Virchows Arch [Pathol Anat] (1983) 400:223–234

Virchows Archiv A

Immunohistological analysis of surfactant-apoprotein in the bronchiolo-alveolar carcinoma

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Summary. Fifty-five cases of bronchiolo-alveolar carcinoma were examined immunohistochemically using mono-specific antisurfactant apoprotein IgG obtained from a rabbit immunized with monkey surfactant preparations. Formalin-fixed and paraffin-embedded lung tissues were stained by an immunoperoxidase method. Antibody stained the normal and hyperplastic alveolar type II pneumocytes, but did not stain bronchial epithelium or other lung cells. In tumor tissue, 26 (47.3%) of 55 cases were positively stained in the cytoplasm, and 15 showed reaction products in both the cytoplasm and intranuclear regions. By electron microscopy osmiophilic lamellar bodies and microvilli on the free surface, thought to be characteristic of type II pneumocytes, were seen in tumor cells (2 cases). In the five cases, the nuclei contained branching tubular inclusions. The results of this study support the idea that certain bronchiolo-alveolar carcinomas originate from type II pneumocytes, the intranuclear inclusions may represent an abnormal proliferation of nuclear membranes containing surfactant-apoprotein.

Key words: Bronchiolo-alveolar carcinoma – Intranuclear inclusion – Surfactant-apoprotein – Type II pneumocyte – Immunoperoxidase method

Bronchiolo-alveolar carcinoma is the least common of the primary pulmonary carcinomata (Liebow 1960). The cellular origin of this carcinoma remains controversial and it has now become apparent that bronchiolo-alveolar carcinomas represent a heterogenous group (Kuhn 1972; Mollo et al. 1973; Bedrossian et al. 1975; Greenberg et al. 1975; Jacques and Currie 1977; Sidhu and Forvester 1977; Sato and Kauffman 1980; Kauffman 1981; Singh et al. 1981; Zolliker and Jacques 1981; Dermer 1982). Several groups have reported that these tumors may be derived from bronchiolar epithelium

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with possible progenitors being ciliated, mucous, Clara, and stem cells of the bronchioles (Kuhn 1972; Greenberg et al. 1975; Sidhu and Forvester 1977; McDowell et al. 1978; Kauffman 1981; Dermer 1982). Recently, type II pneumocytes have been considered as the origin of this tumor, on the basis of electron microscopic appearances (Jacques and Currie 1977) and immunoperoxidase staining (Singh et al. 1981).

Intranuclear eosinophilic inclusions in the tumor cells of bronchiolo-alveolar carcinoma have been reported (Coalson et al. 1970; Flaks and Flaks 1970; Kuhn 1972; Torikata and Ishiwata 1977; Tsumuraya et al. 1981). Apart from so-called pseudo-inclusions containing intracytoplasmic organelles, true intranuclear inclusions consist of a mass of whole or branching structures. Singh et al. (1981) demonstrated that the eosinophilic inclusion was positively stained with rabbit antimonkey surfactant-apoprotein anti-serum, and suggested that the nuclear contents with antigenical similarity to surfactant-apoprotein represented a viral inclusion with a protein cross-reactive with surfactant-apoprotein. However, the significance of the intranuclear inclusions is unclear.

We examined 55 cases of bronchiolo-alveolar carcinoma using an indirect immunohistochemical method and antimonkey surfactant-apoprotein mono-specific IgG, and 8 cases electron microscopically. Our observations are reported here.

Materials and methods

1. Preparation of monkey surfactant

Pulmonary surfactant was prepared according to the method of King et al. (1973). In brief, saline endobronchial lavage fluids obtained from adult monkeys (*Macaca fuscata* and *Macaca fascicularis*) were centrifuged at 800 rpm for 10 min to remove cells and tissue debris. The surfactant was isolated by a series of differential and continuous density gradient centrifugations in sodium bromide solutions. The proteins of the surfactant was analyzed by polyacrylamide gel electrophoresis in 5% gel with sodium dodecyl sulfate (SDS), according to the method of Weber and Osborn (1969). In some experiments, aliquots of the surfactant were reduced with 1% 2-mercaptoethanol prior to electrophoresis. The molecular weights of the proteins were determined by comparing their electrophoretic mobilities with those of various standard proteins. Human surfactant was obtained from healthy volunteers and prepared according to the same method as that of monkey surfactant.

2. Preparation of antibody

The antisurfactant antiserum was obtained from a rabbit subcutaneously immunized with monkey surfactant preparations containing 0.5 mg of protein in complete Freund's adjuvant. After the first innoculation, booster injections of the same amount were repeated three times, at 10 day intervals. The antiserum was absorbed by affinity chromatography coupled with normal human serum, to remove antibodies against serum proteins such as albumin and IgG. The specific IgG against monkey surfactant apoprotein with a monomeric molecular weight of about 36,000 was obtained by affinity chromatography using Sepharose conjugated with proteins in monkey surfactant. The purity of the antimonkey surfactant-apoprotein mono-specific IgG was examined by immunoelectrophoretic and double immunodiffusion methods.

