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Morphometric analysis of the immunohistochemical expression of Clara cell 10-kDa protein and surfactant apoproteins A and B in the developing bronchi and bronchioles of human fetuses and neonates

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Abstract Morphometric analyses of the immunohistochemical expression of the Clara cell secretory 10-kDa protein (CC10) and surfactant apoproteins A and B (SP-A and -B) were carried out on the developing bronchi and bronchioles of human fetuses and neonates. We analysed the ratio of the number of CC10-positive cells per subepithelial length of the bronchial or bronchiolar basement membrane and found that both the bronchial and the bronchiolar population of CC10-positive cells was significantly higher than that of either SP-A or SP-B. In addition, CC10 was found to be distributed mainly in the bronchiole. CC10-positive cells began to be recognized in the late pseudoglandular phase (15 weeks of gestation) and thereafter gradually increased in the canalicular and terminal sac phases, which correspond to the active development period of the acini or peripheral airways. The earliest expression of SP-A was also noted at 15 weeks of gestation, but its positive epithelial cells were present mainly in the larger bronchi. Double immunohistochemical staining for CC10 and SP-A revealed that the CC10-positive cells lining both the bronchi and bronchioles were different from the SP-A-positive cells. This finding suggests that CC10-positive cells are functionally and developmentally heterogeneous in both fetal and neonatal lungs in humans

Key words Clara cells · CC10 · Fetus · Bronchiole · Apoproteins

Introduction

Nonciliated and nonmucous bronchiolar cells (Clara cells) are the predominant epithelial cell type in the distal airways of most mammalian species, in spite of some inter- and intra-species variations. They have been shown to participate in the maintenance of physiological functions, including secretory and metabolic activities and in maintaining progenitor cells capable of dividing and redifferentiation into lining bronchiolar cells. They are also involved in pathobiological conditions, including malignant transformation, leading to the development of peripheral lung adenocarcinoma and in the inflammatory repair process at the critical junction between the conducting airways and the alveoli [6, 10, 27, 35]. Their role in developing lungs remains to be clarified [8, 12, 18, 32, 39].

Several ultrastructural characteristics of this cell type, such as abundant rough endoplasmic reticulum and numerous membrane-bound, electron-dense secretory granules, suggest the vigorous synthesis and storage of proteins and other materials. Recently, a protein with a molecular weight of 6 or 12 kDa has been shown to be a specific protein for Clara cells, and this protein has been called Clara cell secretory protein (CC10) [10, 19, 35]. Clara cells or Clara cell-like cells have been confirmed to localize in both smaller and larger airways, including the trachea [8, 10, 40]. It has yet to be established, however, whether or not these Clara cell-like cells fulfil a function similar to that of Clara cells in the bronchioles. The contents of the Clara cell secretory granules and the nature of the secretory products have yet to be determined, but Clara cells produce other proteins, such as surfactant proteins (SP-A, B and D) [7, 26, 30], cytochrome P-450 monooxygenases [11], leucocyte protease inhibitor [44], β-galactoside-binding lectin [43], trypsin-like protease [33] and others [38]. Some studies have shown that the amino acid sequence of CC10 has a close similar to that of uteroglobin in rabbits, and as both CC10 and uteroglobin inhibit phospholipase A_2 they may play an important part in regulating the inflammatory response [36].

Pulmonary surfactant, which reduces the surface tension in the air–liquid interface of the alveoli, is synthesized and secreted mainly by type II pneumocytes, and partly by Clara cells. The surfactant consists primarily of

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about 90% lipids and 10% proteins. To date, four distinct surfactant-associated proteins (SPs) have been identified, namely, hydrophilic SP-A and -D, and hydrophobic proteins SP-B and -C [45]. SP-A is a variably glycosylated protein of with a nominal molecular weight of 29–35 kDa and is the most abundant and so far the best characterized. This protein is known to regulate surfactant phospholipid secretion and re-uptake by type II cells [47]. SP-B is initially isolated as a protein with low molecular weight of 8–15 kDa and has been shown to have a major role in the surface activity of surfactant as it promotes the spreading of the surfactant layer onto the air–liquid interface [37, 45]. In addition, SP-A and -B appear to play an important part in the formation of tubular myelin while also participating in the host defence mechanism against microorganisms [1, 21, 23, 37, 42, 45]. By immunohistochemistry and in situ hybridization, SP-A and -B proteins and their mRNAs have been detected in the Clara cells of humans [2, 3, 30, 37], dogs [24], rabbits [7, 46] and rats [31].

In the present study, we examined the immunohistochemical distribution and density of CC10 and SP-A and -B, while comparing the temporal and spatial changes of these protein expressions in the bronchioles and bronchi of developing human fetal and newborn lungs. In addition, we also used a double immunostaining technique to further clarify whether the expression of both SP-A, which has been reported to be a major SP expressed by Clara cells, and CC10, which is expressed by nonciliated cells, is homologous in the bronchi and bronchioles of developing human lungs.

