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CELL KINETICS IN THE RESPIRATORY TRACT OF HAMSTERS EXPOSED TO CIGARETTE SIDESTREAM SMOKE

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Male Syrian golden hamsters were exposed for 6 h/day, 7 days/wk for up to 3 wk to sidestream smoke generated from Kentucky 1R4F reference cigarettes. Monitoring showed that the chamber atmosphere contained 1 mg/m^3 of total suspended particulate, 385 μg/m^3 of nicotine, and 4 ppm carbon monoxide. Cumulative labeling indices were measured in the pulmonary parenchyma, intrapulmonary airways, trachea, and nasal passages with the bromodeoxyuridine osmotic minipump technique. Cells were labeled during wk 1, 2, and 3 of smoke exposure and for a 1-wk recovery period in air after 1, 2, or 3 wk of smoke exposure. In the airway epithelia, exposure to sidestream smoke did not increase cell proliferation as long as the animals were inhaling smoke, but did so upon removal of the animals from the chamber. In the nasal respiratory epithelium, cell proliferation was initially increased, but then, after removal of the animals into air, was inhibited relative to clean air controls. No changes in labeling index were seen in the trachea or in the pulmonary parenchyma. Although changes seen were only minimal, they nevertheless represent a biological response to the inhalation of sidestream smoke.

In 1992 the U.S. Environmental Protection Agency published a document that analyzed the impact of passive smoking on human health. It was concluded that exposure to environmental tobacco smoke (ETS) is accompanied by an increased risk to develop lung cancer and, in children, respiratory tract infections. ETS is commonly defined as being a mixture of mainstream cigarette smoke (MS) that is exhaled by active smokers and of sidestream smoke (SS), the smoke curling off the glowing tip of a lit cigarette. There are several differences in chemical composition between MS and SS due to variations in burning temperature and to the aging of the SS (Guerin et al., 1992). The major feature, however, that distinguishes active from passive smoking is the much higher concentrations of smoke constituents that are inhaled with each breath by an active smoker compared to an individual exposed to ETS (Gori & Mantel, 1991).

Under laboratory conditions, SS is often used to study and to characterize the biological effects of ETS. At present, comparatively few animal

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inhalation studies are available that describe the effects of SS at concentrations that might mimic exposure to ETS in the working environment or at home. From a recent series of experiments it was concluded that an airborne concentration of 1 mg/m^3 of respirable particles in SS generated from Kentucky 1R4F reference cigarettes might represent a no-observable-effect level (NOEL) in rats (Coggins et al., 1992, 1993). However, in strain A mice that inhaled a similar concentration of total suspended particulates generated from 1R4F, we found signs of increased cell proliferation in the intrapulmonary airways. In C57BL/6 mice, the SS produced no such changes (Rajini & Witschi, 1994). The observations suggest that there might be strain and species differences in response to SS inhalation.

Syrian golden hamsters have been used extensively to characterize the effects of mainstream cigarette smoke, and the resulting histopathological changes in the respiratory tract are well documented (Dontenwill et al., 1973; Bernfeld et al., 1974, 1979; Ketkar et al., 1977). Data on the effects of SS are limited. No histopathological alterations were found in the respiratory tract of hamsters exposed for 90 days to SS generated from 2R1F cigarettes at a concentration of 4.3 mg/m^3 of particles (Von Meyerinck et al., 1989). Given the possibility that evaluation of the cell labeling index (LI) in respiratory tract epithelia might be a more sensitive indicator to SS exposure than descriptive histopathology alone, we decided to investigate the effects of SS exposure on cell proliferation in the respiratory tract of hamsters.

MATERIALS AND METHODS

Materials

Alzet osmotic minipumps, model 2ML1 with a nominal pumping rate of 10 μl/h, were purchased from Alza Corporation, Palo Alto, Calif., and 5-bromo-2'-deoxyuridine (BrdU) from Sigma Chemical Co., St. Louis, Mo. Anti-BrdU antibody was obtained from Boehringer-Mannheim, Indianapolis, Ind., and the Peroxidase Vecto Stain Elite ABC Kit, mouse immunoglobulin G (IgG), from Vector Laboratories, Burlingame, Calif. Immuno-Bed was purchased from Polysciences, Inc., Warrington, Pa. All other chemicals and reagents used were of the highest quality available.

