Number and Proliferation of Clara Cells in Normal Human Airway Epithelium

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Experimental pathologic studies suggest that Clara cells are one of the types of airway stem cells but the proliferation of Clara cells in human lungs has not yet been examined. The purpose of this study was to assess in conducting airways of normal human lungs: (*1*) the number of Clara cells; and (*2*) the contribution of Clara cells to the proliferation compartment. Samples of histologic normal tissue were taken from seven lungs obtained by autopsy. A triple sequential (immuno)histochemical staining was performed, using MIB-1 as a proliferation marker and anti-CC10 for the identification of Clara cells; subsequently, a PAS stain was carried out as a marker for goblet cells, as these cells were reported to be CC10-immunoreactive in an unknown proportion. Clara cells were virtually absent in the proximal airway epithelium. The number of Clara cells in the terminal bronchioles was 11 ± 3% (mean ± SD) and in respiratory bronchioles 22 ± 5%. The overall proliferation compartment of the conducting airway epithelium was 0.83 ± 0.47%; the contribution of Clara cells was 9%. In the terminal bronchioles 15% of proliferating airway epithelial cells were Clara cells, and in the respiratory bronchioles this number increased to 44%. The contribution of Clara cells to the proliferation compartment of normal human tracheobronchial epithelium is substantial, demonstrating a role of the Clara cell in the maintenance of the normal epithelium of the distal conducting airways in humans.

Boers JE, Ambergen AW, Thunnissen FBJM. Number and proliferation of Clara cells in normal human airway epithelium. AM J RESPIR CRIT CARE MED 1999;159:1585–1591.

Over 60 years ago, a nonciliated, nonmucous cell was described in the human respiratory epithelium of the peripheral conducting airways (1). The eponymous histologist Max Clara defined these cells by their distinctive cytoplasmic granules, indicating a secretory function. Since then numerous electron microscope studies performed in mammals have shown considerable interspecies heterogeneity in both morphology and distribution of these cells (2). An ultrastructural characteristic of Clara cells is the presence of electron-dense membrane-bound granules, which immunolocalize with rat Clara cell 10-kilodalton (kD) protein (CC10), one of the secretory products of Clara cells (3). Antibodies raised against human CC10 (4, 5), later also isolated and described as protein P1 (6), have been used as immunohistochemical markers of Clara cells. CC10 immunoreactive cells are described in the human respiratory epithelium of both peripheral and central conducting airways (4, 7, 8). Though the distribution of Clara cells has been examined in the peripheral conducting airways of lung cancer patients (5, 9, 10), no comprehensive quantitative study of the Clara cell population has been performed of the entire tracheobronchial tree of normal lungs.

Clara cells contribute to cell renewal in hamster bronchial epithelium in the steady state (11). The proliferative response of the bronchiolar epithelium after exposure of rats to NO_2 (12) or O_3 (13) gases is predominantly due to Clara cell division. Grafting isolate cell fractions of rabbit bronchiolar epithelium highly enriched in Clara cells onto denuded tracheas results in an epithelium containing both Clara cells and ciliated cells, resembling bronchiolar epithelium (14). Thus, the Clara cell is an important cell type regarding cell renewal in rodent conducting airway epithelium in both health and disease (15).

In humans, the overall proliferation compartment of normal conducting airway epithelium and the contribution of neuroendocrine, basal, and parabasal cells to the proliferation compartment has been determined (16, 17), but the extent to which Clara cells contribute to cell renewal is unknown. The architecture of terminal conducting airways shows species-specific variations in addition to a highly diverse epithelial composition prohibiting extrapolation of data obtained in animal models to the human lung (2, 18). Because Clara cells play a plausible if undefined role in both neoplastic and non-neoplastic lung disease (8, 9, 19–22), knowledge of Clara cell distribution and proliferation in the normal human lung is of importance when disease is to be defined morphologically and by cell kinetics.