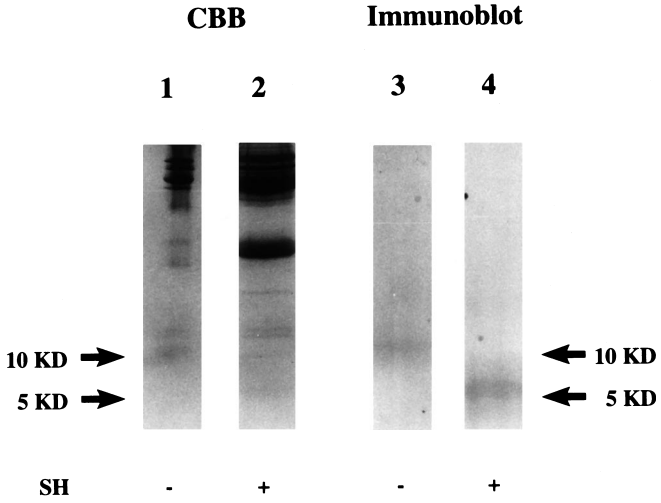

Fig. 1 Immunochemical specificity of rabbit anti-human CC10 antibody. SDS-PAGE and Western blotting were carried on using bronchoalveolar lavage obtained from an alveolar proteinosis patient, as described in "Materials and methods". *Lanes 1, 2* SDS-PAGE shows many protein bands, including a faint band with molecular weights of about 10 kDa and 5 kDa under nonreducing and reducing conditions, respectively. *Lanes 3, 4* Western blotting using a polyclonal antibody to human CC10-peptide antigen shows a single band with molecular weights of about 10 kDa and 5 kDa under nonreducing and reducing conditions, respectively. About 20 μg of proteins, including partially purified CC10, was applied to each lane (*SH* 2-mercaptoethanol, *CBB* Coomassie Brilliant Blue)

Materials and methods

Autopsies were performed on 28 fetuses and 20 neonates at Kyushu University Hospital. No respiratory, central nervous system or infectious illnesses had been present in any of these cases. The gestational age of each was confirmed by the mother's menstrual history or ultrasonographic crown–rump measurement at an early stage of pregnancy. We also excluded the lungs of neonates and fetuses with the following abnormalities: abnormally low or high birth weight, lung weight-to-body weight ratio below 0.015 (< 28 gestational weeks) or 0.012 (≧ 28 gestational weeks) [5], hydrops fetalis, and histological evidence of showing extreme congestion and a massive haemorrhage. The tissue samples were fixed with 10% formaldehyde and embedded in paraffin. The sections were cut in slices 4 μm thick and were then stained with haematoxylin and eosin, elastica-Van Gieson and Masson trichrome staining.

The antibodies used in this study were the rabbit anti-SP-A IgG reported previously [13, 29, 41], mouse monoclonal anti-SP-B IgG (HS-2) [25, 41] kindly donated by Dr. Y. Suzuki (Department of Molecular Pathology, Chest Disease Research Institute, Kyoto University), and rabbit anti-human CC10 IgG prepared with a synthetic multiple antigen peptide (MAP) composed of the sequence ELFSPDQDMREAG, corresponding to amino acid residues 26–38 of human CC10. The specific anti-CC10 IgG was isolated by a synthetic MAP-Sepharose (Pharmacia, Uppsala, Sweden) column chromatography. The immunospecificity of antibody against CC10 was confirmed by ELISA and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting using lung lavage obtained from a patient with idiopathic pulmonary alveolar proteinosis. Figure 1 show the protein profiles based on staining with Coomassie Brilliant Blue after SDS-PAGE and Western blotting for human CC10 fractions partially purified by both Cibacron Blue- and DEAE-Sepharose (Pharmacia) column chromatography. Western blotting following SDS-PAGE revealed a single band with an apparent molecular weight of about 10 kDa under nonreducing conditions and 5 kDa under reducing conditions. The immunospecificity of antibody against SP-A was confirmed in a similar way to those of anti-CC10 antibody, using surfactant fractions prepared from monkey bronchoalveolar lavage by the method of King et al. [19, 24]. The immunospecificity of these antibodies was also confirmed by the demonstration of an immunohistochemical reaction specific to the respective cells, namely, type II pneumocytes and Clara cells.

Immunohistochemical staining was performed by the streptavidin–biotin peroxidase complex method [4] using the Histofine SAB-PO (M,R) Kit (Nichirei, Tokyo, Japan). All sections were deparaffinized and washed with 0.1 M phosphate-buffered saline (PBS), pH 7.4 and then incubated with 10% normal goat or rabbit serum for 30 min at room temperature to block any nonspecific binding of the primary antibodies. Each section was incubated with the primary antibody against CC10 (5 μg/ml), SP-A or -B (5 μg/ml) at 4°C overnight. After washing with PBS, the sections were incubated for 30 min with biotinylated rabbit anti-mouse IgGs for SP-B reaction or goat anti-rabbit IgG for SP-A and CC10. In order to inhibit the endogenous peroxidase activity, the sections were soaked in methanol containing 0.3% hydrogen peroxidase for 30 min. After the sections had been treated with avidin-biotin peroxidase complex for 30 min, the peroxidase activity was visualized using PBS containing 0.1% (W/V) 3,3′-diaminobenzidine hydrochloride and 0.01% (V/V) hydrogen peroxide. Lastly, the sections were counterstained with methyl green or haematoxylin.

As negative controls, nonimmunized rabbit and mouse IgGs were used instead of the primary rabbit anti-SP-A or CC10 IgG and mouse monoclonal anti-SP-B IgG, respectively. In addition, the preincubated antibody to SP-A or CC10 with an excessive amount of the respective antigen was also applied to the parallel sections.

In addition to comparing the immunohistochemical distribution of CC10 and SP-A using serial sections, the double staining technique was also used to visualize these two different antigens in the same section simultaneously. The antigens were detected by the dark brown reaction products using the peroxidase reaction with diaminobenzidine and blue reaction products using the alkaline phosphatase reaction with the alkaline phosphatase substrate Kit (Vector Laboratories, Burlingame, Calif.) according to previously reported procedures [34].

