Role of Nonciliated Cells in Renewal of the Bronchial Epithelium of Rats Exposed to NO_2

MICHAEL J. EVANS, PhD, SUSAN G. SHAMI, DSc, LINDA J. CABRAL-ANDERSON, BS, and NUSI P. DEKKER, MS

From Life Science Division, SRI International, Menlo Park, California, and Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico

The purpose of the present study was to identify the proliferative cell types in the nonciliated cell population of the upper airways and determine the capacity of each to act as progenitor cells. Sprague–Dawley rats (30 days old) were exposed to 20 ppm NO_2 for 24 hours to stimulate cell division, given injected tritiated thymidine (^{3}H-TdR), sacrificed 1 hour and 1, 3, 5, and 7 days later, and prepared for light- and electron-microscopic autoradiography. One hour after injection of ^{3}H-TdR, the mean labeling index (LI) was 1.6% in control animals and 5.2% in exposed animals. Mean grain counts per cell decreased from 15.6 at 1 hour after ^{3}H-TdR to 6.9 on the third day, indicating that the labeled cell population had divided. Labeled cells in the control and exposed cell populations were identified with electron microscopy. At 1 hour after injection of ^{3}H-TdR, basal cells and nonciliated columnar cells were labeled. However, only nonciliated columnar cells were stimulated to divide by NO_2. The labeled nonciliated columnar cell population was made up of serous, "intermediate" and goblet cells. Each of these cell types was stimulated to divide to the same degree. After cell division (1–7 days) labeled cells of all types were observed with labeled ciliated cells appearing on the third day. It was concluded that the basal cell is not a primary progenitor cell. The primary progenitor cell for epithelium in the upper airway is the total columnar secretory cell population (serous, "intermediate," and goblet cells). (Am J Pathol 1986, 123:126–133)

EPITHELIUM lining the upper airways consists of basal, nonciliated columnar, and ciliated cells.[1–6] Both the basal and nonciliated columnar cells are capable of cell division.[7–18] Traditionally the basal cell was thought to be the primary progenitor cell for the epithelium.[7,10,11,14] However, recently, Keenan et al[15–18] pointed out that the cell primarily responsible for the proliferative response following mild mechanical injury to the tracheal epithelium is the nonciliated columnar cell, and not the basal cell. The results of other studies in which airway cell proliferation has been stimulated are in agreement with this observation.[12–14,19–22] The only exception occurs when the nonciliated columnar cells have been completely removed from the surface of the airway, in which case basal cells begin proliferating.[23] From the research to date, McDowell et al[21] advanced the hypothesis that nonciliated columnar cells were the primary progenitor cell of airway epithelium, rather than the basal cell. In support of this hypothesis, Plopper et al[24] concluded that basal cells do not play a role in the formation of ciliated or secretory cells in the airways of the developing fetus.

The nonciliated columnar cell population of the upper airway in most animal species can be separated into three groups according to the type of secretory granule present.[3,5,19,25] Cells with no obvious secretory granules have been called intermediate, indifferent, indeterminate, preciliated, or presecretory cells. Cells with dense secretory granules are classified as serous, transitional, or Clara cells. Cells with lucent secretory granules are classified as goblet or small mucous granule cells. From the existing data it is clear that most of these cell types are capable of cell division. However, it is not clear what role each cell type plays in the mechanism for renewal of airway epithelium. Part of this uncertainty is due to the difficulty in identifying cells in the airway epithelium with the light microscope.[25] The pur-

Research conducted at SRI International under National Institutes of Health Grants ES00842, HL16330 and at Inhalation Toxicology Research Institute under Department of Energy Contract Number DE-AC04-76EV01013.

Accepted for publication November 15, 1985.

Address reprint requests to Dr. Michael J. Evans, Division of Pulmonary Disease, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

pose of the present study was to identify the proliferative cells that make up the nonciliated columnar cell population in the upper airways with electron microscopy and to evaluate the role of each cell type as a progenitor cell.

Materials and Methods

Thirty-day-old, specific-pathogen-free, male, Sprague-Dawley rats (Hilltop Laboratory Animals, Inc., Scottdale, Pa) weighing approximately 100 g each were used in this study. Upon arrival from the supplier, 5 rats chosen at random were tested and found to be free of CRD by standard pathologic processes. They were housed 1 per polycarbonate cage with hard wood-chip bedding for 1 week before exposure. They were fed standard laboratory chow and water *ad libitum*. The light cycle was 12 hours on and 12 hours off, and the temperature was 68–72 F. They were exposed to 18–20 ppm of NO_2 for 24 hours to injure the epithelium and ensure a maximal number of dividing cells in the airways. Details of the exposure methods and chambers have been described elsewhere.[26] The rats were then removed to a normal ambient atmosphere and given intraperitoneal injections of tritiated thymidine (^{3}H-TdR), 1.0 μCi/g (specific activity, 6.7 Ci/mM). At intervals of 1 hour and 1, 3, 5, and 7 days after the injection of ^{3}H-TdR, 3 rats were killed with an overdose of sodium pentobarbitol. The lungs were fixed *in situ* by airway perfusion of 2% glutaraldehyde in 0.1 M cacodylate buffer at a pressure of 25 cm of water. After 1 hour the left lobe was removed, sliced, washed in 0.05 M Veronal acetate buffer, postfixed in 1% OsO_4 in Veronal acetate buffer for an additional hour, and dehydrated in alcohol. Ten samples, each containing a cross-section of an intrapulmonary bronchus, were selected and embedded in epoxy resin (Araldite). The diameter of the bronchi studied ranges from 1.47 to 1.89 mm, with a mean of 1.76 mm. Differential cell counts of these bronchi with the light microscope were as follows: basal, 20–22%; nonciliated, 24–31%; ciliated, 30–42%; not categorized, 14–18%. These results are equivalent to airway level 4 in the rat described by Jeffery and Reid.[3]