Experimental Design

Male LVG Syrian golden hamsters, 8 wk old, were purchased from Charles River, Wilmington, Del. Upon arrival, the animals were randomly assigned to two groups; animals to be exposed to sidestream smoke (SS), and control animals to be kept in filtered air. All experimental protocols were approved by the institutional review committee. After a 10-day acclimatization period, the animals were placed, within plastic shoebox cages and 5 to a cage, into Hinners-type, 0.44-m^3 stainless steel and glass

chambers. Before placing all animals into the chambers, experimental groups 1A and 1S were identified as follows: Five animals were designed to serve as filtered air controls for wk 1 of exposure (group 1A) and 5 animals were designed as smoke-exposed during wk 1 (group 1S). Immediately before the exposure was started, the hamsters in groups 1A and 1S received an Alzet model 2ML1 minipump filled with bromodeoxyuridine (BrdU, 20 mg/ml in sterile saline) implanted under the skin of the back. The implantation of the pump was done under light methoxyflurane anesthesia. Table 1 gives an overview over experimental design.

One week later, the animals carrying the pumps (groups 1A and 1S) were killed by intraperitoneal injection of a pentobarbital overdose. Lung and nose tissues were fixed for histopathology. At the same time, group 2 was identified as follows: Group 2A animals were air controls; in group 2S were animals that continued to be exposed to SS; and in group 2R were animals that had been exposed to SS during wk 1 and were now removed, for recovery, into air. All animals in group 2 received a minipump and were killed 1 wk later (Table 1).

Group 3 was formed and received minipumps sc at the beginning of wk 3 of the experiment. It was similar to group 2; group 3A, air controls; group 3S, animals that continued to be exposed to smoke; and group 3R, animals that had been exposed to SS for 2 wk and were now removed into air for recovery. The animals were killed 1 wk after pump implantation (Table 1).

At the beginning of wk 4, the remaining animals that had been exposed to SS for 3 wk were removed from the smoke chamber into a chamber ventilated with fresh air (group 4R). They received a minipump implanted at the same time as did group 4A, animals that had been kept in filtered air throughout. One week later, the animals were killed (Table 1).

TABLE 1. Experimental Protocol

Group	Week 1	Week 2	Week 3	Week 4
1A	Filtered air[a]	—	—	—
1S	Sidestream smoke[a]	—	—	—
2A	Filtered air	Filtered air[a]	—	—
2S	Sidestream smoke	Sidestream smoke[a]	—	—
2R	Sidestream smoke	Filtered air[a]	—	—
3A	Filtered air	Filtered air	Filtered air[a]	—
3S	Sidestream smoke	Sidestream smoke	Sidestream smoke[a]	—
3R	Sidestream smoke	Sidestream smoke	Filtered air[a]	—
4A	Filtered air	Filtered air	Filtered air	Filtered air[a]
4R	Sidestream smoke	Sidestream smoke	Sidestream smoke	Filtered air[a]

Note. $n = 5$ For each experimental group.

[a]Minipumps were implanted subcutaneously on the first day of the week indicated in the top row. Seven days later, the animals were killed for collection and analysis of tissue for cumulative labeling indices.

Exposure System

The SS exposure system was identical to the one described in detail by Teague et al. (1994) and used by us in previous studies (Witschi et al., 1994; Rajini et al., 1994; Rajini & Witschi, 1994). Briefly, SS was generated by burning Kentucky 1R4F reference cigarettes (purchased from the Tobacco and Health Research Institute, Lexington, Ky.) in a smoking machine. The cigarettes were stored at 4°C until needed, and at least 48 h prior to use were placed in a closed chamber at 23°C along with a mixture of glycerin/water to establish a relative humidity of 60%. The cigarettes were smoked with standardized 35-ml puffs of 2 s duration, once every minute, for a total of 8 puffs per cigarette. The SS given off the tip of the smoldering cigarette between puffs was drawn, after dilution and aging (2 min) in a conditioning chamber, into a glass and stainless steel Hinners-type exposure chamber (volume 0.44 m^3). The chamber atmosphere was continuously monitored for CO, nicotine, and total suspended particulate matter (TSP).