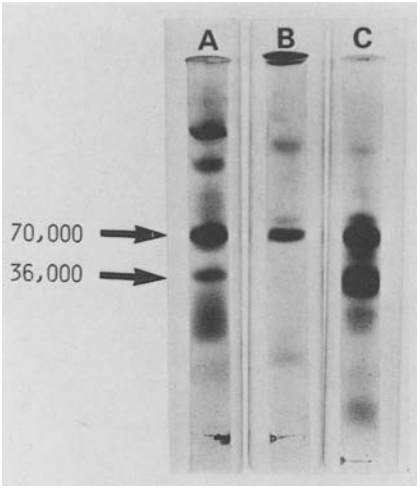

Fig. 1A–C. SDS-polyacrylamide gel electrophoresis of surfactant proteins. The major protein revealed an apparent molecular weight (M.W.) of about 70,000 daltons without reducing agent (**B**). Under reducing condition, a major protein band with an apparent M.W. of about 36,000 daltons was appeared (**C**). Standard proteins (**A**) consisted of trypsin-inhibitor (M.W.: 21,500), BSA (M.W.: 68,000) and RNA-polymerase (M.W.: α; 39,000, β: 155,000, β': 165,000)

3. Preparation and staining of lung tissue

Formalin-fixed and paraffin-embedded blocks of lung tissue were obtained from Kyushu University Hospital, Fukuoka Higashi Hospital, Kyushu Cancer Center Hospital and Minami Kyushu Hospital. Fifty-five cases of surgical or autopsy material assessed histopathologically as bronchiolo-alveolar carcinoma were investigated. The sections were routinely stained with haematoxylin and eosin, and alcian-blue. All the bronchiolo-alveolar carcinomas were stained using an immunoperoxidase technique and rabbit antimonkey surfactant-apoprotein mono-specific IgG. The method used was that described by Nakane and Pierce (1967). In brief, sections were incubated in 0.005 M periodic acid solution for 10 min and then in 0.003 M sodium borohydride solution for 30 min at room temperature to inhibit endogenous peroxidase (Isobe et al. 1977). After washing with 0.01 M PBS, pH 7.4, the sections were incubated with normal goat serum for 30 min, with antimonkey surfactant-apoprotein mono-specific IgG, overnight at 4° C and then washed repeatedly with PBS. The sections were further incubated with goat antirabbit IgG antiserum for 1 h, and then with peroxidase-antiperoxidase complex (Sternberger et al. 1970) for 1 h. After this treatment, peroxidase activity was revealed by incubating the sections in 0.05% 3,3′-diaminobenzidine in 0.1 M Tris-HCl buffer, pH 7.6, containing 0.001% H_2O_2. After washing with PBS, the sections were counterstained with methylgreen and mounted.

For *electron microscopic observation,* fresh tumor tissue (8 cases) was cut into small pieces, fixed in 3% glutaraldehyde buffered with 0.1 M phosphate (pH 7.4) at 4° C for overnight, fixed in 1% OsO_4 for 1 h, dehydrated with a graded series of alcohol and embedded in Epon 812. Ultrathin sections were cut using a LKB-8800 ultratome and observed using a JEM 100C electron microscope.

Results

1. Properties of antigens and antibody

SDS-polyacrylamide gel electrophoresis of the surfactant without the reducing agent revealed a major protein with an apparent molecular weight of about 70,000 daltons plus other minor proteins. Under conditions of reduction, a major band with an apparent molecular weight of about 36,000

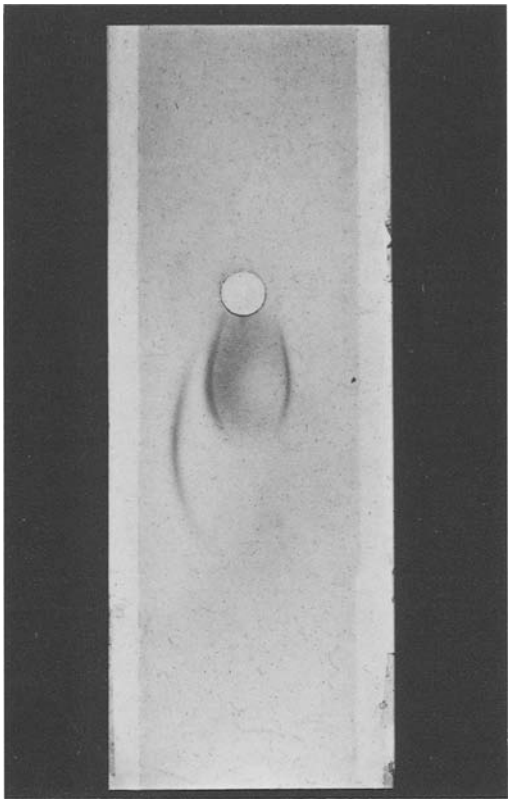

Fig. 2. Immunoelectrophoretic examination. Rabbit antimonkey surfactant-apoprotein serum (*left groove*) and rabbit antimonkey surfactant-apoprotein serum after full absorption with normal monkey serum (*right groove*) against monkey surfactant (well). Rabbit antimonkey surfactant-apoprotein serum produced at least two bands against monkey surfactant, but a single precipitation line remained after full absorption with monkey serum