For light-microscopic autoradiography, 1-μ sections of the airways were cut, coated with Ilford L-4 emulsion, exposed for 3 weeks, developed, and stained with toluidine blue. A cell was considered labeled if it had four or more grains over the nucleus. Labeling and mitotic indices were determined from counts of at least 2000 nucleated cells in each animal. Grain counts were made by counting the number of silver grains over the nucleus on labeled cells in 10 cross-sections of intrapulmonary bronchi for each animal. The results were expressed as the mean grain count per cell. An example

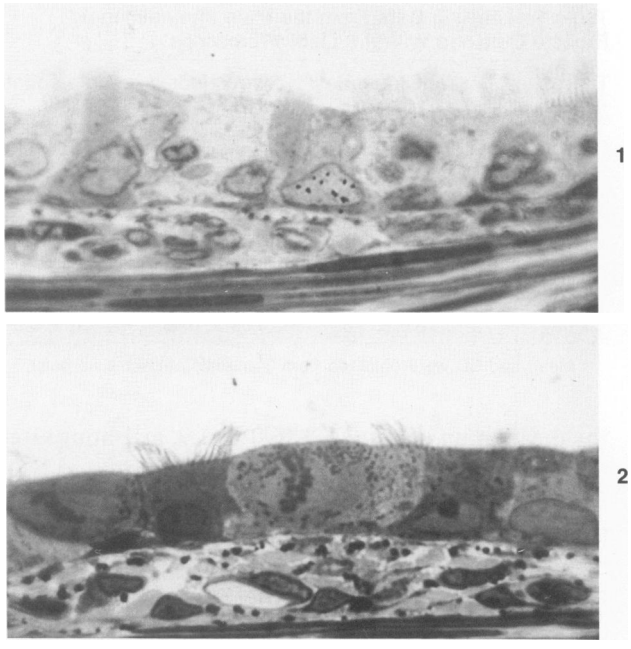

Figure 1—Labeled nonciliated columnar cell 1 hour after ^{3}H-TdR. (×1250) **Figure 2**—Nonciliated columnar cell during mitosis after 24 hours' exposure to 18–20 ppm NO_2, (×1250)

of a labeled cell and one in mitosis are presented in Figures 1 and 2. The labeling and mitotic indices and mean grain counts obtained in this study represent the combined basal and columnar cell populations.[26,27]

For electron microscopy, pale gold sections were cut, picked up on nickel grids, double-stained with uranyl acetate and lead citrate, and coated with carbon on both sides. The grids were then covered with Ilford L-4 emulsion, exposed for 8 weeks, developed, and viewed with Philips EM-200 electron microscope.[26,27]

The labeled epithelial cell population of the intrapulmonary bronchi was analyzed by scanning a grid with the electron microscope and photographing each labeled cell in the airway epithelium from one section on the grid. Ten samples from each rat were studied. All of the labeled epithelial cells collected were separated according to cell type and expressed as a percentage of the total labeled population for each animal. The frequency of cell types in the unlabeled cell populations was determined from counts of 120 cells from four different sections in each animal with the electron microscope. Data are expressed as the mean and standard deviation of three animals.

Results

Light Microscopy

In control rats the mean labeling index (LI) was 1.6 ± 0.3, the mean mitotic index (MI) 0.10 ± 0.06, and

Table 1—Labeling Data From the Main Intrapulmonary Bronchi Obtained With the Light Microscope*

Time after ^{3}H-TdR	Labeling index/ 100 cells	Mitotic index/ 100 cells	Grain count/ cell
Control			
(1 hour)	1.6 ± 0.3	0.10 ± 0.6	16.0 ± 1.9
Experimental			
1 hour	5.2 ± 2.9	0.51 ± 0.09	15.6 ± 1.6
1 day	3.9 ± 1.2	0.14 ± 0.10	9.2 ± 1.5
3 day	4.4 ± 2.6	0.07 ± 0.06	6.9 ± 1.1
5 day	4.9 ± 1.1	0.06 ± 0.04	7.6 ± 0.7
7 day	6.0 ± 1.5	0.08 ± 0.06	7.6 ± 0.5

* Mean and SD were obtained from 3 animals at each time point.

the mean grain count (GC) 16.0 ± 1.9, at 1 hour after injection of ^{3}H-TdR (Table 1). In rats exposed to NO_2 for 24 hours, given ^{3}H-TdR, and sacrificed 1 hour later, the mean LI and MI were dramatically increased. The mean LI was 5.2 ± 2.9, the MI 0.51 ± 0.09, and the GC/6.6 ± 1.6. At 1–7 days after injection of ^{3}H-TdR the mean LI was not significantly different from that at 1 hour. Due to the wide range of individual LI making up the means at each time point, it was not possible to determine whether the labeled cells had divided by analysis of the mean LI. However, the mean GC declined to about one-half by the third day, which indicated that the labeled cell populations had undergone cell division. The MI also returned to normal within 1 day, which indicated that the increased cell proliferation associated with exposure to NO_2 had ceased.

Electron Microscopy

Results of cell labeling obtained with the electron microscope are presented in Table 2. Identification of cell types in the rat was based on the criteria of Jeffery and Reid.[3] Basal cells were identified by their basal position in the airway and low triangular shape (Figure 3). They made up 27.7% ± 8.3% of the labeled cell population in control rats. After exposure to NO_2 they made up 5.2% ± 3.9% of the labeled cells 1 hour after injection of ^{3}H-TdR. At 1–7 days after injection of ^{3}H-TdR the frequency of labeled basal cells remained low.