For semiquantitative assessment of the development of the immunohistochemical expression of CC10, SP-A and SP-B, the histological images were recorded on an Optiphot-2 microscope with a 40 × plane objective lens connected to a television camera (NEC, Tokyo, Japan). Next, using a personal computer PC-9801 RA (NEC) equipped for the image analysis system (Cosmozone-1S image analyser, Nikon, Tokyo, Japan), we measured both the length of the subepithelial basement membrane of the bronchus or bronchiole (the perimeter of the bronchi and bronchioles) and the numbers of immunohistochemically positive cells and the surface epithelial cells within the respective bronchus or bronchiole, and then calculated the ratios of the number of either positive cells or surface epithelial cells per unit length of the subepithelial basement membrane of the bronchus or bronchiole. At least 20 sites of the bronchi or bronchioles were examined in each case.

The morphometric results were expressed as a scattergram of the ratio of the number of immunohistochemically positive cells for either CC10, SP-A or -B and the surface epithelial cells to the unit length of bronchial or bronchiolar basement membrane versus gestational weeks. The cases could not be assessed for the expression of each antigen in the bronchioles at 15–17 weeks of gestation, because it was difficult to define the bronchioles in such cases. The scattergram for the number of each target protein-positive cells was based on the mean values obtained from each case and then analysed by a one-way analysis of covariance to compare the three simple regression lines [16]. All calculations were worked with the computer package program, BMDP1V on an IBM system 3090 [16]. The number of surface epithelial cells was expressed as the mean ± SD. Significant differences between the various groups of gestational weeks were thus determined using the unpaired Student's *t*-test for equal or unequal variances. A value of $P<0.05$ was considered to be statistically significant.

Results

The time-sequential expressions of CC10, SP-A and SP-B in the bronchioles and bronchi are shown in Figs. 2 and 3.

The CC10-positive cells were more frequently noted in the bronchioles than the bronchi, and their prevalence in the bronchioles was about three times higher than that in the bronchi after 25 weeks of gestation (Figs. 2, 3). The expression of CC10 protein was first detected in the primitive nonciliated epithelial cells lining the peripheral bronchi at 15 weeks of gestation (Fig. 4), corresponding to the late pseudoglandular phase. CC10-positive cells occasionally showed an intraluminal projection composed of a few cells forming small clusters. However, scattered expression of CC10 began to be recognized at 18 weeks of gestation in the bronchiolar epithelium. From 23 to 40 weeks of gestation the population of CC10-positive cells and their reaction intensity in both the bronchi and the bronchioles gradually increased as gestational age advanced (Figs. 2–6). However, the CC10-positive cell ratio in bronchioles tended to increase rapidly from 24 to 34 gestational weeks and appeared to reach a plateau after 33–34 gestational weeks (Fig. 2).

In addition to CC10 expression in the bronchial and bronchiolar epithelial cells, some epithelial cells lining the excretory ducts of the bronchial glands also expressed CC10 (Figs. 5a, 6a) throughout the entire gestational period examined, but the nonciliated cells lining the ends of the respiratory bronchioles and the alveolar type II pneumocytes demonstrated no such immunoreactivity (Fig. 11).

The earliest expression of SP-A was detected in the epithelial cells of the main bronchi at 15 weeks' gestation, when CC10 was also first expressed. The number of

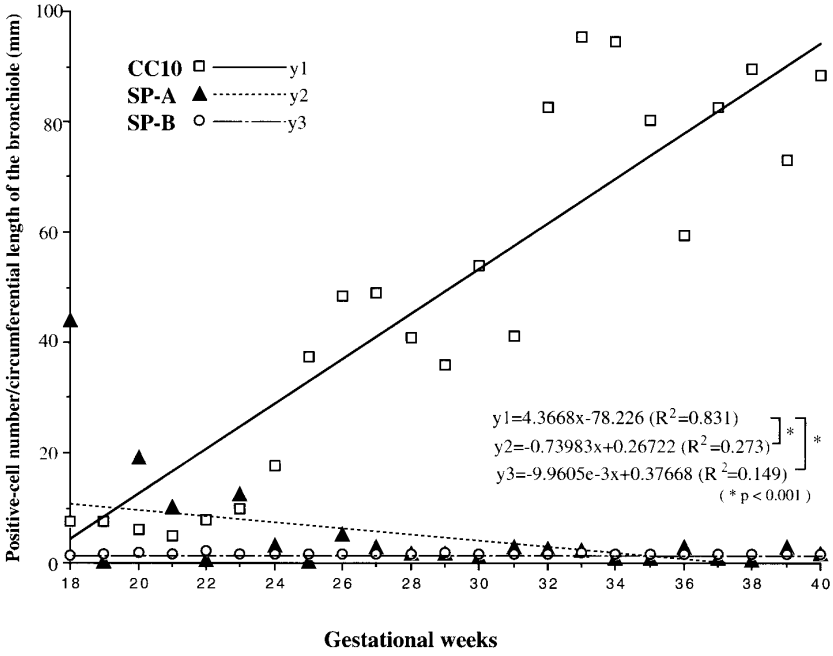

Fig. 2 Incidence of epithelial cells immunoreactive for CC10, SP-A and SP-B in the bronchioles. The circumferential length of the bronchiole was measured morphometrically as described in the "Materials and methods". Each *square*, *triangle* or *circle* represents the mean value obtained in a particular case