Total suspended particulate matter was monitored in the conditioning chamber and within the exposure chamber by a piezobalance (TSI Instruments, St. Paul, Minn.) and a PDM-3 MiniRam forward light-scattering device (MIE, Inc., Billerica, Mass.), calibrated by gravimetric method (weight of particles collected on Teflon filters). Particle size distribution was measured using a Royco 236 laser particle counter (HIAC/Royco Instruments, Menlo Park, Calif.). In experiments conducted so far, the mass median diameter was 0.33 ± 0.03 μm with a geometric standard deviation of 2.52 ± 0.08. When the exposure system was installed, empty cages with bedding material were placed into the chamber. Air was sampled through plastic tubing from inside the cages, below the rim and directly above the bedding material. The TSP measurements taken at this breathing zone location gave the same value as did measurements on air concentrations taken at the sampling port used when the cages within the chamber contained animals.

Carbon monoxide was monitored with a model 880 nondispersive infrared (NDIR) analyzer (Beckmann Industries, La Habra, Calif.). Nicotine concentrations in the exposure chamber were measured by drawing air samples every 15 min through sorbent tubes. The tubes were extracted with HPLC-grade ethyl acetate containing 0.1% triethylamine, and the extract was analyzed in a gas chromatograph (Varian 3740) equipped with a DB-5 30 m × 0.53 mm column (film thickness 1.5 μm) and a nitrogen-selective thermionic specific detector. Temperature and relative humidity within the chambers were monitored with an appropriate probe located within the chamber (Rustrack, St. Paul, Minn.). Actual exposure data measured over the 3-wk period are given in Table 2.

Tissue Preparation

The lungs were inflated through the trachea with 1% paraformaldehyde/0.1% glutaraldehyde solution. One hour later the lungs and the trachea were transferred into 70% ethanol, dehydrated in 95% and 100% ethanol,

TABLE 2. Exposure Conditions

Parameter	Average	SD	*n*
Relative humidity (% RH)	34.3	9.3	21
Temperature (°C)	23.7	1.0	21
Carbon monoxide (ppm)	4.1	0.6	21
Nicotine ($\mu g/m^3$)	385	81	20
Total suspended particulates (mg/m^3)	1.03	0.04	21

Note. Chamber concentrations of CO, nicotine, and TSP were measured daily while the animals were exposed to SS.

placed in Immuno-Bed (all lung lobes in 2 blocks and several cross sections of the trachea into another block), and cut on a Sorvall JB 4 microtome in 1- to 2-μm thin sections. The sections were mounted on glass slides precoated with poly-L-lysine. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide in water, followed by an incubation with pronase (0.1 mg/ml for 30 min at 37°C) until the activity was stopped with undiluted calf serum. The sections were washed, incubated with anti-BrdU antibody (1 : 50), with biotinylated secondary antibody, and finally with diaminobenzidine substrate at concentrations and times given in the manufacturer's instructions. The slides were counterstained with methylene blue and basic fuchsin. All slides included a section of small-intestinal epithelium as a positive control for the immunostain.

For examination of the nasal cavities the procedure described by Henderson et al. (1993) was followed. The nasal passages were flushed retrograde through the nasopharyngeal office with Carnoy's fixative (60% ethanol, 30% chloroform, 10% acetic acid v/v). The tissue was fixed for at least 24 h, decalcified in 13% formic acid for approximately 1 wk, and rinsed thoroughly in tap water. The nasal cavity was sectioned transversely immediately posterior to the incisor teeth and 1–2 mm anterior to the incisive papilla. The tissue was embedded in paraffin, and 5-μm sections were cut on a conventional microtome. After deparaffinizing, the sections were stained essentially as described for the lungs, except that endogenous peroxidase activity was blocked with 1% hydrogen peroxide in methanol.

Evaluation of Cell Labeling

All slides were coded and counted without knowing the treatment. Labeling indices were determined for four defined regions in the respiratory tract: terminal bronchioles, intrapulmonary large airways, trachea, and nasal passages (where the nasal septum and the maxillary and nasal turbinates were evaluated separately). Terminal bronchioles were identified by their opening into alveolar ducts and intrapulmonary large airways by their diameter (0.5–1.5 mm). In the airways, all cells that form the pseudocolumnar epithelium were counted as in our previous studies (Rajini et al., 1993), and no attempt was made to further differentiate between basal cells

and other cell types. A minimum of 500 cells per slide was counted in each of the large and peripheral airways and trachea. The labeling index was calculated as the percentage of labeled epithelial cells of the total number of epithelial cells counted. In the alveolar zone, 1000 cells per lung were counted in randomly selected fields and cells were differentiated into type II epithelial cells (identified by their cuboidal shape and location mostly in the corner of the alveoli) and cells in the alveolar wall (endothelial and interstitial cells; Witschi & Morse, 1983). In the nasal passages, 500 cells each were counted in the respiratory epithelium of the nasal septum, the nasal turbinates, and the maxillary turbinates.