The nonciliated cell population made up 44.8% ± 2.9% of the labeled cells in control rats. After exposure to NO_2, they increased to 73.2% ± 8.1% of the labeled cell population. They maintained a high frequency in the labeled cell population at 1–7 days after ^{3}H-TdR. The labeled nonciliated cell population was separated according to the presence or absence of secretory granules and their morphology in Table 3. Cells with no obvious secretory granules made up 12.2% ± 6.4% of the labeled nonciliated cells in control rats (Figure 4). After exposure to NO_2, the frequency increased to 29.3% ± 3.8%. At 1–7 days after ^{3}H-TdR the frequency declined and was not different from that of the control rats. Most of these cells appeared to be presecretory cells.[25] The nonciliated cell population containing dense secretory granules was made up of cells containing one or more such granules. Dense secretory granules were of varying sizes and number in the cells and included transitional granules that appeared to be differentiating into lucent secretory granules (Figures 5 and 6). They made up 82.2% ± 24.0% of the labeled nonciliated cells in control rats. After exposure to NO_2, their frequency decreased to 61.5% ± 12.9%. At 1–7 days after injection of ^{3}H-TdR, the labeled cell frequency returned toward control levels. Nonciliated cells containing lucent secretory granules made up 5.6% ± 5.1% in the control rats (Figure 7). They were observed labeled at all times after exposure to NO_2 at various frequencies but at no time were different from controls. The frequency of unlabeled nonciliated cell types in the control population and after a 24-hour exposure to NO_2 was similar to that of the labeled cell populations (Table 3).

Preciliated cells were identified by the absence of cilia and the presence of fibrogranular bundles, or basal bodies. They were not labeled at 1 hour after injection of

Table 2—Labeled Cell Types in the Main Intrapulmonary Bronchi Identified With Electron Microscopy*

Time after ^{3}H-TdR	Basal	Nonciliated	Preciliated	Ciliated	Migratory	Other†	Total number: of cells
Control							
(1 hour)	27.7 ± 8.3	44.8 ± 2.9	0	0	17.0 ± 14.5	10.4 ± 6.5	25 ± 7
Experimental							
1 hour	5.2 ± 3.9	73.2 ± 8.1	0	0	9.2 ± 7.7	7.7 ± 7.1	34 ± 5
1 day	9.8 ± 3.9	64.8 ± 8.3	5.7 ± 0.8	1.0 ± 1.6	4.2 ± 4.0	14.5 ± 6.1	48 ± 14
3 days	5.3 ± 6.3	70.4 ± 6.4	3.7 ± 4.5	13.2 ± 4.9	1.8 ± 1.6	5.6 ± 6.0	34 ± 7
5 days	5.6 ± 1.0	65.4 ± 10.9	0	12.4 ± 5.6	9.6 ± 8.4	7.1 ± 3.5	41 ± 3
7 days	10.7 ± 2.4	60.6 ± 11.9	0.3 ± 0.6	20.6 ± 10.2	0	7.7 ± 3.0	55 ± 38

* Data are presented as the percent of each cell type in the total labeled population. Mean and SD were obtained from 3 animals at each time point.
† This category includes all other cell types in the airway epithelium. However, the cells appear to be mainly ciliated and nonciliated cells that could not be identified because the way they were cut or aligned in the tissue.

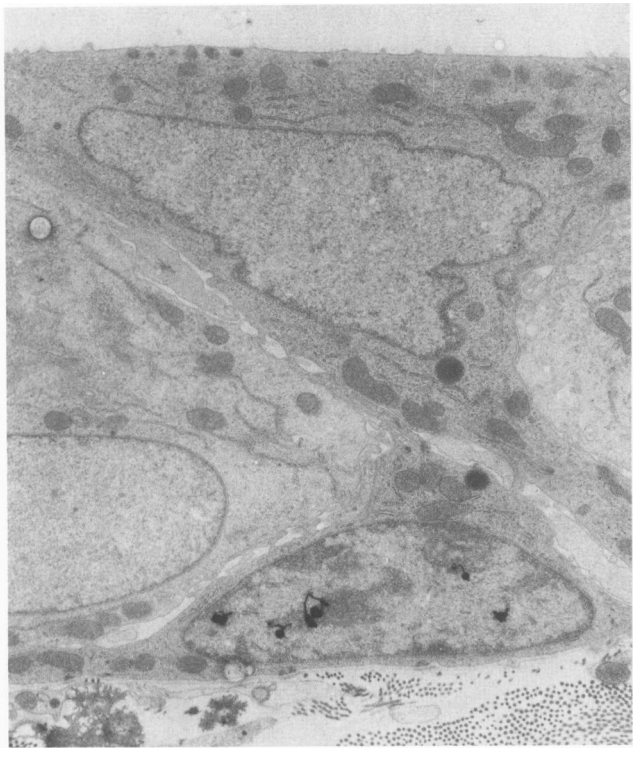

Figure 3—Basal cell 1 hour after ^{3}H-TdR. (×7100)