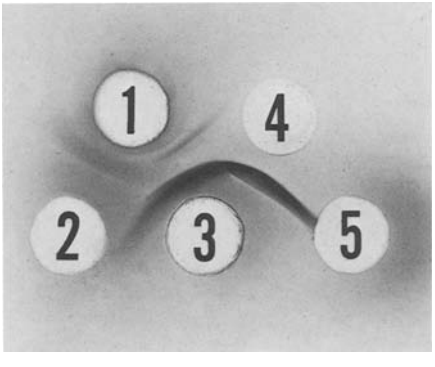

Fig. 3. Double immunodiffusion. Rabbit antimonkey surfactant-apoprotein serum (*1*) produced several precipitation lines against monkey serum (*2*) and pulmonary surfactant (*3*). Rabbit antimonkey surfactant-apoprotein serum after full absorption with normal monkey serum (*4*) produced a single precipitation line against monkey pulmonary surfactant (*3*). There was no precipitation line between rabbit antimonkey surfactant-apoprotein serum after absorption (*4*) and human serum (*5*)

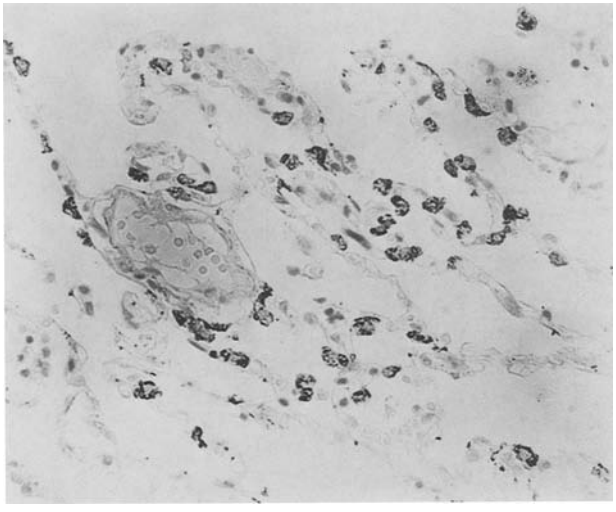

Fig. 4. Immunohistochemistry of normal human lung tissue. Intense granular staining was exhibited only in the cytoplasm of the several type II pneumocytes. Immunoperoxidase, ×320

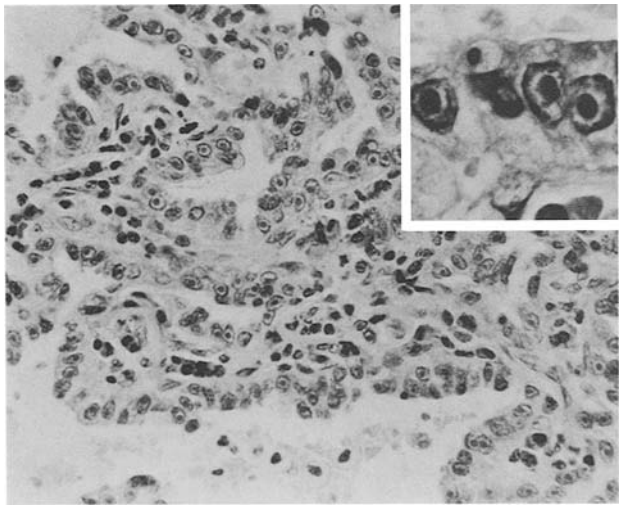

Fig. 5. Light microscopic examination of bronchiolo-alveolar carcinoma. The columnar and cuboidal tumor cells lined the stalks singly. The pleomorphism in some of the nuclei was apparent. Many of the tumor cell nuclei were vacuolated and had thick nuclear membranes. Discrete eosinophilic inclusions were present in several vacuolated nuclei. HE, ×290; *Inset*, ×920

daltons was observed (Fig. 1). Immunoelectrophoretic and double immunodiffusion data are shown in Figs. 2 and 3. A single precipitation line was recognized between human and monkey surfactant(s) and rabbit antimonkey surfactant-apoprotein mono-specific IgG. We also confirmed the absence of immunological reactivity between surfactant-apoprotein and human