Statistical Analysis

For each experimental group, the labeling indices found in any given tissue compartment were calculated as mean and SEM. Comparisons between different treatment groups and corresponding controls were analyzed by ordinary analysis of variance (ANOVA), followed by Dunnett's posttest. Controls were defined as concomitant controls, that is, animals kept in filtered air and carrying a minipump at the same time as did the animals exposed to SS or recovering from smoke exposure. A p value of .05 or less was considered to be significant.

RESULTS

Hamsters were exposed daily, 6 h/day, 7 days/wk for up to 3 consecutive weeks to SS generated from Kentucky 1R4F reference cigarettes. The chamber atmosphere contained 1 mg/m^3 of TSP. Some animals were given an additional week of time in which to recover from smoke exposure. The animals tolerated the smoke atmosphere well and did not show any signs of discomfort. Histological evaluation of tissue slides prepared from the rostral end of the nose, the trachea, and all lung lobes did not reveal any abnormalities that could have been attributed to SS exposure. When the cumulative labeling indices for 1-wk periods were measured, a few changes in labeling indices of the epithelia lining the respiratory tract were observed over the course of the 4-wk study. The cell kinetic findings may be described as follows.

During wk 1 of exposure to SS, a significantly increased labeling index (LI) was found in the respiratory epithelium that lines the nasal septum (Fig. 1). In the maxillary turbinates, a trend towards such an increase was found, whereas in the nasal turbinates and trachea labeling indices in smoke exposed animals were similar to the ones found in controls (Table 3). In the terminal bronchioles, the number of epithelial cells that incorporated BrdU into their DNA appeared to be slightly lower, but the difference to the controls was statistically not significant. In the alveolar zone, overall labeling indices were comparable in controls and SS-exposed animals (Table 4). No differences between smoke-exposed and control animals were also seen

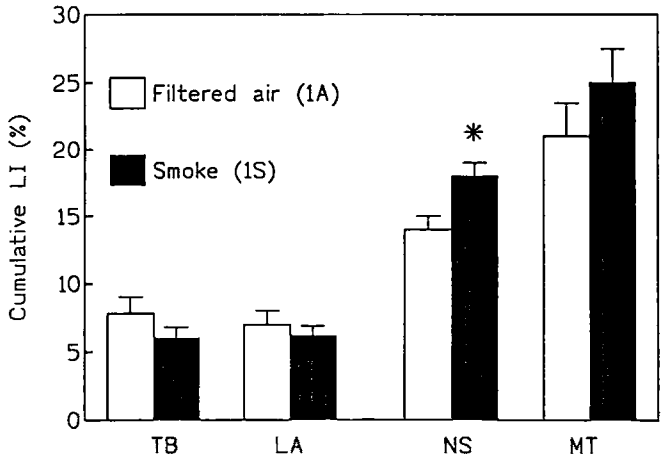

FIGURE 1. Cumulative labeling indices in the respiratory tract of hamsters exposed to SS. Syrian golden hamsters were exposed for 1 wk to SS at a chamber concentration of 1 mg/m^3 of total suspended particulates (1S). Controls were kept in filtered air (1A). Cumulative labeling indices were measured over this period in the terminal bronchioles (TB), large intrapulmonary airways (LA), the respiratory epithelium of the nasal septum (NS), and that of the maxillary turbinates (MT). Data are plotted as means ± SEM, and the number of animals was $n = 5$ per group throughout. The asterisk denotes that the difference between smoke-exposed and control animals was significant at the level of $p < .05$.