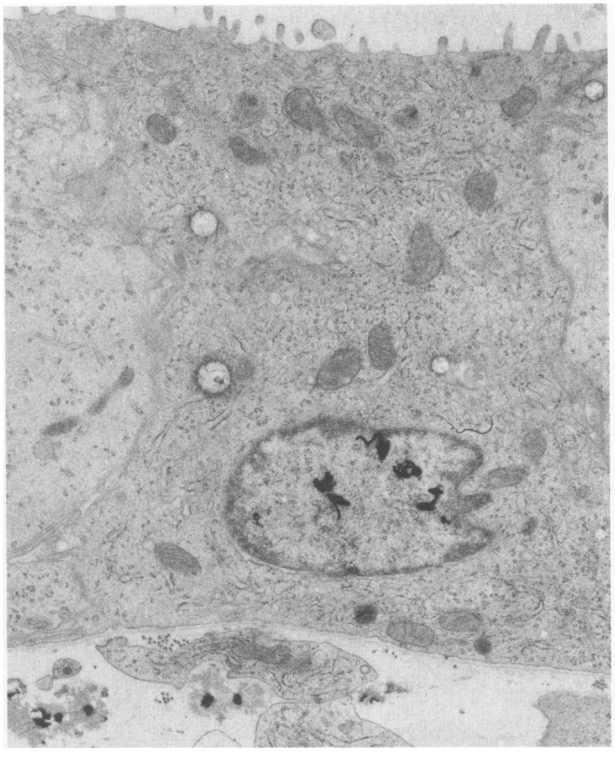

Figure 4—Nonciliated cell containing no obvious secretory granules 1 hour after ^{3}H-TdR. (×7100)

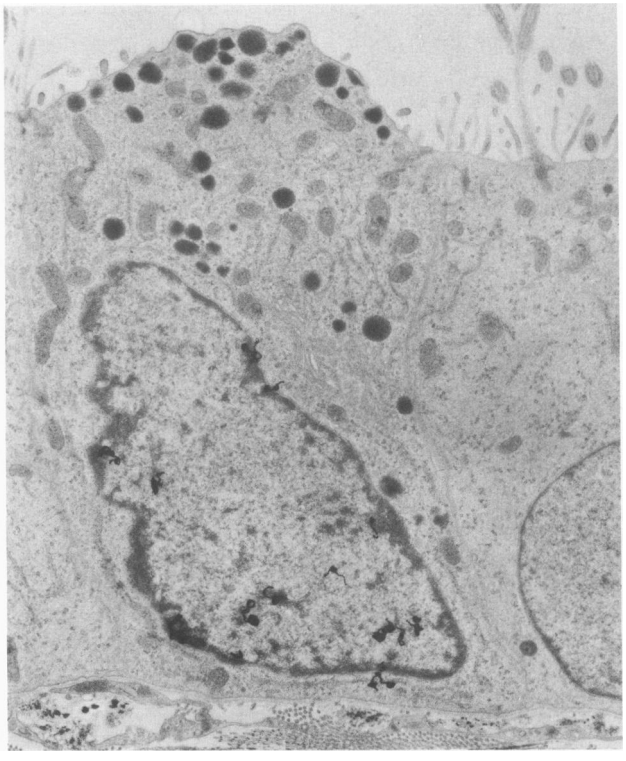

Figure 5—Nonciliated cell containing dense secretory granules 1 hour after ^{3}H-TdR. (×6000)

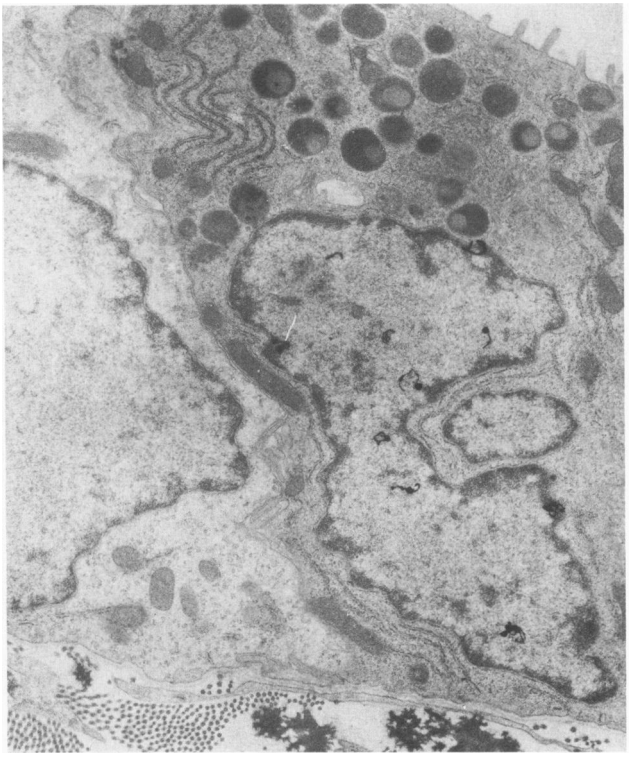

Figure 6—Nonciliated cell containing dense transitional granules 1 hour after ^{3}H-TdR. (×6000)

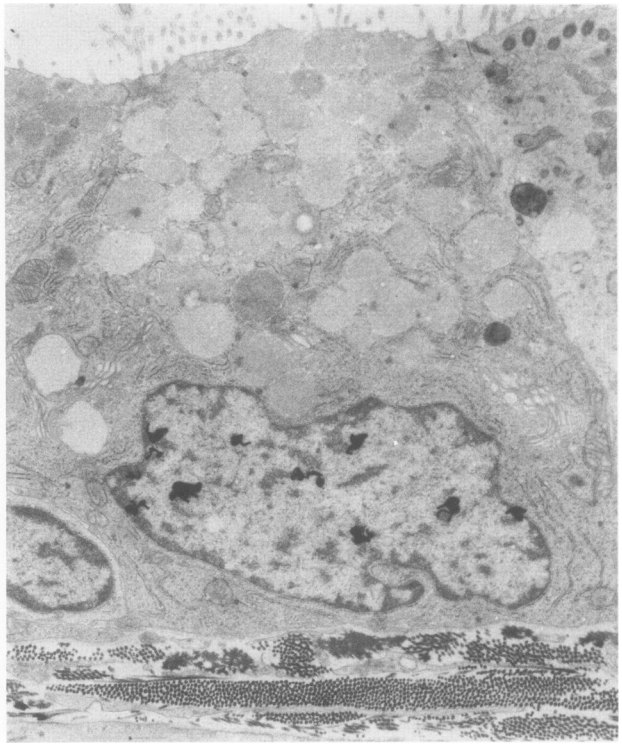

Figure 7—Nonciliated cell containing lucent secretory granules 1 hour after ^{3}H-TdR. (×6000)

