REGIONAL DIFFERENCES IN DNA REPLICATION IN NASAL EPITHELIUM FOLLOWING ACUTE OZONE OR CIGARETTE SMOKE EXPOSURE

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Abstract — The epithelium of the anterior nasal cavity is composed of four cell types, squamous, respiratory, cuboidal, and olfactory cells. We monitored proliferation in these tissues by bromodeoxyuridine (BrdUrd) incorporation; the labeled cells were identified by using a monoclonal antibody that recognizes BrdUrd. The respiratory, cuboidal and olfactory epithelia had low cell turnover (1-labeled cell/mm basal lamina). Squamous epithelium contained 40-labeled cells per mm basal lamina. Following exposure to diluted mainstream cigarette smoke, a transient, but marked increase in DNA replication was seen in the cuboidal epithelium. In contrast, ozone exposure was associated with DNA replication in the olfactory and respiratory epithelium, as well as in the cuboidal epithelium. These studies show that the sensitivity of nasal epithelium to irritants can be assayed by measuring DNA replication.

Exposure of the respiratory epithelium to inhaled irritants is frequently accompanied by an initial wave of cell proliferation, which is dependent on the concentration, dose, and duration of exposure to the irritant. Initiation of DNA replication/cell proliferation can be used as an indicator of toxicological damage. Little attention has been paid to the nasal epithelium in terms of its proliferative response to inhaled toxicants, in spite of the fact that the nose is one of the major portals of entry for inhaled materials.

Cell proliferation/DNA replication have been traced in normal nasal epithelium, and following exposure to either cigarette smoke or ozone, by using BrdUrd incorporation. BrdUrd is an alternative tracer to tritiated thymidine for studying DNA replication. It is more commonly used for examination of sister chromatid exchanges in metaphase spreads and for the flow cytometric analysis of cells in the S phase of the cell cycle. The commercial availability of monoclonal antibodies which recognize BrdUrd in single-stranded DNA has allowed it to be used in a fashion analogous to tritiated thymidine. Immunohistochemical detection, using BrdUrd and the appropriate monoclonal antibody, of cells synthesizing DNA, is a rapid, non-radioactive approach to studying cell proliferation in sectioned material.

METHODS

Cigarette Smoking Study

Male and female Fischer F344/N rats from the Institute's breeding colony were randomized into 5 treatment groups and housed in inhalation chambers (HC-1000, Hazleton Systems, Aberdeen, MD). Three groups were exposed 6 h/day, 5 day/wk to smoke from 1R3 research cigarettes (Tobacco Health Research Institute, Lexington, KY), to achieve a daily concentration x time product of 600 $mg \cdot h \cdot m^{-3}$ total particulate matter (TPM) for 1 wk, followed by 1200 $mg \cdot h \cdot m^{-3}$ for either 1 or 4 wk. The different smoke generation and exposure methods were similar to those described previously (1986-87 Annual Report, LMF-120, pp. 68-71). The nose-only, intermittent (NOI) group received 10,

10-min exposures at 360 or 720 mg TPM/m^3 continuously during each 6 h exposure period. The nose-only, continuous (NOC) and whole-body, continuous (WBC) groups received 100 or 200 mg TPM/m^3 continuously for 6 h. Nose-only (NOS) and whole-body (WBS) sham-exposed groups received clean air. The nose-only groups were removed from their housing chambers and exposed using commercial units (Mark III, AMESA, Geneva, SW). The whole-body groups were exposed in their housing chambers to smoke generated by identical units. Smoke was generated using 2 sec, 35 mL puffs, once per min for a total of 6 puffs per cigarette.

Ozone Study

Male and female Fischer F344/N rats from the Institute's breeding colony were used. The animals were exposed to nominal ozone concentrations of 0.0 (air controls), 0.12, 0.27, or 0.8 ppm in whole-body inhalation chambers (HC-1000, Hazleton Systems, Aberdeen, MD). The equivalent sea-level ozone concentrations used in this study would be 0.0, 0.1, 0.22, and 0.66 ppm (ITRI is located at 1728 m, where the average barometric pressure is 625 mm Hg, equivalent to 0.82 sea level atmosphere).

The rats were randomized by body weight and conditioned in exposure chambers supplied with filtered air for 3 days prior to O_3 exposure. The animals were housed individually in wire mesh cages. Rats were exposed 6 h/day and sacrificed immediately after 3 or 7 days of exposure, or 3 or 7 days after 7 days of exposure.