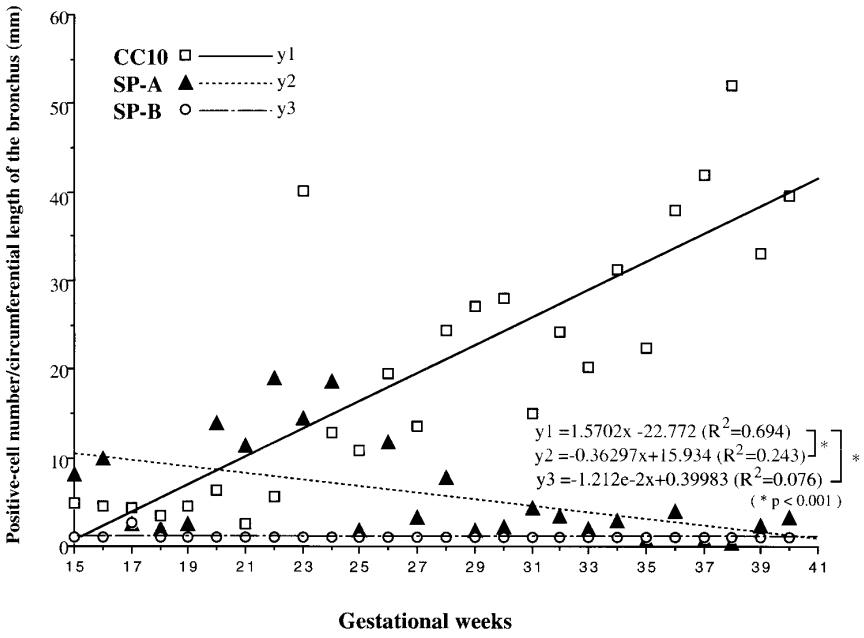

Fig. 3 Incidence of epithelial cells immunoreactive for CC10, SP-A and SP-B in the bronchi. The circumferential length of the bronchus was measured morphometrically ("Materials and Methods"). Each *square*, *triangle* or *circle* represents the mean value obtained in a particular case

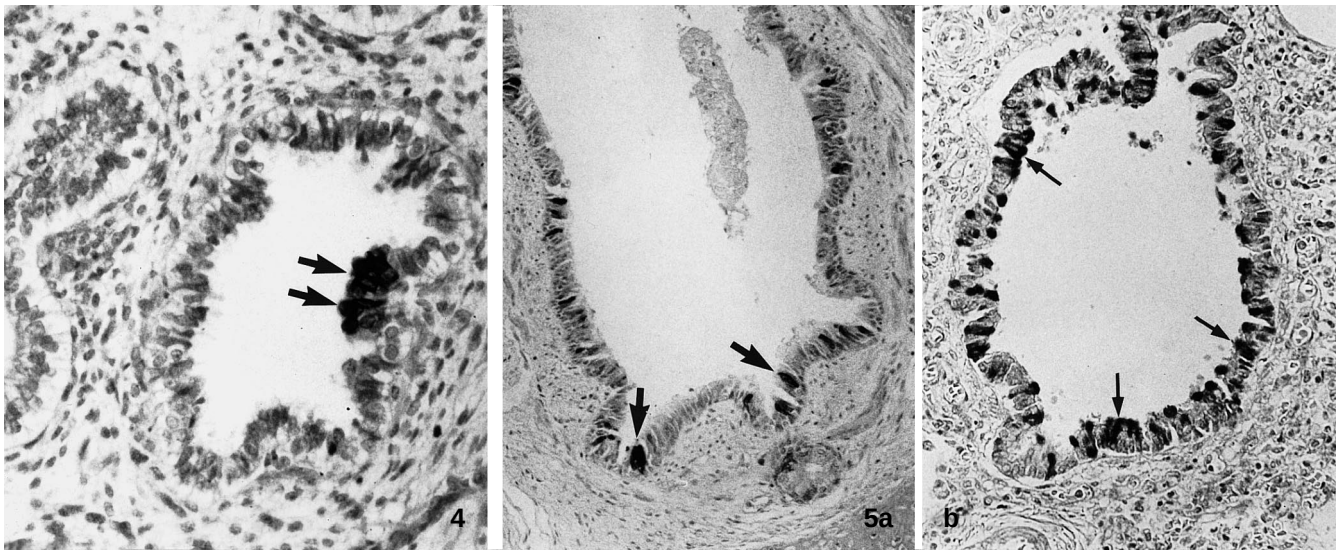

Fig. 4 Immunohistochemical expression of CC10 in nonciliated bronchial cells (15 weeks of gestation). A few cells (*arrows*) forming small clusters are immunoreactive for CC10 protein. ×320

Fig. 5 Immunohistochemical expression of CC10 in **a** the bronchus and **b** the bronchiole (28 weeks of gestation). Some bronchial (**a** *large arrows*) and many bronchiolar (**b** *small arrows*) epithelial cells are immunoreactive for CC10 protein. These findings were obtained in the same case. **a** × 160, **b** × 200

SP-A-positive cells in the bronchial epithelium varied considerably until 25–27 weeks (Figs. 3, 7), and thereafter seemed to decrease gradually (Fig. 3). In the late terminal sac phase only a few bronchial epithelial cells were detected. Like the expression of SP-A in the bronchi, a few bronchiolar epithelial cells also showed a weak immunoreactivity for SP-A (Figs. 2, 11) and these bronchiolar SP-A-positive cells were localized mainly in the respiratory bronchioles (Fig. 11).