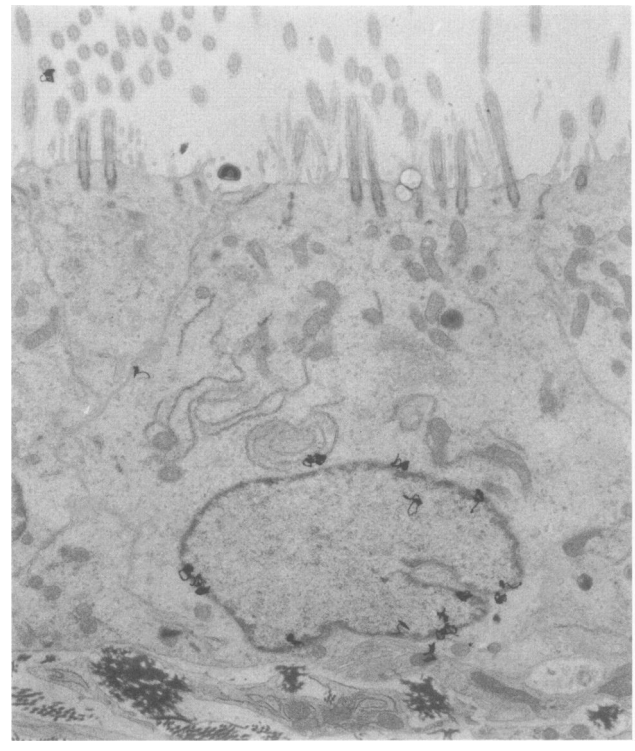

Figure 8—Ciliated cell 3 days after ^{3}H-TdR. (×6000)

^{3}H-TdR in the controls or the rats exposed to NO_2. Several labeled preciliated cells were observed at 1, 3, and 7 days after labeling with ^{3}H-TdR (Table 2).

Ciliated cells were not labeled 1 hour after ^{3}H-TdR in both the control rats and rats exposed to NO_2 (Table 2). However, they were observed labeled at 3, 5, and 7 days after injection of ^{3}H-TdR (Figure 8).

Migratory cells, which appeared to be mainly small lymphocytes, were observed labeled at all times except 7 days (Figure 9). The remaining cells in the labeled cell population (category "other") appeared to be mainly nonciliated cells; however, because of the way these cells were cut or aligned in the tissue, they could not be positively identified. Such labeled cells were present at all time intervals studied (Table 2).

Discussion

This study describes the ^{3}H-TdR-labeled cell population in the intrapulmonary bronchi of the rat exposed to NO_2 with the use of both the light and electron microscopes. After NO_2 exposure there is mild damage to the

Table 3—Labeled Nonciliated Cell Types (Based on the Type of Secretory Granule Present) in the Main Intrapulmonary Bronchi Identified With Electron Microscopy*

Time after ^{3}H-TdR	Type of secretory granules present			Average number of cells/animal
	None	Dense	Lucent	
Control				
(1 hour)	12.2 ± 6.4	82.2 ± 24.0	5.6 ± 5.1	11 ± 3
	(20.1 ± 8.1)†	(73.6 ± 12.4)	(6.3 ± 3.1)	(120)
Experimental				
1 hour	29.3 ± 3.8	61.5 ± 12.9	9.1 ± 9.4	25 ± 5
	(35.0 ± 6.2)	(55.6 ± 14.7)	(9.4 ± 4.0)	(120)
1 day	18.6 ± 2.2	75.2 ± 12.8	5.3 ± 9.1	25 ± 11
3 days	16.3 ± 15.3	63.3 ± 13.3	20.4 ± 11.2	24 ± 3
5 days	11.2 ± 10.1	81.1 ± 14.9	7.6 ± 4.0	18 ± 5
7 days	20.6 ± 10.2	63.4 ± 10.6	16.0 ± 9.4	31 ± 15

* Data are presented as a percentage of the labeled nonciliated cell population. Mean and standard deviation were obtained from 3 animals at each time point.

† Percentage of cell types in the unlabeled nonciliated cell population.

airway epithelium, a decrease in the number of cells containing dense secretory granules, and a large increase in ^{3}H-TdR-labeled cells.[6,28,29] The ^{3}H-TdR-labeled cells were shown to have undergone cell division by the third day of recovery in air. They represent a G_1 population of cells stimulated to divide following injury.[12] Electron-microscopic study of the labeled cell population revealed that all cell types commonly present in the airway epithelium were labeled 1 hour after injection of ^{3}H-TdR, with the exception of preciliated and ciliated cells. The labeled airway epithelial cells at 1 hour after ^{3}H-TdR represent those preparing to divide and, thus, the progenitor cell population. Comparing their relative frequencies in the labeled cell population illustrates the significance of each cell type as a progenitor cell.

The large increase in labeled cells observed after exposure to NO_2 was confined to the nonciliated columnar cell population (Table 2). The basal cell population was not stimulated to divide. In the rat, the nonciliated columnar cell population consists of cells with no obvious secretory granules ("intermediate" cells), with dense secretory granules (serous and transitional cells), and with lucent secretory granules (goblet cells). All three of these nonciliated columnar cell populations were found labeled 1 hour after ^{3}H-TdR in the control rats and in those exposed to NO_2, which indicates that they are capable of acting as progenitor cells.