Ozone was generated by an OREC Model 03VI-0 Ozonizer (Ozone Research and Equipment Corp., Phoenix, AZ). Dilution air was mixed with ozone to bring the total airflow through the exposure chambers to approximately 15 chamber air exchanges per hour. The chamber ozone concentration was monitored throughout the exposure with two Dasibi 100 Ozone monitors (Dasibi Environmental Corp., Glendale, CA) and recorded.

Histology

Two hours prior to sacrifice, all animals were injected intraperitoneally with 50 µg/g body weight bromodeoxyuridine (5-bromo-2'-deoxyuridine, Sigma, St. Louis, MO) made up in buffered saline.

All animals were sacrificed using an overdose of sodium pentobarbital administered intraperitoneally. After death, the head of each rat was removed and the nasal cavity flushed through the nasopharyneal orifice with 10 mL Carnoy's fixative. The lower jaw, skin and musculature were removed, and the head was immersed in a large volume of Carnoy's fluid for at least 24 h. Following fixation, the heads were decalcified in 13% formic acid for 4 days. The nasal cavity of each rat was transversely sectioned at the level immediately posterior to the upper incisor teeth. The tissue was embedded in paraffin and sectioned at 5 µm. The endogenous peroxidase activity of the deparaffinized sections was blocked prior to immunohistochemical staining for BrdUrd-labeled cells (Vector Laboratories, Burlingame, CA). The monoclonal antibody recognizing BrdUrd was obtained from Becton Dickinson (Mountain View, CA). The sections were counter stained with Diff-Quik (American Scientific Products, McGaw Park, IL). The number of positively stained cells per mm basal lamina was determined using a Zeiss Videoplan image analysis system (Carl Zeiss Inc., New York, NY); the data were tested for equality of group means using the Tukey Studentized Range Method. The criterion for statistical significance was set at $p < 0.05$.

RESULTS

Control Animals

The epithelium of the anterior nasal cavity was composed of 4 basic types: squamous, cuboidal/transitional, respiratory and olfactory epithelium. Cells positive for BrdUrd incorporation were seen in all four types of epithelium. The number of positive cells/mm basal

lamina were similar (approximately 1-5 cells/mm) for the olfactory, cuboidal and respiratory epithelium, while the squamous epithelium contained approximately 25-40 positive cells per mm of basal lamina (Figs. 1 and 2). The majority of the positive cells within the olfactory and respiratory epithelium were basally located.

Cigarette-Exposed Animals

Cuboidal epithelia of the smoking rats were hyperplastic after both smoke exposure periods, however after 1 wk of full smoke exposure, mitotic figures were commonly seen within the epithelial lining. Significant increases in cell proliferation were seen in the cuboidal/transitional epithelium (Fig. 1A) after 1 wk of full exposure. This response was diminished after 4 wk of smoke exposure. No significant changes were observed in the respiratory epithelium (Fig. 1B), olfactory epithelium (Fig. 1C) or squamous epithelium (Fig. 1D).