At 20 gestational weeks some cuboidal epithelial cells lining the primitive alveoli began to show immunoreactivity for SP-A. After 32 weeks of gestation, almost all of the type II pneumocytes became positive for SP-A (Fig. 8), and their number and immunoreactive intensity increased as gestational age advanced. Some epithelial cells lining the excretory ducts of the bronchial glands (Fig. 7) and some alveolar macrophages also showed immunopositivity for SP-A protein.

The expression of SP-B protein became apparent in the cuboidal epithelial cells of the bronchi and the primitive bronchioles at 16 weeks of gestation. In all cases examined, however, the expression of SP-B protein in the bronchial and bronchiolar epithelium was much less than that of CC10 or SP-A (Figs. 2, 3). A few bronchial and bronchiolar cells showed immunoreactivity for SP-B in only some sections throughout gestation, while type II pneumocytes were apparently positive for SP-B at 30 ge-

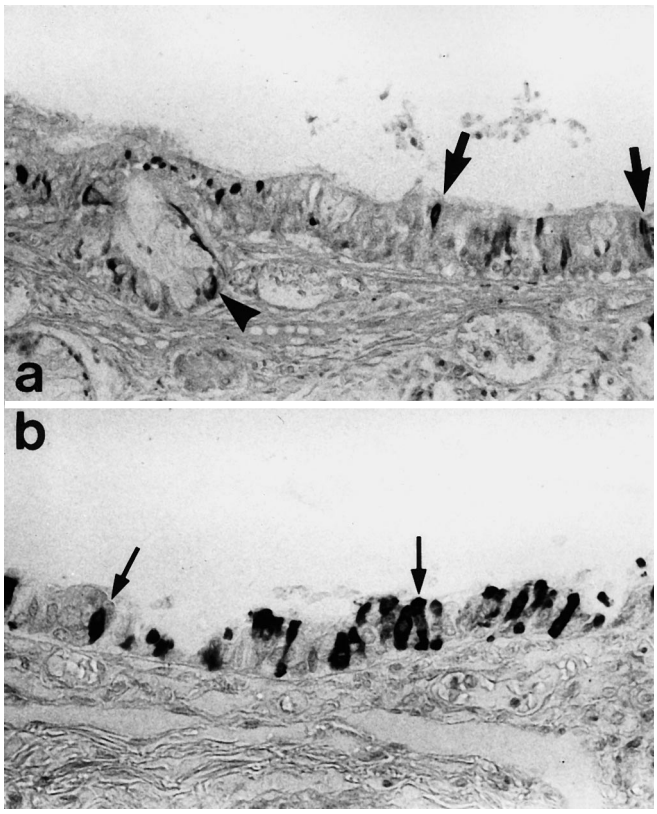

Fig. 6 Immunohistochemical expression of CC10 in **a** the bronchus and **b** the bronchiole (40 weeks of gestation). The occurrence of CC10-positive cells in the bronchiole (**b** *small arrows*) is greater than that in the bronchus (**a** *large arrows*). A few ductal epithelial cells of the bronchial gland are also positive for CC10 (**a** *arrowheads*). These findings were obtained in the same case. ×240

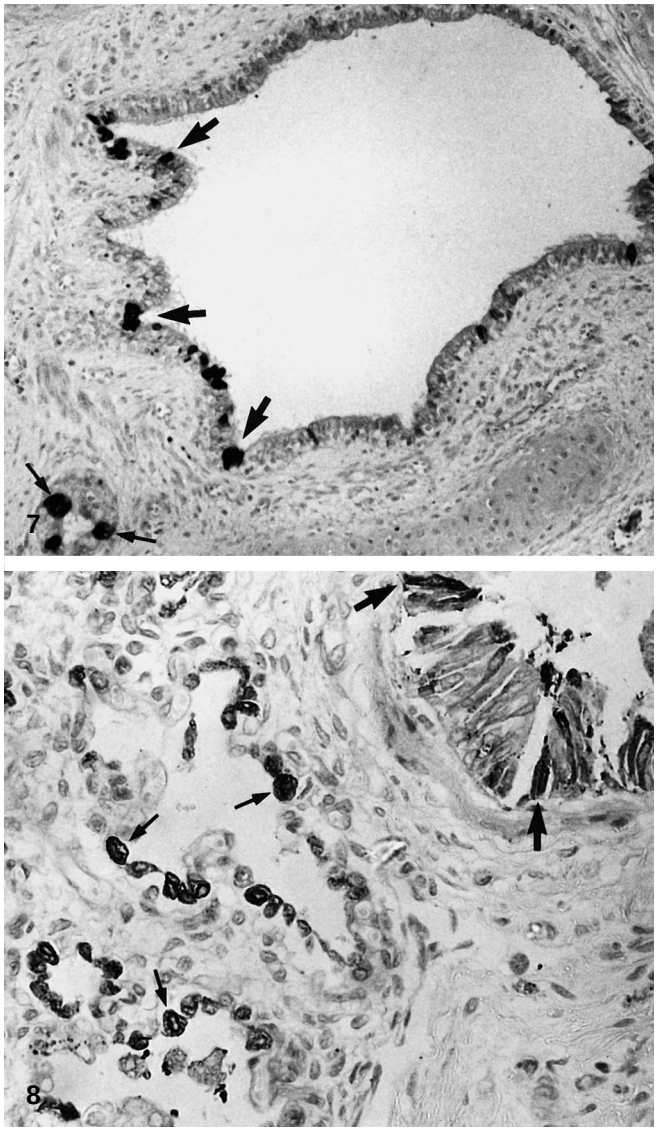

Fig.7 Immunohistochemical expression of SP-A in bronchial epithelial cells (*large arrows*) and ductal glandular epithelial cells (*small arrows*) at 23 weeks of gestation. ×160

Fig. 8 Immunohistochemical expression of SP-A in bronchial epithelial cells (*large arrows*) and type II alveolar epithelial cells (*small arrows*) at 40 weeks of gestation. ×240

stational weeks and thereafter their immunohistochemical reactivity became more intense with advancing gestational age. A few alveolar macrophages also revealed immunopositivity for SP-B.