Most of dividing nonciliated cells were those containing dense secretory granules. The dense secretory granules were observed in varying sizes and numbers in the cells. Cells containing transitional granules were also observed labeled but not in sufficient number to make a separate category. Cells containing dense secretory granules comprise the largest proportion of cells in the nonciliated cell population of the rat and have been reported by other investigators to be capable of cell division.[6,12-14,30]

The second largest population of labeled cells occurred in nonciliated cells containing no obvious secretory granules ("intermediate"). The nature of the nonciliated cell without secretory granules has recently been studied in the hamster by McDowell et al.[25] They showed that most such cells were not a distinct "intermediate" cell type but instead were presecretory and preciliated cells. A small population of cells having no secretory or ciliated cell characteristics was classified as indeterminate cells and thought to represent undifferentiated cells. In the present study preciliated cells were placed in a separate category, and no attempt was made to differentiate between indeterminate and presecretory cells. Thus, the cell population with no obvious secretory granules is made up mainly of presecretory cells. Traditionally, cells without obvious secretory granules (presecretory cells) have been considered the main

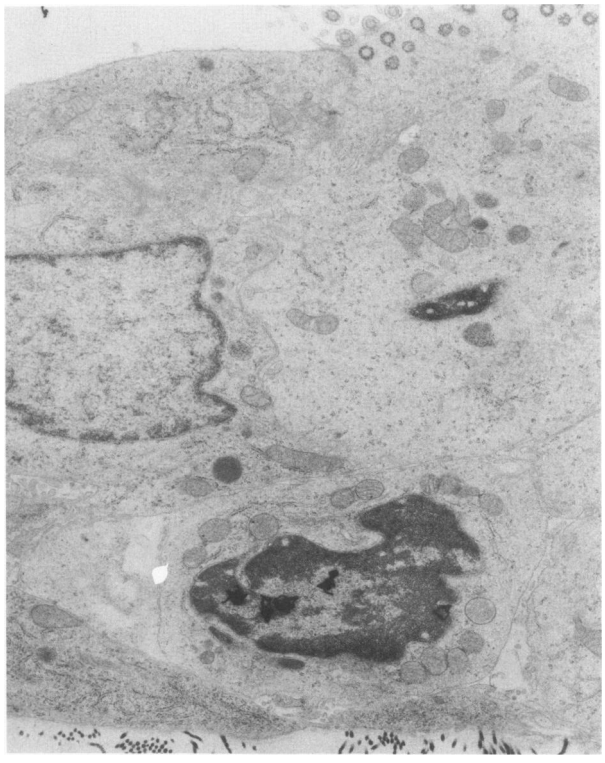

Figure 9—Cell classified as migratory 1 hour after ^{3}H-TdR. (×7100)

columnar nonciliated progenitor cell.[6,7,10,11,14] However, in this and other recent studies,[14-18] they have been shown to make up a small proportion of the total labeled nonciliated cell population and thus cannot be considered of primary importance as progenitor cells.

The smallest proportion of labeled nonciliated cells were those with lucent secretory granules (goblet cells). They were observed labeled in control and experimental rats 1 hour after ^{3}H-TdR, which indicates that they can divide. Recently, Jeffery et al[14] also reported that goblet cells were capable of cell division. Although long considered a terminally differentiated cell, these data indicated that goblet cells could divide and must be considered as part of the progenitor cell population of airway epithelium.

The purpose of the present study was to evaluate the role of each cell type as a progenitor cell. It was shown that each of the nonciliated columnar cell populations could be stimulated to divide. However, the number of dividing cells each population contributed to the total dividing population was different. This difference exists because each cell population is present in the airway epithelium in different numbers. When the frequency of each cell population in the total labeled cell population was compared with their frequency in the total unlabeled cell population, it was found that the frequencies were approximately the same (Figures 10

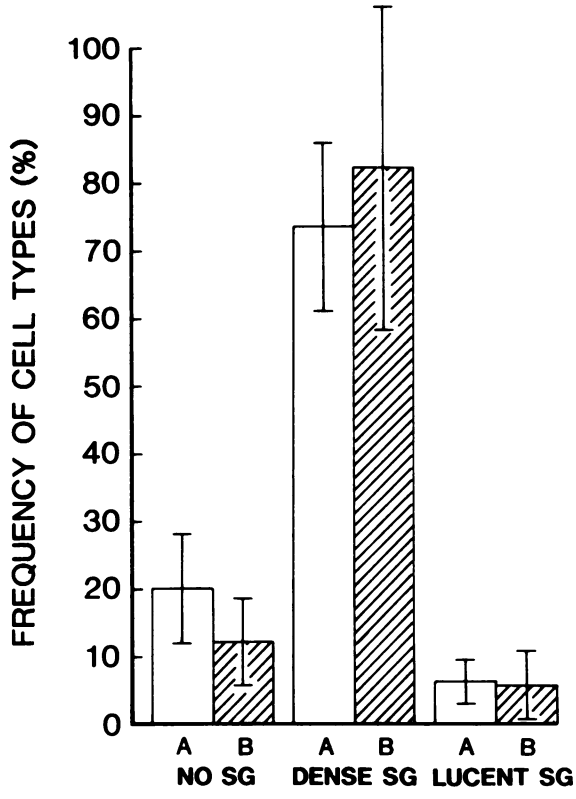

Figure 10—Cell frequencies in the unlabeled (**A**) and labeled (**B**) nonciliated cell populations of control animals 1 hour after ^{3}H-TdR.