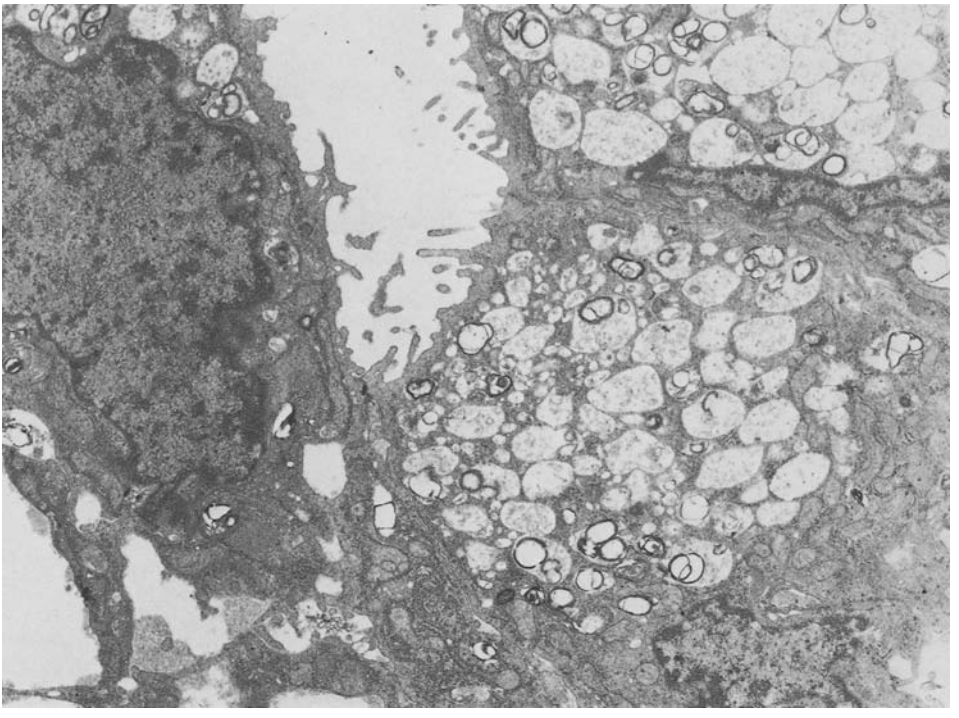

Fig. 6. Electron microscopic examination of tissues in case of bronchiolo-alveolar carcinoma. The tumor cells exhibited osmiophilic lamellar inclusions in the cytoplasm and surface microvilli, and which were similar to those seen in normal type II pneumocyte. × 7,760

Table 1. Immunoperoxidase and alcian-blue stainings in bronchiolo-alveolar carcinomas

	Alcian-blue staining		
	Negative	Positive	Total
Immunoperoxidase method			
Negative	11	18	29
Positive	17	9	26
(Intranuclear)	(11)	(4)	(15)
Total	28	27	55

plasm or anti-CEA IgG (DAKO Co. Santa Barbara CA, USA). In addition, when human adult lung tissue was immunohistochemically examined with rabbit antimonkey surfactant-apoprotein mono-specific IgG, granular staining was evident only in the cytoplasm of the type II pneumocytes (Fig. 4) and some alveolar macrophages. There were no reaction products in any other cells such as type I pneumocytes, bronchiolar epithelial cells and others. Using normal rabbit IgG as the primary reactant, no reaction products were evident in the lung tissues.