The aim of the present study was to determine, in normal human airway epithelium, the number of Clara cells, the overall proliferation compartment, and the contribution of Clara cells to the proliferation compartment. To this end, human lungs obtained by autopsy were fixed by tracheal infusion of formalin, and cross-sections from central to peripheral airway were stained sequentially with an antibody to the proliferation-associated protein Ki-67 (MIB-1), followed by a Clara cell marker (CC10 antibody).

(*Received in original form June 10, 1998 and in revised form October 22, 1998*)

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Am J Respir Crit Care Med Vol 159. pp 1585–1591, 1999
Internet address: www.atsjournals.org

METHODS

Material

In De Wever Hospital, Heerlen, and Maastricht University Hospital, Maastricht, The Netherlands, perfusion fixation of lungs was performed if deceased patients were available for autopsy within 6 h after death. Perfusion fixation of lungs in 275 cases was done. After fixation, seven samples were taken from the trachea down to peripheral lung tissue, and were routinely processed. A standard hematoxylin and eosin (H&E) stain was used for histologic assessment. In order to obtain a more or less normal subject population, all cases with primary or secondary pulmonary diseases were excluded. After these selection procedures, a total pathologic score (TPS) was established for each slide. Four variables—inflammatory cellular infiltrate, pigmentation, fibrosis, and muscle hypertrophy—were scored in small airways with an internal diameter of 2 mm or less using H&E and elastica-von Gieson–stained sections. The four variables were graded from 0 (= normal) to 3 (= severely abnormal) for each airway, after which an average score was calculated for each variable for each slide. From the average score of the slides, a TPS was calculated as follows: the average score for each variable was expressed as a percentage of the maximum score. Next, the four percentages were added. The maximum theoretical TPS was 400.

Seven cases (six nonsmoking, one with unknown smoking history) with a TPS of less than 100 remained for further study. In five cases the patients were female and in two male. The average age was 61 yr (range: 24–84 yr). The causes of death were myocardial infarction (three patients), cerebral infarction (one patient), saddle embolus of the lung (one patient), sudden death with aortic stenosis (one patient), and sick sinus syndrome (the 24-yr-old patient).

Immunohistochemistry

A sequential double indirect immunohistochemical staining procedure followed by the periodic acid-Schiff (PAS) stain was performed. Three-micrometer thick tissue sections were placed on 3-amino propyltriethoxysilane-coated slides (Sigma Chemical Co., St. Louis, MO) and dried overnight in a convection oven (56° C). Slides were dewaxed in xylene, rehydrated in graded alcohols, and rinsed in distilled water. Subsequently, the slides were placed in plastic jars containing 500 ml of 10 mM citrate buffer in distilled water, pH 6. Two jars were placed in a 650-watt microwave oven and irradiated to the boiling point. This was followed by 15 min of uninterrupted boiling, using full-power irradiation. After the jar was removed from the microwave oven, cooling to room temperature was achieved in about 20 min. The first immunostaining was an incubation with the MIB-1 antibody (23) (Immunotech, Marseille, France; dilution 1:25) for 45 min at room temperature. Slides were washed three times with phosphate-buffered saline (PBS), incubated with biotinylated rabbit antimouse antibody (Dako, Glostrup, Denmark; 1:500) and subsequently with peroxidase-conjugated streptavidin label (Dako; 1:1,000). As a chromogen, diaminobenzidine (Sigma) was used, producing a dark brown nuclear immunostain. Subsequently, slides were incubated with 20% normal swine serum (Dako) in distilled water for 20 min at room temperature, followed by the second primary rabbit polyclonal antibody CC10 (4) (a generous gift from Dr. G. Singh, Pittsburgh, PA; dilution 1:2,500) for 45 min at room temperature. After washing with PBS, incubation of biotinylated swine antirabbit (Dako; 1:1,000) and alkaline phosphatase-conjugated streptavidin label (Dako; 1:200) were used as subsequent steps. The slides were rinsed in PBS and incubated with Fast Blue (Sigma) for 10 min, producing dark blue cytoplasmic immunostain, and were then were rinsed in PBS, and the PAS stain (24) was carried out, which stains neutral glycoproteins bright red. Finally, slides were lightly counterstained with Mayer's hematoxylin and placed in tap water for 15 min. Three drops of Imsol/Mount (Klinipath, Zevenaar, The Netherlands) were applied to the tissue section. Slides were then allowed to dry, and mounted in Entellan (Merck, Darmstadt, Germany).

