens. Histologically normal tissues were obtained from 6 patients undergoing lobectomy or pneumonectomy for bronchogenic carcinoma at Nagoya University Hospital. The tissues were promptly fixed in periodate-lysine-4%paraformaldehyde (PLP) (McLean and Nakane 1974), for 6 h, washed in phosphate-buffered saline (PBS) containing increasing concentrations of sucrose, and frozen in OCT compound (LAB Tek products, Naperville IL, USA).

Antibodies. Rabbit anti-human SC and anti-human α chain were purchased from Dakopatts (Kyowa-Medics, Tokyo, Japan). Rabbit anti-human J chain was produced in our laboratory (Kobayashi et al. 1973). Antibody specificity was established by immunoelectrophoresis and immunodiffusion in agarose gel as previously described (Nagura et al. 1981; Nakamura et al. 1982). The Fab' fragments of γ-globulin fractions were labeled with horseradish peroxidase (HRP) (Toyobo, Tokyo) according to the method of Wilson-Nakane (Wilson and Nakane

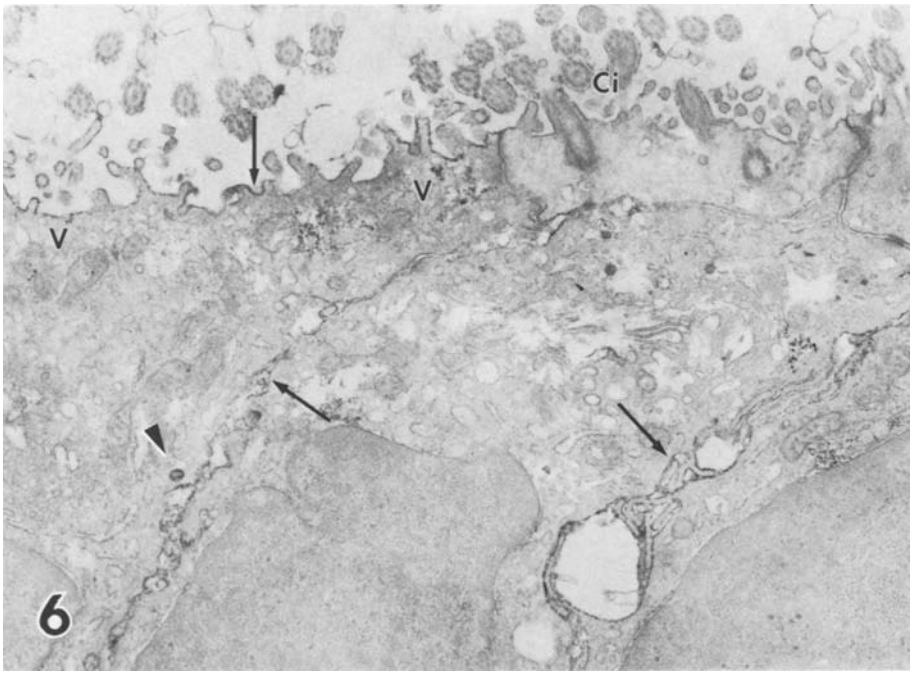

Fig. 6. Electron micrographs of the epithelial cells in a bronchiole reacted with HRP-anti-IgA. IgA is present on the lateral and apical plasma membranes (*arrows*) and in cytoplasmic vesicles (*V*) of non-ciliated cells. IgA is also present in the endocytic invaginations just inside the lateral plasma membrane (*arrow head*). IgA is not observed in a ciliated cell (*Ci*). (× 14,000)

1978). For use in control experiments, Fab' fragments of non-immune rabbit γ-globulin were conjugated with HRP.

Immunohistochemistry. SC, IgA and J chain were localized in the lung tissues by the direct peroxidase-labeled antibody technique used previously in intestine and biliary tract (Nagura et al. 1979a; Nagura et al. 1981; Nagura et al. 1983).