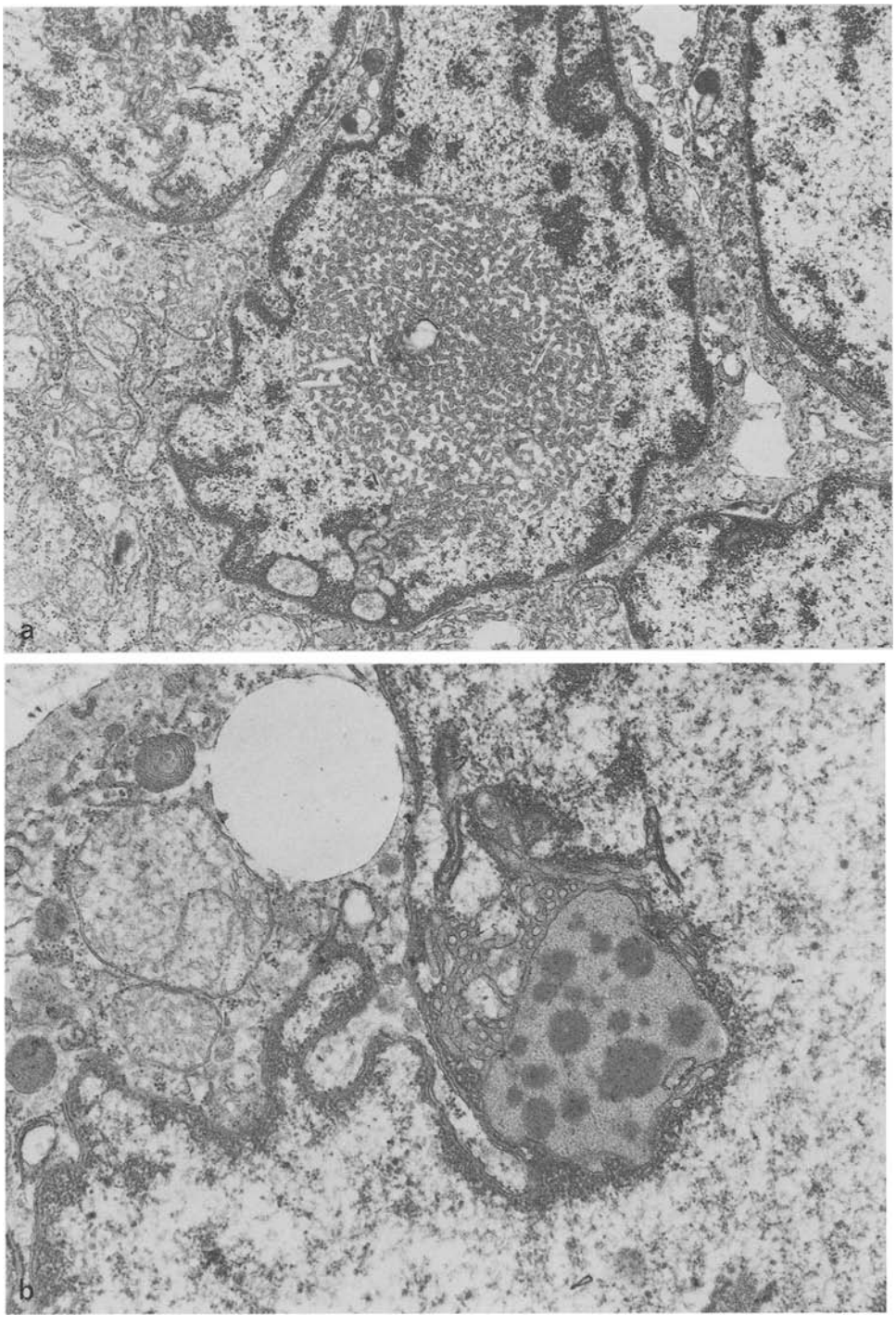

Fig. 7a, b. Electron microscopic appearance of intranuclear inclusion. Intranuclear inclusion consisted of a mass of branching tubular structures. Some branching tubules were directly connected to the inner nuclear membrane. **(a)** ×13,600, **(b)** ×22,800

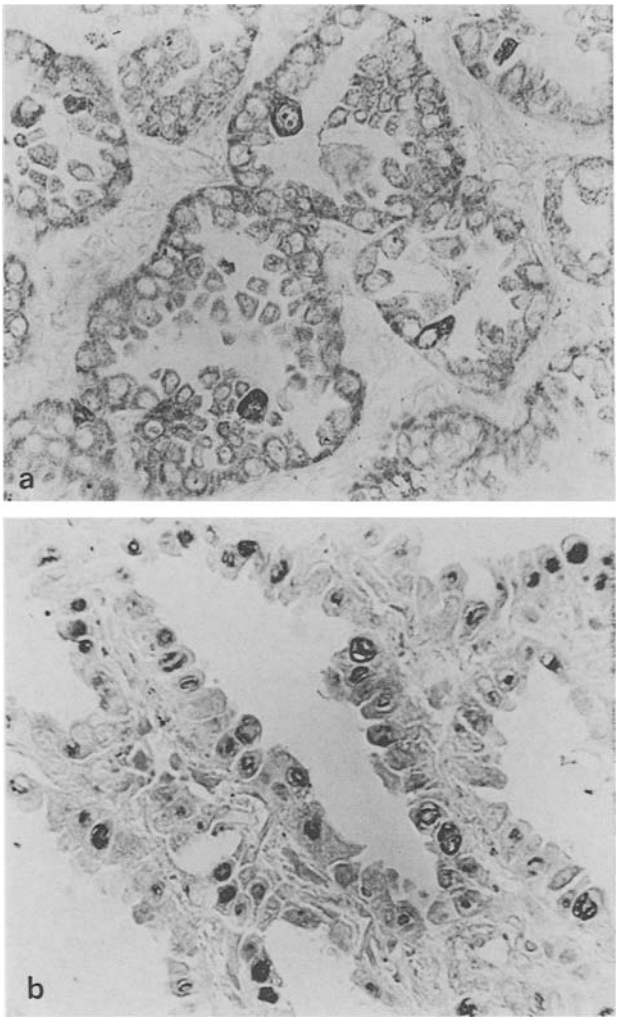

Fig. 8a, b. The cytoplasmic and intranuclear stainings, using an immunoperoxidase method. The cytoplasmic staining was generally granular and faint. The intranuclear staining was intense. Immunoperoxidase, (**a**) × 300, (**b**) × 510