For the detection of goblet cells, additional slides containing both large and small airways were stained with the following histochemical stains: PAS, staining neutral mucins red; Alcian blue (pH = 2.5), staining acidic mucins blue; both PAS and Alcian blue (AB-PAS). In a pilot study comprising two large (internal diameter $>$ 4 mm) and two small (internal diameter: 1 to 2 mm) airways, the three histochemical methods were compared. Since the differences in assessed goblet cell numbers were less than 5% between these methods, the PAS stain with the red color was used for further study.

Negative control slides were stained with omission of the primary antibodies MIB-1 and/or CC10. As a positive control, a slide was used containing several respiratory bronchioles from a pneumonectomy specimen showing many Clara cells, and large bronchi with squamous metaplasia with readily apparent MIB-1 staining (16). A fixation delay study performed on a pneumonectomy specimen showed no impact of fixation delay up to 12 h on either MIB-1 or CC10 immunoreactivity.

Image Analysis and Measurements

An interactive image analysis system consisting of the Quantimet 570C and a Leitz microscope was used to investigate the specimens and controls. The conducting airway epithelium of the slides was assessed as follows: first, the internal diameter of the airway was measured, using a measuring rule at low power. Next, digitized video images were made of an epithelial area at high power ($\times$40 objective, numerical aperture = 0.65) with an internal magnification between the microscope and the digital camera of 50%. One video image showed on average 20 to 40 airway epithelial cells on 100 μm basement membrane. Only epithelium with an orientation perpendicular to the basement membrane was evaluated. A line was drawn manually over the image of the basement membrane using the computer mouse, and the luminal side of the epithelium was marked. The length of basement membrane was automatically determined. Subsequently, only nucleated epithelial cells were counted manually, and these were divided into the following categories: total number of epithelial cells; number of Clara cells; number of goblet cells; number of indeterminate cells; number of MIB-1–immunoreactive epithelial cells; and number of MIB-1 positive Clara, goblet, or indeterminate cells. The total number of epithelial cells was assessed by counting all nuclei located above the basement membrane. A cell was identified as a Clara cell when a typical dome-shaped cytoplasm was distinctly immunostained with CC10 without PAS positivity. Goblet cells were identified by a flask-shaped cytoplasm with large PAS-positive cytoplasmic vacuoles and ovoid nuclei below the cytoplasmic vacuoles. A proportion of the goblet cells demonstrated CC10 immunoreactivity and were included as a separate category of epithelial cells. A small proportion of epithelial cells had CC10 immunoreactivity and indistinct PAS positivity, not fulfilling the criteria of either Clara cells or goblet cells; these cells were designated as indeterminate cells. Epithelial cells with nuclei that stained unequivocally dark brown with diaminobenzidine were regarded as MIB-1 positive cells. Reproducibility of the number of all airway epithelial cells, Clara cells, goblet cells, indeterminate cells, and MIB-1 positive cells was greater than 90%.

Data Handling and Statistics

Determination of length of basement membrane and cell numbers was performed for a series of subsequent epithelial areas until 400 to 450 epithelial cells were assessed. The total number of epithelial cells per airway required for quantification was based on the following assumption: when the standard error of proportion SEprop = sqrt($p(1 - p)/n$) of a given cell type is 0.025 or less, the 95% confidence intervals of a proportion are $\pm$ 0.05. As the expected proportions (n) of the cell types to be determined were expected to be 0.5 or less, the equation SEprop $\leqslant$ 0.025 requires a maximum number (n) of 400 cells.