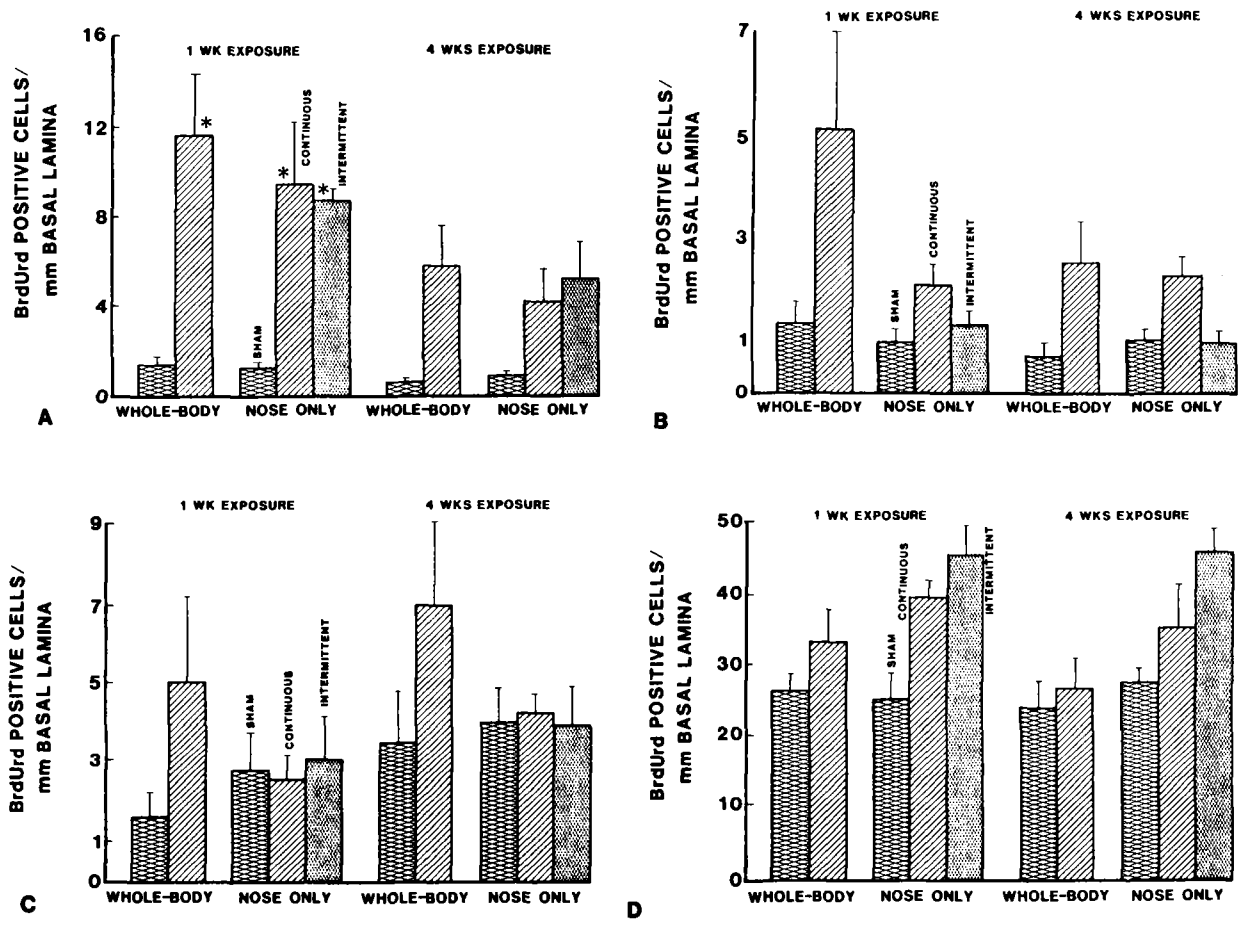

Figure 1. Histograms showing the number of BrdUrd positive cells in the four epithelial cell types; cuboidal (A), respiratory (B), olfactory (C) and squamous (D) following 1 and 4 wk exposure to cigarette smoke. * Denotes significant change in cell labeling ($p < 0.01$).

Ozone-Exposed Animals

Significant increases, compared to the controls, in BrdUrd labeling were seen in the cuboidal/transitional and respiratory epithelium after 3 days exposure to 0.8 ppm ozone. This response was elevated, but not significantly, after 7 days of exposure (Figs. 2A and 2B), and was absent post exposure. A significant depression in DNA replication was seen in the squamous epithelium 7 days after exposure to 0.8 ppm ozone (Fig. 2D). Significant changes in DNA

replication were not seen in the olfactory epithelium (Fig. 2C). Exposure to 0.8 ppm ozone was associated with the development of a hyperplastic lesion of the cuboidal cells lining the lateral wall and naso- and maxillo-turbinates after 7 days, but not after 3 days, of exposure.