TABLE 3. Cumulative Labeling Indices in Nasal Turbinates and Trachea

Group	Nasal turbinate (LI)	Trachea (LI)
Week 1		
Controls (1A)	20.0 ± 2.7	10.0 ± 1.1
Smoke (1S)	20.0 ± 2.0	12.2 ± 0.9
Week 2		
Controls (2A)	19.3 ± 1.0	11.5 ± 0.9
Smoke (2S)	21.2 ± 2.3	11.9 ± 0.9
Recovering (2R)	15.8 ± 1.4	9.1 ± 1.4
Week 3		
Controls (3A)	20.8 ± 3.1	12.7 ± 0.8
Smoke (3S)	19.1 ± 2.6	10.8 ± 1.3
Recovering (3R)	16.7 ± 1.8	10.5 ± 0.3
Week 4		
Controls (4A)	17.7 ± 1.9	11.4 ± 1.4
Recovering (4R)	15.2 ± 1.4	11.4 ± 1.7

Note. All values are expressed as mean ± SEM; the number of animals per group was $n = 5$ throughout.

TABLE 4. Alveolar Labeling Indices

Group	Alveolar labeling index	Number of labeled type II cells/ 10,000 parenchymal cells
Week 1		
Controls (1A)	25.0 ± 1.4	642 ± 30
Smoke (1S)	27.3 ± 1.7	702 ± 73
Week 2		
Controls (2A)	27.5 ± 2.0	689 ± 101
Smoke (2S)	26.8 ± 1.2	720 ± 50
Recovering (2R)	24.8 ± 0.5	590 ± 70
Week 3		
Controls (3A)	25.7 ± 0.7	608 ± 23
Smoke (3S)	27.9 ± 1.4	610 ± 70
Recovering (3R)	26.6 ± 2.2	574 ± 40
Week 4		
Controls (4A)	24.8 ± 2.6	666 ± 101
Recovering (4R)	26.7 ± 1.0	608 ± 81

Note. The number of labeled type II alveolar epithelial cells was calculated from the overall labeling index in the pulmonary parenchyma and the percentage of labeled type II cell (Haschek et al., 1983). All values represent mean ± SEM with the number of animals per group $n = 5$ throughout.

when the data were calculated as the number of labeled type II cells per 10,000 cells in the pulmonary parenchyma (Table 4), a procedure that often is a more sensitive indicator of epithelial cell repair than is the LI (Haschek et al., 1983; Witschi, 1988).

During wk 2, there were again no significant differences between controls and smoke-exposed animals in any of the regions of the respiratory tract that were examined (Fig. 2 and Tables 3 and 4). However, the epithelium lining the terminal bronchioles of animals who had been exposed for 1 wk only to SS showed a significantly increased incorporation of BrdU into nuclear DNA during the recovery period than was found in the concomitant controls. A comparison of the LI in the terminal bronchioles of the animals that recovered during wk 2 with the LI found in the same place in smoke-exposed animals during wk 1 (Fig. 1) showed the difference to be highly significant. A pattern similar to the one seen in the terminal bronchioles was observed in the large airways, except that differences between controls and exposed were not significant at the .05 level. On the other hand, the LI in the maxillar turbinates of the same animals was significantly lower than in the controls. In the nasal septum and in the nasal turbinates the pattern was repeated, although statistically the differences between exposed and control animals were not significant for these anatomical locations.

During wk 3, similar observations were made: In animals that had been removed into filtered air after 2 wk of SS exposure, there was an increased labeling index in the terminal bronchioles compared to controls, although it was statistically not significant. In the maxillar turbinates, the LI was again significantly lower than in the controls, and in the nasal septum and nasal turbinates a similar although not significant pattern was seen (Fig. 3 and Table 3). No changes were found in trachea or alveolar region. In wk 4 there was a suggestion that incorporation of BrdU into DNA was lowered in the animals that had been exposed to SS during the 3 previous weeks and then were allowed to recover for another week in filtered air (Fig. 4). However, statistical significance was not reached.