The airways were classified into four categories in two different ways. The first approach was to classify the airways solely on the basis of internal airway diameter. Four airway diameter groups were created: group 1 (diameter $\geqslant$ 4 mm), group 2 (diameter $\geqslant$ 2 mm but less than 4 mm), group 3 (diameter $\geqslant$ 0.5 mm but less than 2 mm), and group 4 (diameter $<$ 0.5 mm). The second approach was based on a combination of airway diameter and morphology, and the airways were classified into four morphologic categories: category I (labeled as bronchi), airways with a diameter of 2 mm or greater; category II (larger nonterminal bronchioles), bronchioli with diameters greater than or equal to 0.5 mm but less than 2 mm and without respiratory bronchioles in the vicinity; category III (terminal bronchioles), small nonalveolarized bronchioles in the vicinity of respiratory bronchioles;

TABLE 1

CALCULATION OF MEAN PERCENTAGES OF CLARA CELLS PER PATIENT AND PER MORPHOLOGIC AIRWAY CATEGORY

	Airway Category			
Patient No.	Bronchi	Bronchioles	Terminal Bronchioles	Respiratory Bronchioles
1	0	0.6	14	22
2	0	0	13	26
3	0	0	14	19
4	0	0	6	34
5	0	0.3	11	21
6	0	0.9	10	18
7	0	0.9	8	17
Mean (± SD)	0	0.4 (± 1.0)	11 (± 5)	22 (± 5)

category IV (respiratory bronchioles), alveolarized bronchioles. Only cylindric epithelium was considered for assessment in the respiratory bronchioles. The individual airways labeled as terminal and respiratory bronchioles (airway categories III and IV) frequently failed to contain a minimum of 400 epithelial cells; the data (i.e., length of basement membrane and the number of the nine cell categories) of several of these small airways in a particular slide were added until 400 to 450 epithelial cells were available for assessment. Such aggregated data were referred to as an "airway" for the purpose of the present study.

The following calculations were performed for both airway classification systems. The data of the described cell types obtained per airway were grouped per patient and per airway category. The number of the individual epithelial cell types was expressed as percentages of total airway epithelial cell count. A mean was calculated for each epithelial cell type per patient and per airway category. This resulted in $7 \times 4 = 28$ means as is shown for Clara cells in Table 1. These mean percentages were used to determine a mean per airway category. The standard deviation (SD) was calculated with the nested ANOVA procedure (25); this approach not only detects variance between patient means but also between the different observations within a patient per airway category.

RESULTS

A total of 51,264 epithelial cells and 198 mm of basement membrane were counted in 111 airways; the number of assessed airways, the range of internal airway diameters, and the total number of epithelial cells counted per airway category are presented in Table 2.

Clara Cells

A total number of 2,950 Clara cells (Figure 1) were counted. Table 1 shows the number of Clara cells expressed as the mean percentage of airway epithelial cells per patient and per morphologic airway category. Clara cells were absent in the bronchi; 0.4 ± 1.0% (mean ± SD) of the epithelium of the larger (nonterminal) bronchioles were Clara cells. Clara cells were predominantly present in the terminal bronchioles (11 ± 5%) and in the ciliated epithelium of the respiratory bronchioles (22 ± 5%). In the respiratory bronchioles a short transitional nonciliated cuboidal epithelium was frequently observed between the bronchiolar and the alveolar epithelium, consistently lacking CC10 immunoreactivity.

The number of Clara cells using the airway classification based solely on internal diameter was as follows: airway diameter groups 1 and 2, 0%; airway diameter group 3 (0.5–2 mm), 5 ± 3%; and group 4 (< 0.5 mm), 18 ± 7%.