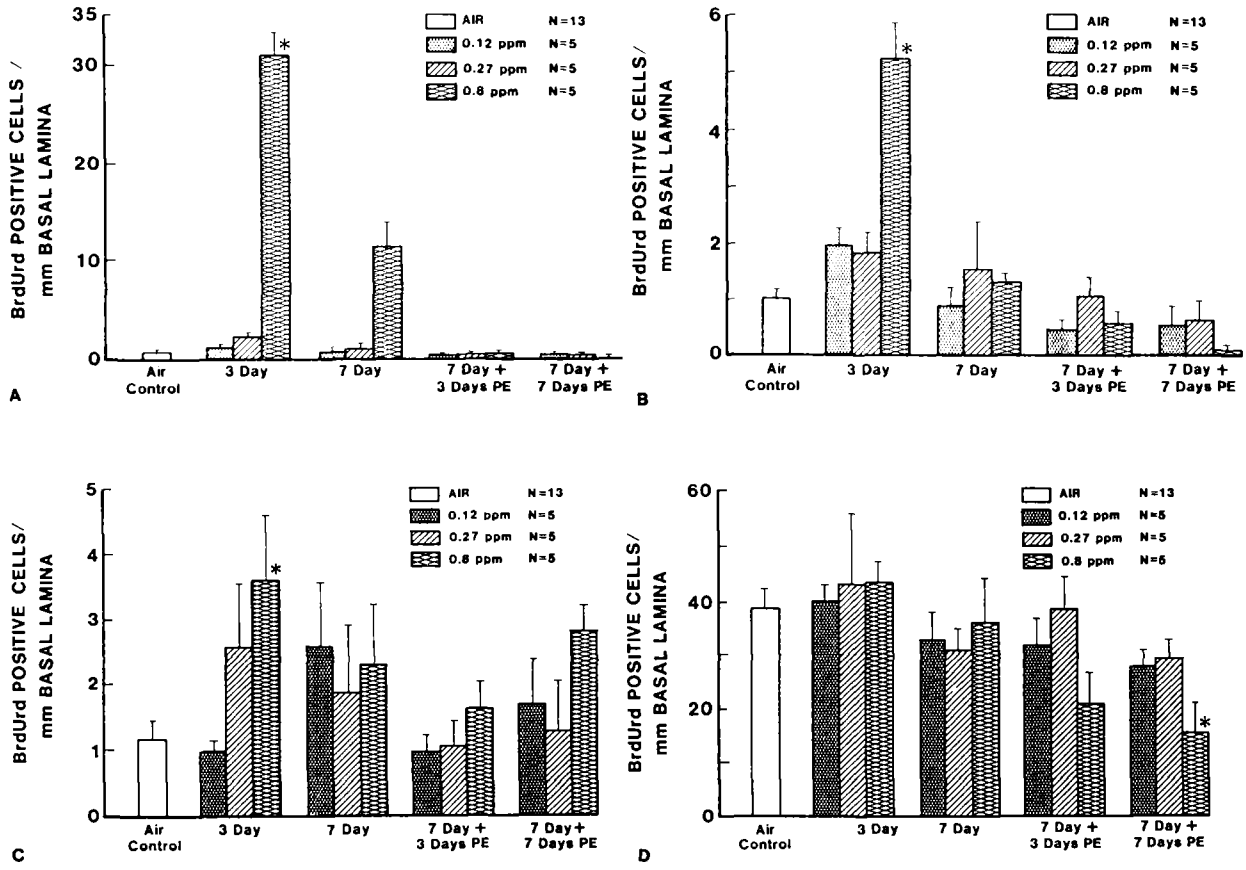

Figure 2. Histograms showing the number of BrdUrd positive cells in the four epithelial cell types; cuboidal (A), respiratory (B), olfactory (C) and squamous (D) following ozone exposure. * Denotes significant change in cell labeling ($p < 0.01$).

DISCUSSION

Histological examination of the normal rat anterior nasal epithelium revealed four distinct morphologic epithelial regions: squamous, respiratory, cuboidal/transitional and olfactory. The respiratory epithelium in rats contains ciliated, basal and secretory cells, and is similar to the lining of the trachea and upper airways. The cuboidal epithelium contains cuboidally shaped cells, showing no differentiation towards cells encountered in the respiratory epithelium. This distinction has not generally been made in previous studies,[1] where these two distinct epithelia are grouped together as respiratory epithelium. This may be an important distinction, as many inhaled toxicants such as formaldehyde[2] and 1,3-dichloropropene[3] preferentially affect the cuboidal epithelium.

Cell proliferation/DNA replication in the rat nasal epithelium under normal circumstances is low. The respiratory, cuboidal/transitional and olfactory epithelium have similar numbers of proliferating cells per mm of basal lamina. In contrast, the squamous epithelium contains markedly more proliferating cells. These results are similar to those previously reported.[2]

A variable response to ozone that was dependent on concentration, duration of exposure and epithelial cell type was encountered. The cuboidal/transitional epithelium exhibited the greatest response to ozone. This response was only evident with 0.8 ppm ozone, and was more marked after 3 days exposure than after 7 days exposure. Development of hyperplastic lesions after 7 days of exposure and 7 days post exposure was associated with the concentration of 0.8 ppm ozone. Thus, there is an immediate proliferative response to ozone exposure, which subsides once the hyperplastic lesion is developed. This response may be missed if appropriate exposure or post-exposure periods are not selected. In addition to the increase in labeling seen in cuboidal/transitional and respiratory epithelium, a decrease in labeling was noted in the squamous epithelium 7 days after 7 days of exposure to 0.8 ppm ozone.

Cigarette smoke induced significant increases in cell turnover only in the cuboidal/transitional epithelia after 1 wk full exposure; 4 wk exposure was associated with an elevated, but nonsignificant response.

CONCLUSION

These data show that the nasal epithelium is sensitive to two common environmental pollutants and that this sensitivity can be assayed by measuring DNA replication. The cuboidal epithelium lining the lateral wall and turbinates showed the maximal response, while the respiratory epithelium was responsive to ozone, but not to cigarette smoke, under the conditions of this experiment. Cell proliferation occurred rapidly after exposure and diminished once the lesion was established, and was not evident during prolonged exposure.

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