2. Light-microscopic examination

Microscopically, the growths consisted of cuboidal, peg-shaped, tall columnar epithelium lining intact but occasionally thickened alveolar walls. The cells were pleomorphic and often formed papillary projections into the alveolar spaces. Some had a thick nuclear membrane and vacuolated centers, with or without eosinophilic inclusions (Fig. 5). In 27 of 55 cases, alcian-blue positive material was present in the cytoplasm and/or the luminal spaces, indicating mucin production and secretion.

3. Electron-microscopic examination

Osmiophilic lamellar inclusions and microvilli on the cell surface similar to those seen in normal type II pneumocytes were evident in 2 of 8 cases (Fig. 6). Intranuclear inclusions consisted of branching tubules (5 cases) and were directly connected to the inner nuclear membrane (Fig. 7).

4. Immunoperoxidase staining

Results of the immunoperoxidase examination and mucin staining in the specimens from 55 cases of bronchiolo-alveolar carcinoma are summarized in Table 1. Tumor cells of 26 (47,3%) cases were positively stained in the cytoplasm, within or without the intranuclear region. The cytoplasmic staining was generally granular and fainter than the nuclear staining (Fig. 8). The distribution of the reaction products was not always uniform in the tumors, some were stained diffusely and others formed several nests with antimonkey surfactant-apoprotein mono-specific IgG. Cytoplasmic reaction products were frequently found in the hyperplastic type II pneumocytes and which were occasionally seen in the border between the tumor and the normal lung tissue. The nuclear staining was usually intense and seemed to correspond to the intranuclear inclusions and vacuoles, under a light microscope (Fig. 8). Nine of 27 tissues stained positive with alcian-blue showed reaction products in immunoperoxidase method.

Squamous cell carcinomas (5 cases), adenocarcinomas (5 cases), large cell carcinomas (5 cases) and small cell carcinomas (4 cases) showed no reactivity to antimonkey surfactant-apoprotein mono-specific IgG.

Discussion

Pulmonary surfactant is thought to be synthesized by type II pneumocytes (Buckingham and Avery 1962; Askin and Kuhn 1971), and is rich in phospholipids, especially desaturated species of lecithins (King 1974; Clements and King 1976). However, lung surfactant also contains a specific apoprotein, a non-serum protein closely associated with isolated surfactant (King et al. 1973). Its antibody might possibly be specific for surfactant-apoprotein (Klass 1973). Sueishi et al. (1977) and Williams and Benson (1981) used electron microscopic immunohistochemistry to define the cellular source of this protein. They suggested that the type II pneumocytes were the exclusive source of this protein. From these reasons surfactant-apoprotein might be a useful marker, by which the type II pneumocytes can be identified.

We obtained monkey surfactant-apoprotein and prepared rabbit antimonkey surfactant-apoprotein mono-specific IgG in order to identify as type II pneumocyte by immunoperoxidase method. The biochemical properties of monkey surfactant-apoprotein are similar to those of the proteins obtained from rats (Katyal et al. 1977), cow (Sawada and Kashiwamata 1977), dogs (King et al. 1973) and humans (Bhattacharyya and Lynn 1979; Shelley et al. 1982). Antiserum against monkey surfactant-apoprotein cross-

reacts specifically with human surfactant-apoprotein (Singh and Katyal 1980). We demonstrated the specificity of the antimonkey surfactant-apoprotein IgG not only biochemically but also immunohistochemically in normal adult lung tissue. We then examined the localization of surfactant-apoprotein using the immunoperoxidase method and have speculated on the cellular origin of bronchiolo-alveolar carcinoma.

Bronchiolo-alveolar carcinoma may represent a heterogenous entity (Bedrossian et al. 1975; Greenberg et al. 1975; Jacques and Currie 1977; Sidhu and Forvester 1977; McDowell et al. 1978; Kauffman 1981; Singh et al. 1981; Zolliker and Jacques 1981). The cellular origin of this carcinoma may by type II pneumocyte, Clara cell or metaplastic mucin secreting cell of the bronchioles. In the present study using antimonkey surfactant-apoprotein mono-specific IgG, we demonstrated the presence of apoprotein in the cytoplasm, with or without the intranuclear region of tumor cells, in 26 of 55 cases of bronchiolo-alveolar carcinoma. Furthermore, as shown in Fig. 6, the microvilli on their free surfaces and osmiophilic lamellar inclusions seem to be characteristic of the type II pneumocyte (Kikkawa et al. 1965; Brumley et al. 1967). These findings add more evidence for the hypothesis that type II pneumocyte is important as one of the cellular origins. We also found mucinous material in 27 of 55 cases of bronchiolo-alveolar carcinoma with alcian-blue staining, and 9 of 27 cases containing mucinous materials showed reactivity to surfactant-apoprotein mono-specific IgG, in some areas. From the point of view of discussion of the cellular origin of this carcinoma, it seems important that cells with a mucin secretion and those closely resembling type II pneumocyte are intermingled in the same tumor. One of these cells of origin may be a primitive one possessing the potential to differentiate to both mucin-secreting cells and type II pneumocytes. However, the possibility that the positive staining in alcian-blue is derived from mucinous degeneration of the tumor cells was not ruled out.