Goblet Cells

A total number of 3,519 goblet cells (Figure 1) were counted. Table 2 shows the mean percentage of goblet cells per morphologic airway category. The number of goblet cells in the bronchi and the larger (nonterminal) bronchioles were 11 ± 6% and 10 ± 5%, respectively. Goblet cells were infrequently present in the terminal bronchioles (2 ± 3%) and absent in the respiratory bronchioles. Using the airway classification based on internal diameter, the number of goblet cells was: group 1 (⩾ 4 mm), 10 ± 6%; group 2 (2–4 mm), 13 ± 5%; group 3, 10 ± 4%, and group 4, 1 ± 2%. Co-expression of CC10 was found in 1,014 goblet cells (Figure 1). Relative to the total number of goblet cells, the proportion of CC10 positive goblet cells in the bronchi was 25%, in the larger nonterminal bronchioles, 34%, and in the terminal bronchioles, 45%, using the morphologic airway classification system. Three different patterns of CC10 immunoreactivity were seen (Figure 1): coarse CC10 positive granules throughout the PAS-positive mucous vacuoles; dense immunostaining on the apical

TABLE 2

NUMBER OF ASSESSED AIRWAYS, RANGE OF INTERNAL AIRWAY DIAMETERS, TOTAL NUMBER OF EPITHELIAL CELLS PER MILLIMETER OF BASEMENT MEMBRANE (BM)*

	Airway Category				
	Bronchi	Bronchioles	Terminal Bronchioles	Respiratory Bronchioles	Total/Mean
Number of assessed airways	45	24	21	21	111
Range of internal airway diameters, mm	2–17	0.5–1.8	0.4–0.9	0.2–0.4	
Number of assessed epithelial cells	23,018	10,102	9,388	8,756	51,264
Mean number of epithelial cells/ mm of bm	349 ± 34	284 ± 27	214 ± 20	184 ± 5	258 ± 16
Clara cells†	0	0.4 ± 1.0	11 ± 5	22 ± 5	
Goblet cells†	11 ± 6	10 ± 5	2 ± 3	0	
Proliferation compartment†	0.91 ± 0.73	0.61 ± 0.91	1.00 ± 0.74	0.81 ± 0.65	0.83 ± 0.77
Contribution of Clara cells to the proliferation compartment, %	0	0	15	44	

* Clara cells, goblet cells and proliferation compartment expressed as percentage of epithelial cells per morphologic airway category.
† Mean ± SD.