Light microscopy. Sections were cut on a cryostat, placed on albumin-coated slides, and dried at room temperature. The sections were treated with 100% methanol and 0.3% hydrogen peroxide in PBS to inactivate endogenous peroxidase. They were then immersed in 10% non-immune rabbit serum, washed in PBS, and reacted either with the HRP-labeled Fab' fragments of the antisera or non-immune rabbit serum. In addition, control sections were reacted with the conjugated antibodies absorbed with the purified relevant antigens. After washing in PBS, the sections were reacted with 0.25% diaminobenzidine (DAB) solution containing 10 mM hydrogen peroxide and 10 mM sodium azide, and then counterstained with methylgreen. For the detection of J chain (the antigenic determinants of which are masked in IgA molecules) cryostat sections were treated with 6 M acid urea in glycine-HCl buffer (pH 3.2) for about 20 h at 4 °C before staining (Brandtzaeg 1976b; Nagura et al. 1979a).

Electron microscopy. Sections to be examined by electronmicroscopy after being reacted with the HRP-labeled antibodies, were postfixed in 1% glutaraldehyde in PBS, and incubated sequentially in 0.25% DAB solution for 30 min and 0.25% DAB solution containing 10 mM hydrogen peroxide for 10 min. The sections were washed, fixed in 2% osmium tetroxide in phosphate buffer (pH 7.6), dehydrated in graded alcohol, and embedded in Quetol 812. Ultrathin sections were viewed with a JEOL 100C electronmicroscope.