As negative controls for CC10, SP-A and -B immunohistochemistry, parallel sections were incubated with normal mouse or rabbit IgG (5 μg/ml) instead of the respective primary antibodies, and these sections also showed no immunoreaction. In addition, when the preabsorbed antibodies were applied to CC10 and SP-A with an excessive amount of the respective antigens, the immunoreactivity of these antibodies was thus completely abolished (Fig. 9).

Double immunohistochemical staining for CC10 and SP-A in the bronchi and bronchioles and parallel staining in serial sections confirmed that the number of CC10-positive cells was greater than that of SP-A-positive cells. In addition, the CC10-positive cells were generally different from SP-A-positive cells and only a few cells in the bronchial lining cells coexpressed both CC10 and SP-A proteins (Fig. 10). Some nonciliated cuboidal cells located in the peripheral respiratory bronchioles were positive for SP-A, but not for CC10 (Fig. 11). Interestingly, the nonciliated and cuboidal cells with scanty cytoplasm, which are thus smaller than Clara cells and are also located characteristically at the bronchiolo-alveolar duct junction, were negative for SP-A, -B and CC10.

Figures 2 and 3 show the incidence of immunoreactive epithelial cells for CC10, SP-A and -B in the bronchus and bronchiole. The expression of CC10 in both the bronchi and bronchioles was significantly higher (P<0.001) than the expression of SP-A or SP-B, while the difference between SP-A and SP-B was not significant. The number of CC10-positive cells gradually increased in both bronchioles (R^2 = 0.85, P<0.001) and bronchi (R^2 = 0.63, P<0.001) as gestational age advanced, and this bronchiolar incidence appeared to be higher than the bronchial one (P<0.001).

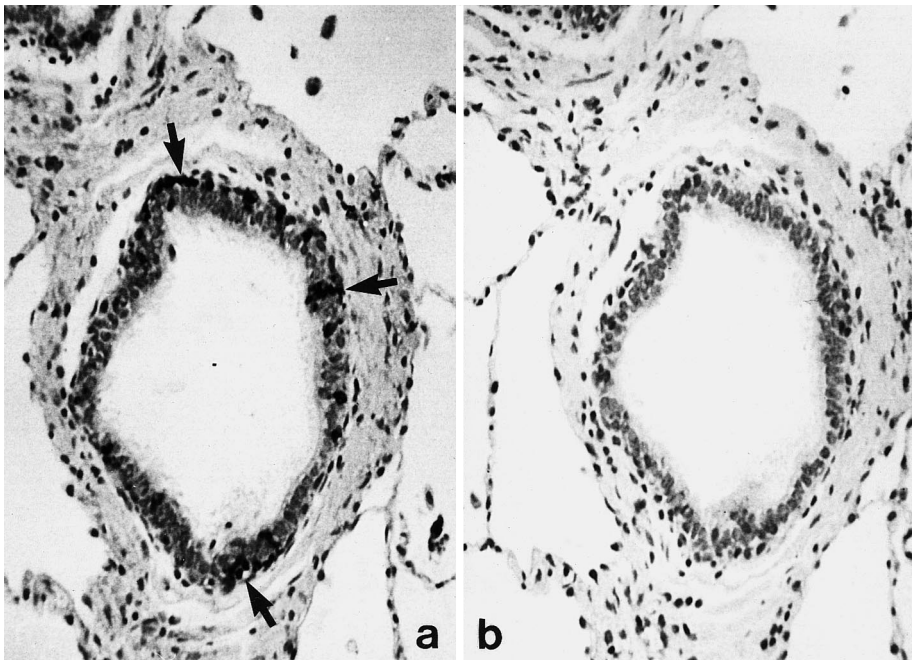

Fig. 9 **a** Immunohistochemical expression of CC10 in the bronchiole (normal adult lung). Nonciliated bronchiolar epithelial cells (*large arrows*) are immunoreactive for CC10 protein. **b** A serial section of **a**: the immunoreactivity of these positive cells has been completely abolished by preincubation of rabbit anti-human CC10 IgG (5 μg/ml) with CC10 MAP (20 μg/ml). ×160