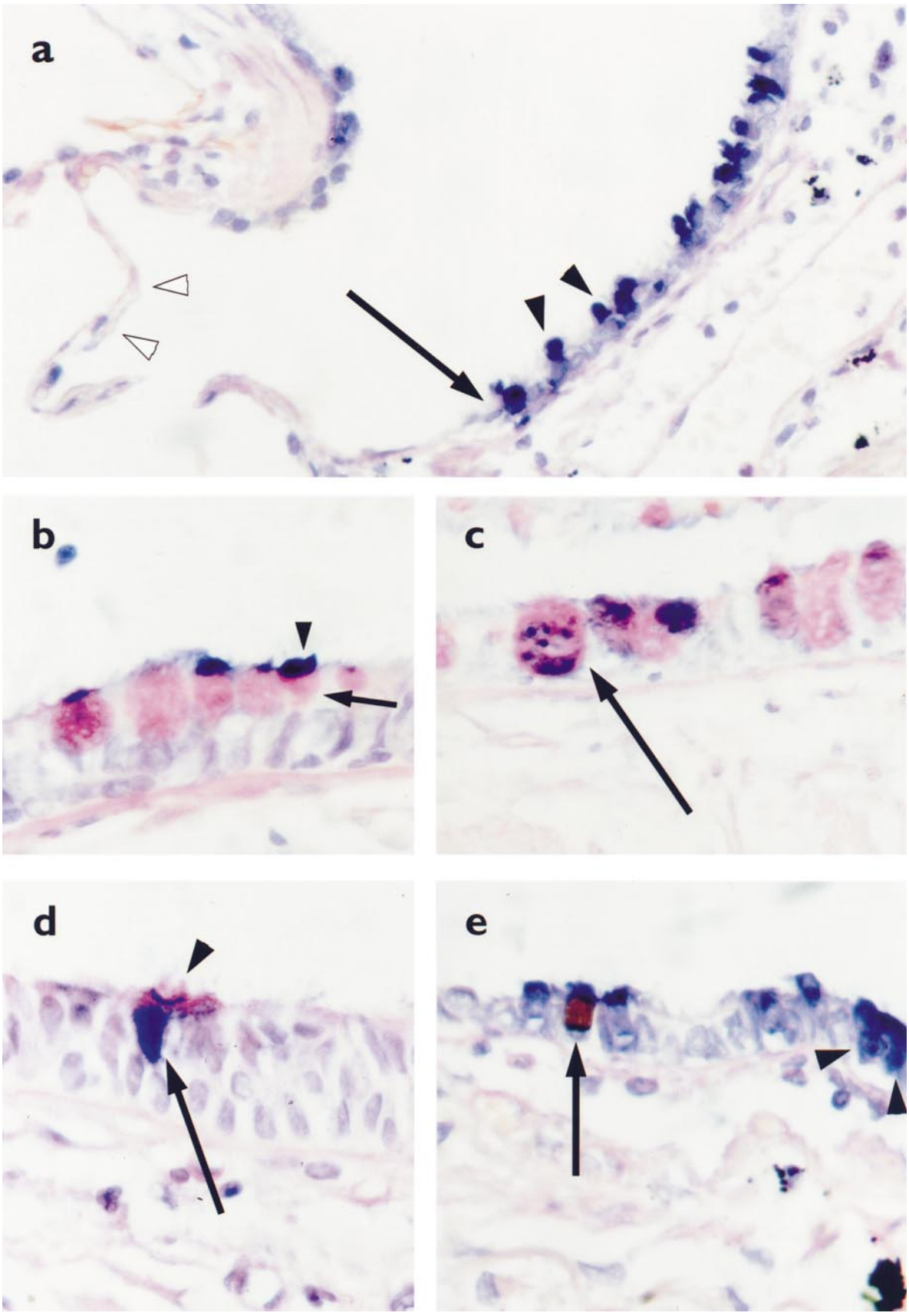

Figure 1. (*a*) The right side of the photograph shows the lightly stained ciliated epithelium of a respiratory bronchiole with intermingled dark blue CC10-immunoreactive Clara cells (*black arrowheads*). The junction between the ciliated conducting airway epithelium and the respiratory zone is indicated (*large arrow*). An alveolar wall is present in the left side of the photograph (*open arrowheads*). (*b*) Several goblet cells with PAS-positive cytoplasm (light red, *arrow*) with dark blue CC10 immunoreactivity of the apical part of the cells (black, *arrowhead*). (*c*) Goblet cell (*arrow*) with PAS-positive cytoplasm (light red) with coarse CC10-positive granules (dark blue). (*d*) Goblet cell with perinuclear CC10-immunoreactive cytoplasm (dark blue, *arrow*) with PAS-positive apex (*arrowhead*). (*e*) Clara cells in a respiratory bronchiole (*arrowheads*). One Clara cell with a MIB-1 positive nucleus (dark brown/black, *arrow*). Original magnification: a, ×500; b–e, ×1,000.

part of the cytoplasm; finely granulated immunostaining of the non-PAS-positive cytoplasm surrounding the goblet cell nucleus.

Indeterminate Cells

In total, 130 indeterminate cells were counted in the nonrespiratory conducting airways, accounting for 3% of the total CC10 immunoreactive cell population. Indeterminate cells were present in the bronchi (72 cells), larger (nonterminal) bronchioles (42 cells), and terminal bronchioles (16 cells). This pattern resembles the distribution of goblet cells and not Clara cells, suggesting that indeterminate cells are part of the goblet cell population that has extruded its mucins.