Surfactant-apoprotein could not be recognized in more than half of cases studied in this paper. Bronchiolo-alveolar carcinoma, therefore, would appear to have heterogenous origins including the type II pneumocyte, Clara cell, metaplastic mucin secreting cell and others (Bedrossian et al. 1975; Greenberg et al. 1975; Jacques and Currie 1977; Sidhu and Forvester 1977; McDowell et al. 1978; Kauffman 1981; Singh et al. 1981; Zolliker and Jacques 1981).

Several investigators have reported intranuclear inclusions in cases of various viral infections of the respiratory tract (Koprowska 1961; Warner et al. 1964; Naib et al. 1968; Jain et al. 1973) and bronchiolo-alveolar carcinoma (Coalson et al. 1970; Flaks and Flaks 1970; Kuhn 1972; Torikata and Ishiwata 1977; Tsumuraya et al. 1981). The ultrastructure of intranuclear inclusions in bronchiolo-alveolar carcinoma does not seem to be compatible with that of any known human pathogens (Singh et al. 1981). Tsumuraya et al. (1981) suggested that true intranuclear inclusions might be derived from intranuclear proliferation of the inner nuclear membrane. However, Singh et al. (1981) reported that the intranuclear contents probably repre-

sented a viral inclusion with a protein crossreactive with surfactant-apoprotein. In our study, nuclear inclusions also reacted strongly with the antimonkey surfactant-apoprotein monospecific IgG in 15 of 55 cases of bronchiolo-alveolar carcinoma. In addition, we demonstrated that intranuclear branching tubular structures connected directly with the inner nuclear membrane. Sueishi et al. (1977) and Williams and Benson (1981) noted a conspicuous accumulation of reaction products of antirabbit and antirat surfactant-apoprotein Fab' conjugated with horseradish peroxidase in membrane structures such as lamellar inclusions, endoplasmic reticulum and others in normal rabbit and rat lung tissues. The perinuclear cisterna is one of the system with a positive reaction to antisurfactant-apoprotein Fab' labelled with horseradish peroxidase. Thus, intranuclear inclusions would represent an abnormal intranuclear proliferation of inner nuclear membranes with surfactant -apoprotein in transformed type II pneumocytes.

Acknowledgement. We thank M. Ohara for reading the manuscript.

References

Askin FB, Kuhn C (1971) The cellular origin of pulmonary surfactant. Lab Invest 25:260–268

Bedrossian CWM, Weilbaecher DG, Bentinck DC, Greenberg SD (1975) Ultrastructure of human bronchiolo-alveolar carcinoma. Cancer 36:1399–1413

Bhattacharyya SN, Lynn WS (1979) Structural characterization of a glycoprotein isolated from alveoli of patients with alveolar proteinosis. J Biol Chem 254:5191–5198

Brumley GW, Chernick V, Hodson WA, Normand C, Fenner A, Avery ME (1967) Correlations of mechanical stability, morphology, pulmonary surfactant, and phospholipid content in the developing lamb lung. J Clin Invest 46:863–873

Buckingham S, Avery ME (1962) Time of appearance of lung surfactant in the foetal mouse. Nature 193:688–689

Clement JA, King RJ (1976) Composition of surface active material. In: Crystal RG (ed) The biochemical basis of pulmonary function, vol 2. Marcel Dekker, Inc, New York, p 363

Coalson JJ, Mohr JA, Pirtle JK, Dee AL, Rhoades ER (1970) Electron microscopy of neoplasm in the lung with special emphasis on the alveolar cell carcinoma. Am Rev Respir Dis 101:181–197

Dermer GB (1982) Origin of bronchiolo-alveolar carcinoma and peripheral bronchial adenocarcinoma. Cancer 49:881–887

Flaks B, Flaks A (1970) Fine structure of nuclear inclusions in murine pulmonary tumor cells. Cancer Res 30:1437–1443

Greenberg-SD, Smith MN, Spjut HJ (1975) Bronchiolo-alveolar carcinoma. Cell of origin. Am J Clin Pathol 63:153–167