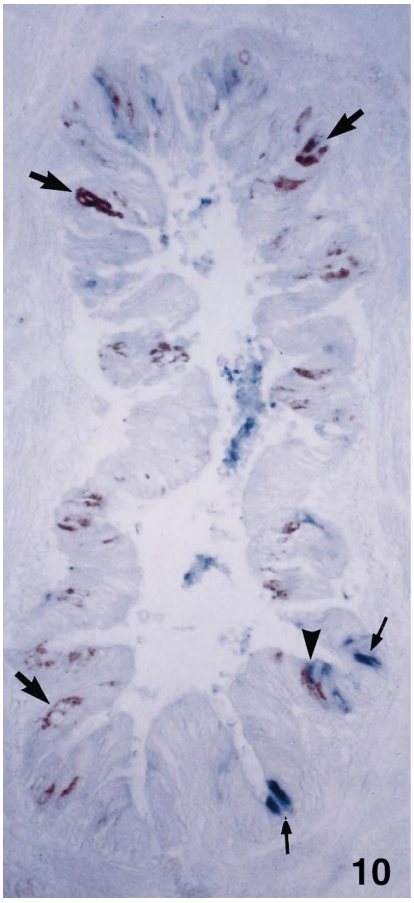

Fig. 10 Double immunostaining for SP-A (*blue*) and CC10 (*brown*) in a bronchus at 40 weeks of gestation. The number of CC10-positive cells (*large arrows*) is greater than that of SP-A-positive cells (*small arrows*), and the CC10-positive cells are largely different from the SP-A-positive cells. Only a few cells (*arrowheads*) coexpress both CC10 and SP-A. ×240

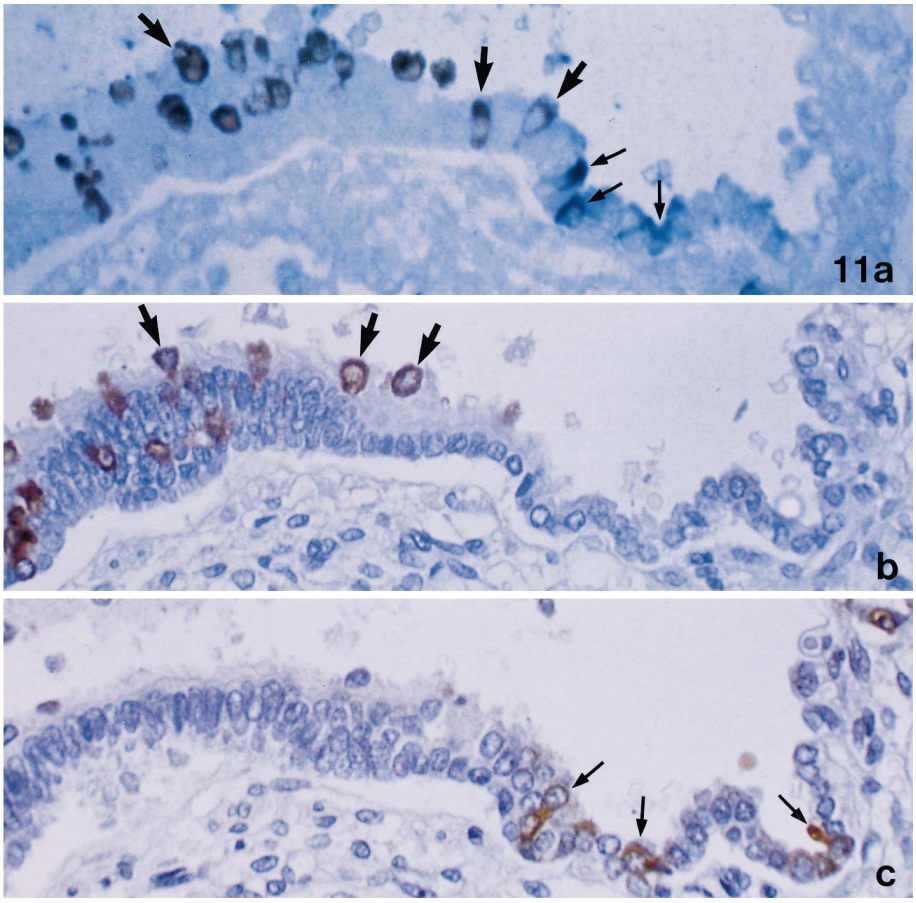

Fig. 11 **a** Double immunostaining for SP-A (*blue*) and CC10 (*brown*) in a respiratory bronchiole at 39 weeks of gestation. **b**, **c** Serial sections immunostained for CC10 (**b**) and SP-A (**c**) proteins. Nonciliated cells lining the respiratory bronchiole are immunoreactive for either SP-A (**a**, **c** *small arrows*), or CC10 (**a**, **b** *large arrows*). The number of CC10-positive cells is greater than that of the SP-A-positive cells, and the latter are located mainly at the end of the respiratory bronchiole adjacent to the alveolar duct (**a–c**). ×320

Table 1 Surface epithelial cell density of the bronchi and bronchioles

Gestational Weeks	Surface epithelial cells/mm (mean±SD)	
Bronchi		
15–24 (*n*=18)	293.15±32.09	*
25–32 (*n*=12)	262.92±24.03	*
33–40 (*n*=14)	257.69±21.02	
Bronchioles		
18–24 (*n*=12)	214.23±11.21	*
25–32 (*n*=12)	236.14±10.51	*
33-40 (*n*=14)	223.97±12.32	

* $P<0.05$

To examine whether this increase in the number of CC10-positive cells in the bronchioles and bronchi correlated merely with the change in the number of surface epithelial cell, we compared the density of the surface epithelial cells with that of the CC10-positive cells. As shown in Table 1, the epithelial density of the bronchi was higher than that of the bronchioles throughout the whole of gestation ($P<0.001$). Comparison of the surface epithelial cell density in the late pseudoglandular and canalicular phase (15–24 gestational weeks), the early terminal sac (25–32 weeks) and the late terminal sac (33 weeks to full term) phases showed that the bronchial epithelial cell density was higher in the late pseudoglandular and canalicular phase than in the others ($P<0.05$), while the bronchiolar epithelial population was the highest in the early terminal sac phase ($P<0.05$). However, the alterations of epithelial density in both bronchi and bronchioles were less than about 1.2 times between these gestational phases. Therefore, the increase in the number of CC10-positive cells in both the bronchioles and bronchi seemed to correlate with the functional maturation of both bronchial and bronchiolar epithelial cells according to the expression of CC10 protein and not merely to the numerical change in the epithelial cells.