Proliferation Compartment

The proliferation compartment was defined by the immunoreactivity of the Ki-67 antigen by the MIB-1 antibody in airway cell nuclei. There were 427 airway cells that were MIB-1 positive. The overall proliferation compartment expressed as the percentage MIB-1 positive epithelial cells per airway category is shown in Table 2. Four of 3,519 goblet cells were MIB-1 positive, representing approximately 0.1% of the proliferation compartment. Thirty-eight of 2,950 Clara cells (1.3%) were MIB-1 immunoreactive; the contribution of Clara cells to the overall proliferation compartment was 9%. The contribution of Clara cells to the proliferation compartment is higher in the respiratory bronchioles (44%) than in the terminal bronchioles (15%), possibly due to the difference of Clara cell numbers.

DISCUSSION

The purpose of the present study was to determine in normal human conducting airway epithelium the distribution of Clara cells and the contribution of these cells to the proliferation compartment. The number of Clara cells in the terminal and respiratory bronchioles was 11% and 22%, respectively. Clara cells were virtually absent in the proximal airways. The contribution of Clara cells to the proliferation compartment was 15% in the terminal bronchioles, and 44% of the ciliated epithelium of the respiratory bronchioles.

The presence in the human distal conducting airways of a nonciliated nonmucous cell with characteristic secretory granules, now commonly known as the Clara cell, has been confirmed by numerous morphologic studies following the original descriptions by Kolliker in rabbits and by Max Clara in human lungs (1, 9, 26–30). The development of antibodies against human CC10 (4, 5), a protein localized in the Clara cell granules (3), and a similar protein (P1) (6) enabled researchers to perform immunohistochemical (4, 7, 8) studies in human lungs in order to visualize Clara cells. These qualitative studies not only reproduced earlier observations that the epithelium of terminal and respiratory bronchioles contains many Clara cells, but also indicated the presence of variable numbers of CC10/P1 immunoreactive cells in the proximal airways. A proportion of these CC10 immunoreactive bronchial cells were demonstrated to be goblet cells (4, 7), a secretory cell commonly found in nonrespiratory airways. For the determination of Clara cell numbers throughout the human bronchial tree, we decided to use CC10 immunohistochemistry simultaneously with the PAS stain as an additional marker for goblet cells. A considerable number of goblet cells, defined by a flask-shaped cytoplasm filled with large PAS-positive mucous vacuoles, showed one of three patterns of CC10 immunoreactivity (Figure 1). The proportion of CC10 positive goblet cells increased with diminishing airway size, reaching 45% of goblet cells located in the terminal bronchioles. The frequent occurrence of CC10 immunoreactive goblet cells puts forward the question of whether an intermediate secretory cell exists with characteristics of both goblet cells and Clara cells. Some evidence indicates that the existence of such cells is not unlikely. Ultrastructural examination of rhesus monkey lungs has revealed considerable morphologic heterogeneity of goblet cell secretory granules. Plopper and colleagues (31, 32) reported electron-dense structures in or adjacent to electron-lucent mucous granules of goblet cells in the terminal and respiratory bronchioles. Secretory cells with this intriguing morphology have also been reported in rats exposed to tobacco smoke and in a human lung resected for carcinoma (33).

Plopper and colleagues (2, 18) concluded that Clara cells are principally located at the centriacinar region in those nonhuman species that have an extensive number of respiratory bronchioles. In his classic paper, Max Clara described a short transitional epithelial zone in the human respiratory bronchiole between the ciliated conducting airway epithelium and the flat alveolar lining (1). In our hands, CC10 immunostaining revealed abundant numbers of Clara cells in the respiratory bronchiolar ciliated epithelium (located frequently but not exclusively adjacent to a pulmonary arteriole [2, 18]), but no immunoreactivity was found in the nonciliated cuboidal cells of the transitional zone. As these cuboidal cells have been shown to be ultrastructurally and immunohistochemically related to type II pneumocytes (7, 27), negative CC10 immunostaining supports the distinction in cell type in the centriacinar zone in the human lung.