DISCUSSION

Continuous labeling of dividing cells in the respiratory tract with a suitable precursor such as [^{3}H]thymidine or BrdU over a 1- to 2-wk period is a useful procedure for the evaluation of acute or chronic lung injury (Haschek et al., 1983; Lindenschmidt et al., 1986). More recently it was shown that extensive cell damage and death is not necessarily a prerequisite in order to cause increased cell labeling in the respiratory tract epithelium (Monticello et al., 1991a; Shami et al., 1986). For example, it is possible to detect and to quantitate with the technique of cumulative cell labeling in the deep lung and in the nasal passages quite subtle consequences of exposure to

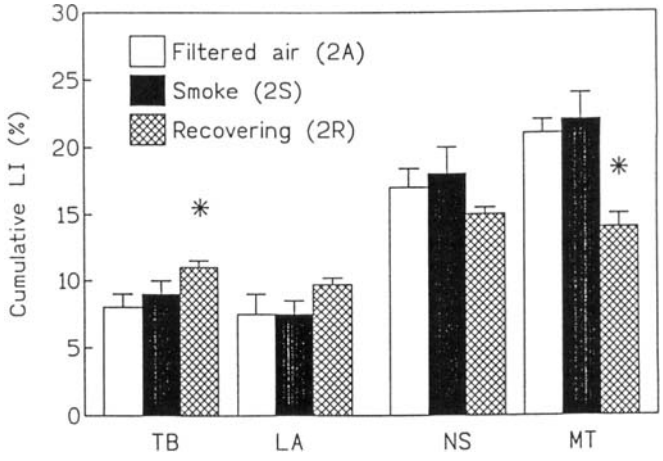

FIGURE 2. Cumulative labeling indices in the respiratory tract of hamsters exposed to SS and allowed to recover in air. Syrian golden hamsters were exposed for 2 wk to SS at a chamber concentration of 1 mg/m^3 of total suspended particulates (2S), or to filtered air (2A). The third group (2R) had been exposed to SS during the previous week and was allowed to recover for another week in air. Cumulative labeling indices were measured during wk 2 of SS exposure in the terminal bronchioles (TB), large intrapulmonary airways (LA), the respiratory epithelium of the nasal septum (NS), and that of the maxillary turbinates (MT). Data are plotted as means ± SEM, and the number of animals was $n = 5$ per group throughout. The asterisk denotes that the difference between smoke-exposed and control animals was significant at the level of $p < .05$.

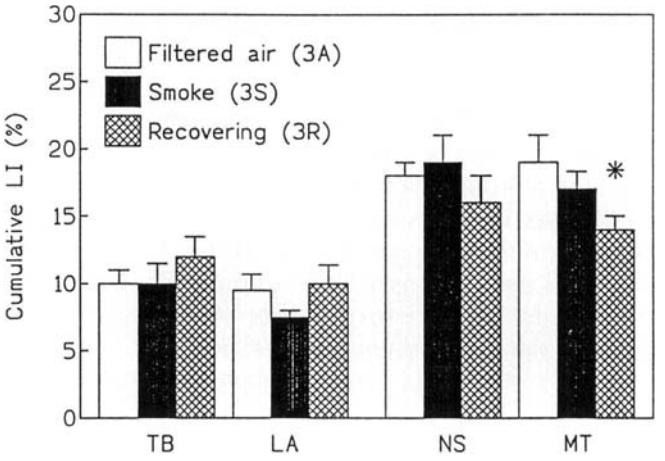

FIGURE 3. Cumulative labeling indices in the respiratory tract of hamsters exposed to SS and allowed to recover in air. Syrian golden hamsters were exposed for 3 wk to SS at a chamber concentration of 1 mg/m^3 of total suspended particulates (3S), or to filtered air (3A). The third group (3R) had been exposed to SS during the previous 2 wk and was allowed to recover for another week in air. Cumulative labeling indices were measured during the third week of SS exposure in the terminal bronchioles (TB), large intrapulmonary airways (LA), the respiratory epithelium of the nasal septum (NS), and that of the maxillary turbinates (MT). Data are plotted as means ± SEM, and the number of animals was $n = 5$ per group throughout. The asterisk denotes that the difference between smoke-exposed and control animals was significant at the level of $p < .05$.