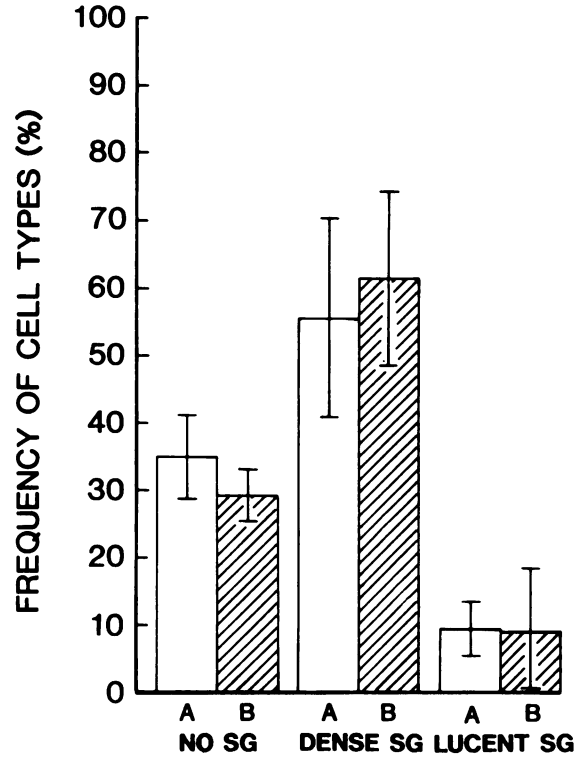

Figure 11—Cell frequencies in the unlabeled (**A**) and labeled (**B**) nonciliated cell populations of exposed animals 1 hour after ^{3}H-TdR.

and 11). This indicates that each nonciliated cell population (serous, presecretory, and goblet) was stimulated to divide to the same degree. Thus, each cell population has the same capacity to act as progenitor cells. These data can also be interpreted to mean that the total nonciliated columnar cell population acted as a unit with respect to a stimulus for cell division. Substantial evidence has accumulated indicating that presecretory, serous, and goblet cell types are subsets of once cell type, columnar secretory cells.[6,14,19,25] The present data support this concept and indicate that the total columnar secretory cell population can act as the primary progenitor cell for airway epithelium.

Traditionally, basal cells were thought to be the primary progenitor cells of airway epithelium.[6,7,10,11,14] If so, mild injury to the epithelium should stimulate basal cell division, as has been reported for the skin.[31] The results of the present study show that after exposure to NO_2, the basal cells were not stimulated to divide above control levels (Table 2). This is in agreement with other studies demonstrating no increase in basal cell division after various forms of stimualtion.[14-18,20,21] Basal cells have been stimulated to divide after severe mechanical injury in which the epithelium had been denuded and the basement membrane exposed.[12,13,23] However, in these studies, nonciliated columnar cells adjacent to the injury also had an increased rate of cell division and were involved in renewal of the damaged epithelium. In most of the studies reported, the rate of basal cell division remains constant after stimulation, whereas the nonciliated cell rate of division increases dramatically. Therefore, in agreement with others,[15-18,21,24] we conclude that basal cells are not of primary importance as progenitor cells of airway epithelium.

The cells classified as "other" were those whose surface did not reach the airway lumen and could not be classified as being ciliated, nonciliated, basal, or migratory. However, most of these cells appeared to have characteristics of secretory cells. Because of our criteria for cell identification, the cells in this category do not represent a unique cell population. Their labeling frequency was generally low and not significantly different at any time except at 1 day after exposure to NO_2.

The appearance of labeled preciliated and ciliated cells at 1-3 days after labeling is consistent with other studies.[7,13,26,27] These cells have been shown to be terminally differentiated and nondividing, derived through differentiation of nonciliated columnar cells.

In summary, this study has shown with electron microscopy that all cell types normally present in the airway epithelium of the rat, except preciliated and ciliated

cells, are capable of cell division. However, only nonciliated columnar cells could be stimulated to divide in numbers above control levels. Each of the nonciliated columnar cell populations (serous, presecretory, and goblet) were stimulated to divide to the same degree. From these data, we conclude that the basal cell is not a primary progenitor cell. The primary progenitor cell of airway epithelium is the total nonciliated columnar cell population (columnar secretory cells), which can divide and form new secretory and ciliated cells. This mechanism for renewal of pulmonary upper airway epithelium is similar to that found in the terminal bronchioles and alveoli, where secretory cells, Clara and Type 2, also, serve as progenitor cells and basal cells are absent.[26,27,32-35]