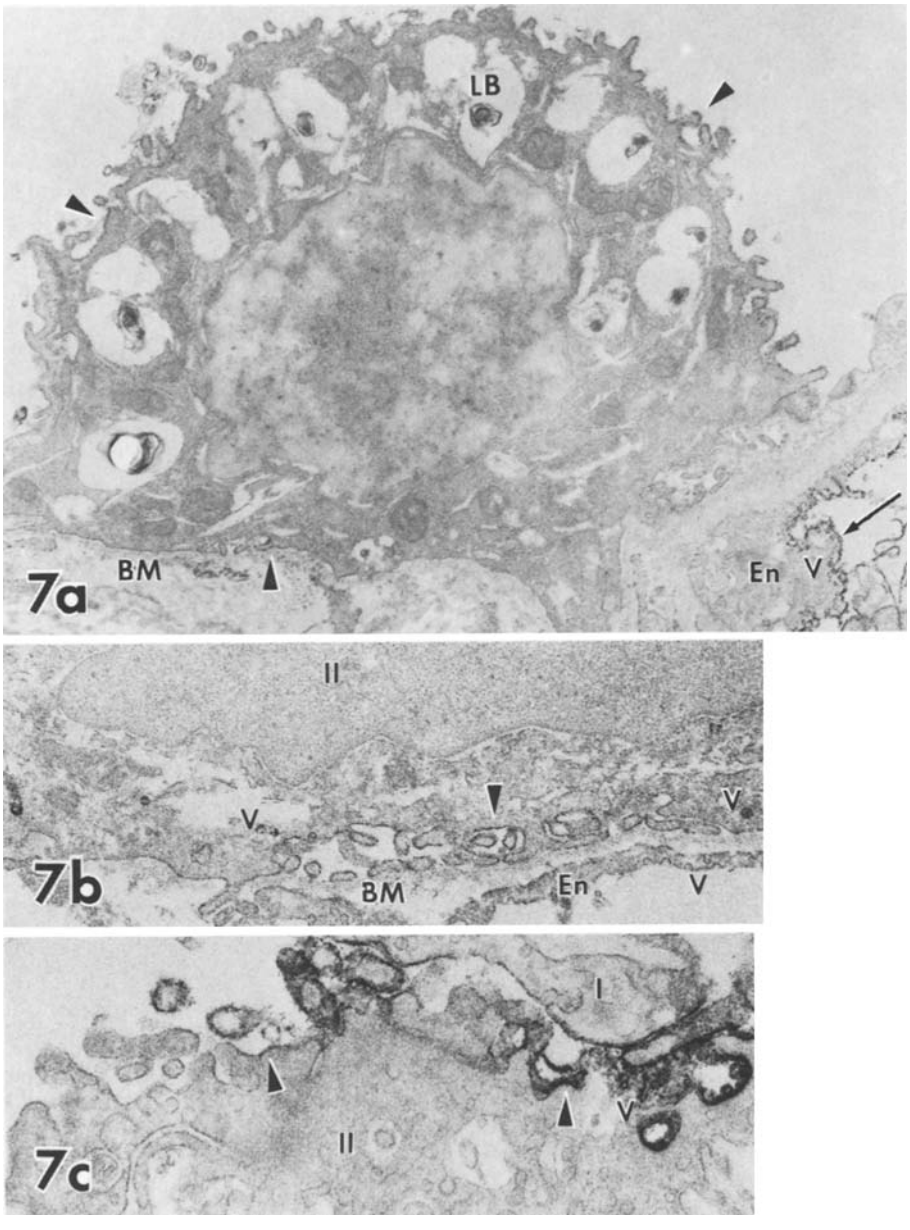

Fig. 7a–c. Electron micrographs of the type *II* alveolar epithelial cells (*II*) reacted with HRP-anti-IgA. In (**a**), (**b**) and (**c**), IgA is present along the external and basal surfaces of the plasma membrane (*arrow heads*), and in endocytic like vesicles (*V*) just inside them. In (**a**) and (**b**), *IgA* is also localized on the luminal surface (*arrow*) and micropinocytic vesicles (*V*) of capillary endothelial cells (En). In (**c**), IgA is found to be associated with the luminal surface and endocytic vesicles of the type *I* alveolar epithelial cell (*I*) adjacent to the type *II* alveolar epithelial cell (*II*). *BM*: Basement membrane; *LB*: Lamellar inclusion body. (**a**: × 12,000, **b**: × 18,000, **c**: × 25,000)

Results

Light microscopy

SC was distributed on the surface and the cytoplasm of some of the epithelial cells lining the bronchioles including respiratory bronchioles (Figs. 1a and 2a). In alveoli, SC was also found along the surfaces and intracellularly in the type II alveolar epithelial cells (Fig. 2a). The type I alveolar epithelial cells were scarcely stained.

IgA was present on the external surfaces of epithelial cells in bronchioles and type II alveolar epithelial cells where SC was located (Figs. 1b and 2b). In addition IgA was found in the interstitial spaces of the bronchioles and alveoli, and along the luminal surface of capillary endothelial cells (Fig. 2b). A few plasma cells containing IgA were found beneath the epithelial cells of bronchioles (Fig. 1b). The distribution of J chain in the lung after the acid urea treatment mostly corresponded to that of IgA (Fig. 2c). The control sections were uniformly negative (Figs. 1c and 2d).

Electron microscopy

Immunoelectronmicroscopy of the specimens corroborated the light microscopic findings. SC was found in non-ciliated cells of the bronchioles including the respiratory bronchioles and in type II alveolar epithelial cells (Figs. 3a, b, 4 and 5), while SC was rarely present in the ciliated cells (Fig. 3c). In both cells, SC was demonstrated within the rough endoplasmic reticulum, perinuclear spaces and Golgi complexes. SC was also identified in association with the plasma membranes, endocytic invaginations and cytoplasmic vesicles of these cells (Figs. 3b, 4 and 5), and occasionally with the luminal surface and endocytic vesicles of the type I alveolar epithelial cells adjacent to the type II alveolar epithelial cells. In the goblet cells of the bronchiolar lining, SC was not found.