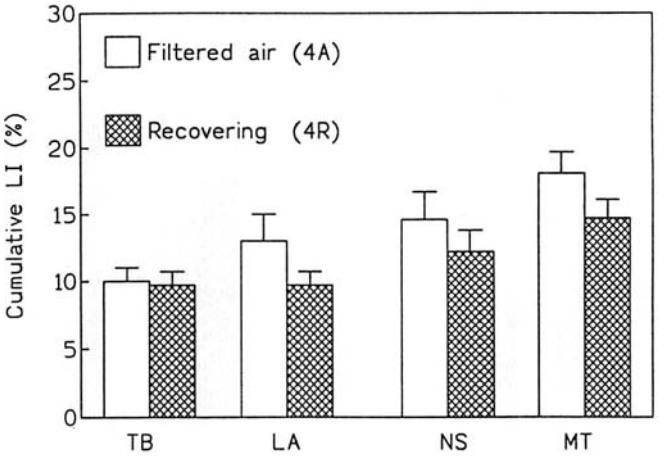

FIGURE 4. Cumulative labeling indices in the respiratory tract of hamsters exposed to SS and allowed to recover in air. Syrian golden hamsters had been exposed for 3 wk to SS at a chamber concentration of 1 mg/m^3 of total suspended particulates. They were then removed and kept, together with control animals (4A), for an additional week in filtered air. Cumulative labeling indices were measured during wk 4 in the terminal bronchioles (TB), large intrapulmonary airways (LA), the respiratory epithelium of the nasal septum (NS), and that of the maxillary turbinates (MT). Data are plotted as means ± SEM, and the number of animals was $n = 5$ per group throughout. The asterisk denotes that the difference between smoke-exposed and control animals was significant at the level of $p < .05$.

low levels of ozone, in the 0.1–0.2 ppm range (Rajini et al., 1993; Henderson et al., 1993). Evaluation of cumulative cell proliferation in the nasal passages may also be a useful tool in predicting potential risk from the inhalation of airborne carcinogens and for making extrapolations from animal data to humans (Monticello et al., 1991b).

Judged by the criterion of increased cell labeling, the effects of SS inhalation on the epithelia that line the hamster respiratory tract were small. Trachea and peripheral lung parenchyma were not affected at all by smoke exposure. In the nasal cavity, there was initially some stimulation of cell proliferation in the septum and to a lesser extent in the turbinates. However, the effect was small and no longer discernible at later time points. Somewhat more pronounced changes in cell labeling were actually seen during wk 2 in animals that recovered from a 1-wk smoke exposure. The changes in cell proliferative patterns appeared to be site specific. In the terminal bronchioles, there was an increased incorporation of BrdU into the epithelium. The change may be interpreted as compensatory hyperplasia, following some slight initial damage. However, in the nasal passages the opposite was observed. At this time, it is not clear whether this decrease in cell labeling reflects a persistent toxic effect of SS or whether this is a common response of nasal respiratory epithelium to cessation of exposure. A similar response pattern as found after 2 wk was also apparent in animals that had been exposed for 2 or 3 wk to SS and subsequently were allowed to recover in air for an additional week.

The modest changes in cell proliferation patterns found in the present experiments agree with the observations that exposure to 1–4 mg/m^3 of SS particulate matter produces only minimal and transient histopathological signs or none whatsoever in the respiratory tract of rats and hamsters (Coggins et al., 1992, 1993; Von Meyerinck et al., 1989). However, whether exposure of rodents to SS at a concentration of 1 mg/m^3 of particulate matter truly constitutes a no-observable-effect level is less certain. In strain A/J mice this concentration of particulate matter was enough to increase cell proliferation substantially over control values in large and small airways, whereas filtered SS without particulate matter had no effect (Rajini & Witschi, 1994). Furthermore, it was found that exposure of rats in utero and during weaning to SS under identical conditions as described in this article alters the pattern of microsomal enzyme activity and of cell proliferation in the developing lungs (Gebremichael et al., 1993; Ji et al., 1993). The SS at a concentration of 1 mg/m^3 also produces a small but statistically significant intrauterine growth retardation (Rajini et al., 1994) and may lead to early pregnancy failure (Witschi et al., 1994) in rats.

The question remains, to what extent will our findings eventually relate to potentially adverse health effects in humans exposed to ETS? The changes seen in the airways of experimental animals exposed to SS at a concentration of 1 mg/m^3 of particulate matter are small. Furthermore, the biological significance of the observations remains uncertain. For example, it is not

clear whether apparently increased (or decreased) cell proliferation without further signs of histopathological damage (this article) or induction of mixed-function oxidases (Ji et al., 1993) represents a mechanism of adaptation rather than a warning sign that precedes the development of serious tissue and organ damage. On the other hand, the findings represent a biological response in the respiratory tract of animals that have been exposed to an inhalant with considerable toxic potential.

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