Discussion

We have described the expression and distribution of epithelial cells immunoreactive for human CC10, SP-A and -B in the developing bronchi and bronchioles of human fetuses after 15 weeks of gestation and neonates without fetal lung diseases or other disorders causing pulmonary hypoplasia. For immunohistochemical study of CC10, we used a specific antibody to a synthetic multiple antigen peptide composed of amino acid residues 26–38 of human CC10. To our knowledge, there has been no previous report using such an anti-peptide for the human CC10 protein antibody.

We found that the expression of CC10 in both bronchi and bronchioles was significantly greater than that of SP-A and -B throughout gestation, and, moreover, the population of CC10-positive cells in the bronchioles was larger than that in the bronchi. This suggests that in the developing conducting airway, especially in the bronchioles, CC10 protein is one of the major secretory proteins of bronchial and bronchiolar epithelial cells. CC10 was first detected in the primitive nonciliated epithelial cells lining the peripheral bronchi at the 15th week of gestation, which corresponds to the pesudoglandular phase. This finding is also similar to a previous report by Barth et al. [8]. In addition, Judy et al. [40] demonstrated that the first expression of CC10 mRNA in rat lungs was noted in the bronchial respiratory tubules in the pseudoglandular phase (the 16th gestational day), when the acinar tubules began to branch out from the bronchial respiratory tubules in the rat [40].

Our study revealed that the density of CC10-positive cells in the bronchioles and bronchi gradually increased after 24 gestational weeks. In particular, the increase in the number of CC10-positive cells in the bronchioles was rapid during the period from 24 to 34 gestational weeks. This phase has also been thought to correspond to the active development period of the acini or peripheral airways, namely, the respiratory bronchioles, transitional ducts and saccules [20, 22]. In addition, CC10-positive cells were mainly located in the terminal bronchioles and it might therefore be assumed that the bronchiolar Clara cells may possibly be stem cells of the peripheral conducting airway. The previous in vivo and in vitro studies showed that Clara cells possess not only self-renewal activity but also a capability of differentiation to ciliated cells [8, 9, 17]. Together with these findings, the increase in the number of CC10-positive cells in bronchi and bronchioles thus supports the correlation of functional maturation with the structural development in the peripheral lung tissue. However, further studies are necessary to determine whether or not the CC10-positive cells lining the bronchial epithelium fulfil a similar stem cell function to that of the Clara cells in the bronchioles.

Some investigators have reported that CC10 can inhibit phospholipase A_2, while Clara cell tryptase has also been shown to cleave the haemagglutinin and activate the infectivity of the influenza A virus [33, 36, 43, 44]. Together with these previously proposed functions, the expression of CC10 in broncho-bronchiolar epithelial cells may thus play a part in the regulation of the inflammatory response as a defence mechanism. Other investigators, however, have suggested that Clara cells may be a heterogeneous population with various ultrastructural phenotypes [27], and thus these CC10-positive cells at different tracheo-broncho-bronchiolar levels may possess different functions in development and host defence.

We also investigated the immunohistochemical expression of SP-A and -B, and carried out a morphometric analysis of their occurrence in the developing bronchi and bronchioles. The number of SP-A- and B-positive cells in both bronchi and bronchioles was significantly lower than that of CC10. SP-A-positive cells were occasionally recognized in the canalicular phase, but thereafter gradually decreased until birth, as reported in earlier papers [2, 7, 14, 15, 28). However, the earlier studies did not clarify whether or not the nonciliated epithelial cells expressed both CC10 and SP-A proteins simultaneously

in the bronchi and bronchioles. To clarify this point, double immunostaining for CC10 and SP-A proteins was performed. SP-A-positive cells in both the bronchi and the bronchioles were largely different cells from the CC10-positive cells, although a few cells coexpressed both SP-A and CC10 proteins. Therefore, the nonciliated secretory cells distributed in the bronchi and bronchioles seem to be heterogeneous for the functional phenotype in the both fetal and neonatal periods. In the late pseudoglandular phase (16th week of gestation) SP-B was recognized in a few bronchial and primitive bronchiolar epithelial cells and, thereafter, was frequently seen in the primitive alveolar epithelium or type II pneumocytes, as reported in previous papers [3, 37]. Therefore, the expression of SP-B was mainly related to the functional development of type II pneumocytes.

The present study has shown that the expression of CC10 in both the bronchi and bronchioles is apparent in the late pseudoglandular phase, gradually increasing as gestational age advances; the bronchial and bronchiolar population of CC10-positive cells is significantly greater than that of SP-A and -B; and as shown by double immunostaining for CC10 and SP-A, the CC10-positive cells in both the bronchi and the bronchioles were largely different cells from the SP-A-positive cells. This suggests that such broncho-bronchiolar secretory cells are functionally and developmentally heterogeneous. The expression of CC10 and SP-A by broncho-bronchiolar epithelial cells may play a part in the regulation of the inflammatory response as a defence mechanism in the human fetal and neonatal lung.

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