In a recent study, Shijubo and colleagues (5) established the proportion of Clara cells in the peripheral conducting airways of lung cancer patients using a different antibody raised against CC10. They reported a somewhat higher number of Clara cells (i.e., 27%) after the examination of a total of 30 bronchioles in the lungs of 10 nonsmoking subjects; in addition, a significant decrease in the proportion of bronchiolar Clara cells was noted in smoking in contrast to nonsmoking patients.

Proliferation in the conducting airway epithelium has been examined predominantly in rodents. Several studies (11, 15, 34) have demonstrated a pivotal role of Clara-like cells in the conducting airway epithelium both in health and disease. Important differences exist in both the tracheobronchial cell composition and the organization of the centriacinar region between man and these laboratory animals (2, 18). Even species with an extensive branching of respiratory bronchioles, e.g., nonhuman primates (35), are far from an ideal model for the human lung, in contrast to the speculation of Plopper and colleagues (2). In the rhesus monkey, the composition of the ciliated epithelium corresponds to its upper airways, with 15% goblet cells. A large transitional zone situated between the ciliated and the alveolar epithelium is lined almost exclusively by Clara-like cells, ultrastructurally identical to human Clara cells (31, 32). In humans, the ciliated columnar epithelium of the respiratory bronchiole is situated not only adjacent to a pulmonary arteriole but also between the alveolar sacs (Figure 1). The columnar epithelium is composed of ciliated cells and Clara cells; goblet cells are absent. The cuboidal cells of the transitional zone, located between the columnar epithelium and alveolar lining, represent presumably type 2 pneumocyte-like cells. Thus, important differences are present in airway organization, epithelial cell composition, and even Clara cell ultrastructure between man and all animals examined hitherto.

The present study demonstrates that Clara cells (where

present) contribute substantially to cell renewal in normal conducting airway epithelium of humans and is consistent with results from experimental pathologic studies performed in animal lungs both in the steady state and following induced injury (11–13, 35). Supplementary evidence pointing toward a credible role of the Clara (-like) cell as a major bronchiolar progenitor cell with limited self-renewal capacity can be inferred from rabbit bronchiolar cell culture experiments (14). While in human lungs no information is available of the proliferative response to injury, we do know that the number of Clara cells is actually reduced in smokers (5, 9, 29). Furthermore, Clara cell secretory proteins CC10 and P1 detected in serum and bronchoalveolar lavage fluids are decreased in smokers (5) and in patients suffering from bacterial pneumonia (19), chronic obstructive pulmonary disease, and lung cancer (21). As the Clara cell is implicated in the carcinogenesis of adenocarcinoma (8), examination of the proliferation of this metabolically active bronchiolar cell in human disease may be of help in the determination of the pathogenesis and biology of lung cancer.

In summary, the Clara cell population as detected by the Clara cell 10-kD protein antibody (CC10) is virtually restricted to the distal conducting airway epithelium in normal human lungs. The number of Clara cells in the terminal and respiratory bronchioles is 11% and 22%, respectively. CC10 immunoreactive cells of the proximal airways were found to be goblet cells. The contribution of Clara cells to the proliferation compartment of normal human conducting epithelium was 15% in the terminal bronchioles and 44% in the respiratory bronchioles, demonstrating a substantial role of the Clara cell in the maintenance of the normal epithelium of the distal conducting airways in humans.

***Acknowledgment*:** The authors thank Dr. Gurmukh Singh, University of Pittsburgh and the VA Medical Center, for kindly providing the anti-Clara cell 10-kD protein antibody, and the Department of Pathology of De Wever Hospital, Heerlen, The Netherlands, for supplying part of the lung material.

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