References

1. Becci PJ, McDowell EM, Trump BJ: The respiratory epithelium. II. Hamster trachea, bronchus, and bronchioles. J Natl Cancer Inst 1978, 61:551-562
2. Breeze RG, Wheeldon EB: The cells of the pulmonary airways. Am Rev Respir Dis 1977, 166:705-777
3. Jeffery PK, Reid L: New observations of rat airway epithelium: A quantitative and electron microscopic study. J Anat 1975, 120:295-320
4. McDowell EM, Barrett LA, Glavin F, Harris CC, Trump BF: The respiratory epithelium: I. Human bronchus. J Natl Cancer Inst 1978, 61:539-549
5. Plopper CG, Mariassy AT, Wilson DW, Alley JL, Nishio SJ, Nettesheim P: Comparison of nonciliated tracheal epithelial cells in six mammalian species: Ultrastructural and population densities. Exp Lung Res 1983, 5:281-298
6. Reid L, Jones R: Bronchial mucosal cells. Fed Proc 1979, 38:191-196
7. Bindreiter M, Schuppler J, Stokinger L: Zellproliferation und Differenzierung im Trachealepithel der Ratte. Exp Cell Res 1968, 50:377-382
8. Blenkinsopp WK: Proliferation of respiratory tract epithelium in the rat. Exp Cell Res 1967, 46:144-154
9. Bolduc P, Reid L: Mitotic index of the bronchial and alveolar lining of the normal rat lung. Am Rev Respir Dis 1976, 114:1121-1128
10. Boren HG, Paradise LJ: Cytokinetics of the lung. Pathogenesis and Therapy of Lung Cancer. Edited by C Harris. New York, Marcel Dekker, 1978, p 369
11. Donnelly GM, Haack DG, Heird CS: Tracheal epithelium: Cell kinetics and differentiation in normal rat tissue. Cell Tissue Kinet 1982, 15:119-130
12. Gordon RE, Lane BP: Wound repair in rat tracheal epithelium: Division of G_1 and G_2 arrested cells following injury. Lab Invest 1980, 42:616-621
13. Gordon RE, Lane BP: Ciliated cell differentiation in rat tracheal epithelium. Lung 1984, 162:233-243
14. Jeffery PK, Ayers M, Rogers D: The mechanisms and control of bronchial mucus cell hyperplasia. Adv Exp Med Biol 1982, 144:399-409
15. Keenan KP, Combs JW, McDowell EM: Regeneration of hamster tracheal epithelium after mechanical injury: I. Focal lesions: Quantitative morphologic study of cell proliferation. Virchows Arch [Cell Pathol] 1982, 41:193-214
16. Keenan KP, Combs JW, McDowell EM: Regeneration of hamster tracheal epithelium after mechanical injury: II. Multifocal lesions: Stathmokinetic and autoradiographic studies of cell proliferation. Virchows Arch [Cell Pathol] 1982, 41:215-229
17. Keenan KP, Combs JW, McDowell EM: Regeneration of hamster tracheal epithelium after mechanical injury: III. Large and small lesions: Comparative stathmokinetic and single pulse and continuous thymidine labeling autoradiographic studies. Virchows Arch [Cell Pathol] 1982, 41:231-252
18. Keenan KP, Wilson TS, McDowell EM: Regeneration of hamster tracheal epithelium after mechanical injury: IV. Histochemical, immunocytochemical and ultrastructural studies. Virchows Arch [Cell Pathol] 1983, 43:213-240
19. McDowell EM, Combs JW, Newkirk C: Changes in secretory cells of hamster tracheal epithelium in response to acute sublethal injury: A quantitative study. Exp Lung Res 1983, 4:227-236
20. McDowell EM, Keenan KP, Huang M: Effects of vitamin A-deprivation in hamster tracheal epithelium: A quantitative morphologic study. Virchows Arch [Cell Pathol] 1984, 45:197-219
21. McDowell EM, Keenan KP, Huang M: Restoration of mucociliary tracheal epithelium following deprivation of vitamin A. Virchows Arch [Cell Pathol] 1984, 45:221-240
22. Wilson DW, Plopper CG, Dungworth DL: The response of the Macaque tracheobronchial epithelium to acute ozone injury: A quantitative ultrastructural and autoradiographic study. Am J Pathol 1984, 116:193-208
23. Lane BD, Gordon RE: Regeneration of rat tracheal epithelium after mechanical injury: I. The relationship between mitotic activity and cellular differentiation. Proc Soc Exp Biol Med 1974, 145:1139-1145
24. Plopper CG, Alley JL, Weir AJ: Differentiation of tracheal epithelium during fetal lung maturation in the rhesus monkey *Macaca Mulatta.* Am J Anat (In press)
25. McDowell EM, Combs JW, Newkirk C: A quantitative light and electron microscopic study of hamster tracheal epithelium with special attention to so-called intermediate cells. Exp Lung Res 1983, 4:205-226
26. Evans MJ, Johnson LV, Stephens RJ, Freeman G: Renewal of the terminal bronchiolar epithelium in the rat following exposure to NO_2 or O_3. Lab Invest 1976, 35:246-257
27. Evans MJ, Cabral-Anderson LJ, Freeman G: Role of the Clara cell in renewal of the bronchiolar epithelium. Lab Invest 1978, 38:648-655
28. Creasia DA, Nettesheim P, Kim JCS: Stimulation of DNA synthesis in the lungs of hamsters exposed intermittently to nitrogen dioxide. J Toxicol Environ Health 1977, 2:1173-1181
29. Hackett NA: Proliferation of airway cells induced by nitrogen dioxide. J Toxicol Environ Health 1979, 5:917-928
30. Wells AB: The kinetics of cell proliferation in the tracheobronchial epithelia of rats with and without chronic respiratory disease. Cell Tissue Kinet 1970, 3:186-206
31. Hennings H, Elgjo K: Epidermal regeneration after cellophane tape stripping of hairless mouse skin. Cell Tissue Kinet 1970, 3:243-252
32. Castleman WL, Dungworth DL, Schwartz LW, Tyler WS: Acute respiratory bronchiolitis: An ultrastructural and autoradiographic study of epithelial cell injury and renewal in Rhesus monkeys exposed to ozone. Am J Pathol 1980, 98:811-840
33. Lum H, Schwartz LW, Dungworth DL, Tyler WS: A comparative study of cell renewal after exposure to ozone or oxygen: Response of terminal bronchiolar epithelium in the rat. Am Rev Respir Dis 1978, 118:335-345
34. Evans MJ, Cabral LJ, Stephens RJ, Freeman G: Renewal of alveolar epithelium in the rat following exposure to NO_2. Am J Pathol 1973, 70:175-198
35. Kauffman SL: Cell proliferation in the mammalian lung. Int Rev Exp Pathol 1980, 22:131-191