Isobe S, Chen ST, Nakane PK, Brown WR (1977) Studies on translocation of immunoglobulins across intestinal epithelium. I. Improvements in the peroxidase-labeled antibody method for application to study of human intestinal mucosa. Acta Histochem Cytochem 16:161–171

Jacques J, Currie W (1977) Bronchiolo-alveolar carcinoma: A Clara cell tumor? Cancer 40:2171–2180

Jain U, Mani K, Frable WJ (1973) Cytomegalic inclusion disease: Cytologic diagnosis from bronchial bruching material. Acta Cytol (Baltimore) 17:467–468

Katyal SL, Estes LW, Lombardi B (1977) Method for the isolation of surfactant from homogenates and lavages of lung of adult, newborn, and fetal rats. Lab Invest 36:585–592

Kauffman SL (1981) Histogenesis of the papillary Clara cell adenoma. Am J Pathol 103:174–180

Kikkawa Y, Motoyama EK, Cook CD (1965) The ultrastructure of the lungs of lambs. Am J Pathol 47:877–903

King RJ (1974) The surfactant system of the lung. Fed Proc 33:2238–2247

King RJ, Klass DJ, Gikas EG (1973) Isolation of apoproteins from canine surface active material. Am J Pathol 224:788–795
Klass DJ (1973) Immunochemical studies of the protein fraction of pulmonary surface material. Am Rev Respir Dis 107:784–789
Koprowska I (1961) Intranuclear inclusion bodies in smears of respiratory secretions. Acta Cytol (Baltimore) 5:219–228
Kuhn C (1972) Fine structure of bronchiolo-alveolar cell carcinoma. Cancer 30:1107–1118
Liebow AA (1960) Bronchiolo-alveolar carcinoma. Adv Intern Med 10:329–358
McDowell EM, McLaughlin JS, Merenyi DK, Kieffer RH, Harris CC, Trump BF (1978) The respiratory epithelium. V. Histogenesis of lung carcinomas in the human. JNCI 61:587–606
Mollo F. Canese MG, Campobasso O (1973) Human peripheral lung tumors. Light and electron microscopic correlation. Br J Cancer 27:173–182
Naib ZM, Stewart JA, Dowdle WR, Casey HL, Marine WM, Nahmias AJ (1968) Cytological features of viral respiratory tract infections. Acta Cytol 12:162–171
Nakane PK, Pierce GB (1967) Enzyme-labeled antibody: Preparation and application for the localization of antigens. J Histochem Cytochem 14:929–931
Sato T, Kauffman SL (1980) A scanning electron microscopic study of type II and Clara cell adenoma of the mouse lung. Lab Invest 43:28–36
Sawada H, Kashiwamata S (1977) Sodium dodecyl sulfate-disk gel electrophoresis patterns of bovine lung surfactant. Biochem Biophys Acta 490:44–50
Shelley SA, Balis JU, Paciga JE, Espinoza CG, Richman AV (1982) Biochemical composition of adult lung surfactant. Lung 160:195–206
Sidhu GS, Forvester EM (1977) Glycogen-rich Clara cell type bronchiolo-alveolar carcinoma. Light and electron microscopic study. Cancer 40:2209–2215
Singh G, Katyal SL (1980) Surfactant apoproteins in nonmalignant pulmonary disorders. Am J Pathol 101:51–62
Singh G, Katyal SL, Torikata C (1981) Carcinoma of type II pneumocytes. Immunodiagnosis of a subtype of "Bronchiolo-alveolar carcinomas". Am J Pathol 102:195–208
Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG (1970) The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex and its use in identification of spirochates. J Histochem Cytochem 14:219–302
Sueishi K, Tanaka K, Oda T (1977) Immunoultrastructural study of surfactant system. Distribution of specific protein of surface active material in rabbit lung. Lab Invest 37:136–142
Torikata C, Ishiwata K (1977) Intranuclear tubular structures observed in the cells of an alveolar cell carcinoma. Cancer 40:1194–1201
Tsumuraya M, Kodama T, Kameya T, Shimosato Y, Koketsu H, Uei Y (1981) Light and electron microscopic analysis of intranuclear inclusions in papillary adenocarcinoma of the lung. Acta Cytol (Baltimore) 25:523–532
Warner NE, McGrew EA, Nanos S (1964) Cytologic study of the sputum in cytomegalic inclusion disease. Acta Cytol 8:311–315
Weber K, Osborn M (1969) The reliability of molecular weight determinations by dodecylpolyacrylamide gel electrophoresis. J Biol Chem 244:4406–4412
Williams Mc, Benson BJ (1981) Immunocytochemical localization and identification of the major surfactant protein in adult rat lung. J Histochem Cytochem 29:291–305
Zolliker AS, Jacques J (1981) Clara cell carcinoma of the lung. Hum Pathol 12:748–750

Accepted March 18, 1983