IgA was localized along the external surfaces of apical and basolateral plasma membranes of epithelial cells, where SC was present (Figs. 6, and 7a–c). There were numerous IgA-containing endocytic invaginations of the IgA positive plasma membranes and cytoplasmic vesicles located beneath the apical surfaces of the non-ciliated cells (Fig. 6). Occasionally the vesicles appeared to open into the lumen. A small amount of IgA was seen also along the external and basal surfaces and in the endocytic invaginations of type II alveolar epithelial cells (Fig. 7a–c). In addition, IgA was found to be associated with the luminal surface and micropinocytic vesicles of capillary endothelial cells (Fig. 7a and b), and occasionally those of the type I alveolar epithelial cells adjacent to the type II alveolar epithelial cells (Fig. 7c). Extracellular IgA staining was observed also in the basement membranes and surrounding interstitium.

Discussion

The majority of investigations of mucosal immunity in the respiratory system have focused on the IgA system of the upper respiratory tract (Martinez-

Tello et al. 1968; Rossen et al. 1968; Soutar 1976), and little is known about IgA in the lower respiratory tract and alveoli. The results of this immunocytochemical study, together with compatible studies previously made on the intestine, biliary tract and salivary glands (Nakamura et al. 1982; Nagura et al. 1979a; Nagura et al. 1979b; Nagura et al. 1981; Nagura et al. 1983) have suggested that IgA is transported into alveolar spaces and the bronchiolar lumen by SC-mediated endocytic transfer across type II alveolar epithelial cells and epithelial cells, particularly non-ciliated cells of bronchioles and respiratory bronchioles other than bronchial glands.

SC was identified in the type II alveolar epithelial cells as well as in the non-ciliated cells ranging from bronchioles to respiratory bronchioles. There was evidence of SC synthesis in these cells; ultrastructurally, SC was found to be present in the perinuclear spaces, rough endoplasmic reticulum and Golgi complexes. SC was also associated with the external surfaces and endocytic invaginations of the basolateral plasma membranes and intracellular vesicles.

IgA and J chain were found in identical sites. These findings are compatible with the transfer of J chain-containing dimeric IgA across the epithelial cells of the intestine (Brandtzaeg 1974; Brown et al. 1976; Nagura et al. 1979a), salivary glands (Brandtzaeg 1977; Nakamura et al. 1982), biliary tract (Nagura et al. 1981; Nagura et al. 1983) and bronchial gland (Goodman et al. 1981) by SC-mediated endocytosis with SC serving as an IgA receptor.

The origin of locally synthesized IgA in the alveoli is not known because few plasma cells are present in the alveolar septa. A vascular source similar to that shown in the biliary tract (Nagura et al. 1981) seems most likely. We have observed that IgA is associated with capillaries that surround the bronchioles and alveoli. Immunoelectronmicroscopically, IgA was present within the lumen of the capillaries, in endocytic vesicles of the endothelial cells and in intercellular spaces between adjoining endothelial cells and on the basement membrane. IgA was also present in the connective tissue between the capillaries and the epithelial cells. J chain was found in the same location as IgA, suggesting that at least some of the IgA was dimeric. Thus the proportion of the dimeric IgA that is derived by transfer from plasma might travel across or between the endothelial cells. On the other hand, there are a few IgA plasma cells in the peribronchiolar area. Thus, in addition to the vascular source, these IgA plasma cells are a likely source of the secreted IgA through the bronchiolar epithelium.

It has been reported that microorganisms fail to demonstrate in normal alveoli and bronchioles (Lees and McNaught 1959; Laurenzi et al. 1960). It is claimed that IgG and IgM are the predominant immunoglobulins having antibacterial activity in the secretions of the lower respiratory tract (Hand and Cantey 1974; Kaltreider et al. 1974). However, IgG can not pass through the tight junctions of the epithelium, nor be transported through the cytoplasm like IgA in normal conditions (Bernaudin et al. 1982). As a result of damage to the epithelial lining of the mucosa, it is supposed that IgG leaks through the interepithelial spaces into the mucosal lumen

(Brandtzaeg 1973). From our observations, it is suggested that sIgA-mediated immunity is required for protection against certain microorganisms in the lower respiratory tract and alveoli in the normal state.

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