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Nasal Epithelium as a Sentinel for Airborne Environmental Pollution

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A wide range of chemicals, particulate matter, and gaseous air pollutants are present in urban atmospheres and may pose a significant health risk for human populations. Nasal passages are the first site of contact of the respiratory tract with the environment and offer significant protection to the lower respiratory tract by conditioning the inspired air. This activity, which includes removal of certain pollutants, places the nose at risk of pathological changes, including cancer. Mexico City residents are exposed to a complex mixture of air pollutants. Based on predicted nasal air flow characteristics, four nasal biopsy sites were selected for study in adult male volunteers from a control low polluted town (n = 12) and southwest metropolitan Mexico City permanent residents (n = 54). Clinical data with emphasis on nasal symptoms and histopathological changes including basal and goblet cell hyperplasia, squamous metaplasia, epithelial dysplasia, and neovascularization were evaluated. Immunohistochemical staining was used to assess accumulation of p53 protein. Control individuals had no respiratory symptoms and their biopsies were unremarkable. Mexico City residents complained of epistaxis, rhinorrea, nasal crusting, dryness, and nasal obstruction. Their biopsies showed patchy shortening of cilia, deciliated areas, basal cell hyperplasia, and squamous metaplasia. Dysplastic lesions were predominantly located on antral squamous epithelium and in squamous metaplastic epithelium of the posterior inferior turbinates and they exhibited p53 nuclear accumulation. Individuals with >10 h of daily outdoor exposure for 5 years or more had the highest rate of dysplasia. Subjects with epistaxis were more likely to have dysplasias and neovascularization. Results of this study suggest: (a) Nasal lesions in Mexico City residents are likely the result of many potentially toxic and/or carcinogenic pollutants, including ozone, aldehydes, particulate matter, and unmeasured pollutants; (b) the alteration of the nasal mucociliary defense mechanisms and the effects of reactive and/or water-soluble materials and particulates could be playing a major role in the nasal pathology; (c) the accumulation of p53 protein in dysplastic nasal lesions in the context of prolonged exposure to air pollutants raises the possibility that p53 mutations are already present and are providing the squamous cells with a selective advantage for clonal expansion; and (d) the nasal passages provide a valuable sentinel tissue for the detection of toxic air pollutants. © 1998 Society of Toxicology.

A complex mixture of chemicals and particulate matter is present in the ambient air in polluted urban and industrial areas. Exposures to these pollutant mixtures may pose a significant health risk for local human populations. The nasal passages provide protection to the lower respiratory tract from certain air pollutants by filtering the inspired air (Proctor *et al.*, 1982). This filtering activity, however, places the nasal epithelium at risk of damage and consequent disease. Experimental animals, including rodents and monkeys, have been used for nasal inhalation toxicologic studies that are directed toward the prediction of human risks to environmental toxicants (Morgan *et al.*, 1990; Morgan, 1991). Several inhaled chemical irritants, including formaldehyde, ozone, and chloroform, have been demonstrated to cause significant injury to experimental animals' nasal surface epithelium upon acute or repeated exposures. Alterations to the nasal mucosa include acute inflammation, degeneration and necrosis of surface epithelium, change of the normal epithelium to a morphologically different, adaptive epithelium, increased cell proliferation, dysplastic lesions, and carcinomas (Mery *et al.*, 1994; Harkema *et al.*, 1987, 1995; Monticello *et al.*, 1996; Morgan *et al.*, 1986; Bermudez, 1991). Epidemiological and environmental chamber studies in humans have provided valuable insights into the nasal responses to critical air pollutants. Nasal lavage studies have shown that short exposures to pollutants such as ozone elicit an acute inflammatory response in the upper respiratory tract of humans (Graham *et al.*, 1990; Koren *et al.*, 1990; Steerenberg *et al.*, 1996). The strongest evidence for the carcinogenic effect of occupational inhalants in the nasal cavity and paranasal sinuses is seen with exposure to hardwood dust, nickel, tobacco smoke, furniture making, and leather tanning (Feron *et al.*, 1991; Leopold, 1992, 1994; Boysen *et al.*, 1994; Holt, 1996; Holmstrom *et al.*, 1989, 1990). Studies of nasal histopathology and

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the use of the single cell gel electrophoresis assay for detecting DNA strand breaks in nasal cells of children and adults exposed to the heavily polluted atmosphere of southwest metropolitan Mexico City (SWMMC) indicate that the nose may provide a valuable indicator of exposure to toxic air pollutants (Calderón-Garcidueñas *et al.,* 1992, 1997).

Multiple factors can influence the distribution of lesions induced by inhaled chemicals in the nasal passages, including the physical and chemical properties of the material, the flow characteristics of nasal mucus and air, local blood flow, and regional xenobiotic metabolism (Morgan, 1994). For highly reactive and water-soluble gases, such as formaldehyde, nasal airflow appears to play the dominant role in determining the location of nasal lesions (Kimbell *et al.,* 1993; Morgan *et al.,* 1991). In the case of ozone (O_3), nasal mucus is probably more important (Pryor, 1992). For the present study, because a significant amount of information is available on nasal airflow in humans (Proetz, 1951; Keyhani *et al.,* 1995; Subramaniam *et al.,* 1998) and much less is known about other factors influencing local dosimetry of inhaled materials, sites for collection of nasal biopsies were selected on the basis of the location of the major inspiratory airflow streams. Reactive gases frequently show an anterior to posterior severity gradient for toxicity in the nasal lining, and a site of potentially high exposure in the nasal antrum and two sites (one anterior and one posterior) on the inferior turbinate were selected to address this issue. An additional site on the middle turbinate which is known to lie adjacent to the major medial and ventral air streams and beneath the major olfactory airstream was selected to provide an indication of potential olfactory mucosal damage.

The present work addresses nasal lesion location and extends the characterization of the nasal responses previously reported in Mexico City inhabitants (Calderón-Garcidueñas *et al.,* 1992), to include consideration of nasal crusting, epistaxis, macroscopically abnormal areas in the nasal mucosa, and dysplastic changes. Concerns with respect to carcinogenesis were focused on characterization of epithelial dysplasia and immunohistochemistry (IHC) analysis was used to assess accumulation of p53 protein. p53 functions as an integrator of stress response signals, via a multiplicity of pathways, inducing a range of alterations in p53 protein (Jacks *et al.,* 1996). p53 protein alterations due to missense mutations and loss of p53 by nonsense or frameshift mutations provide a selective advantage for clonal expansion of preneoplastic and neoplastic cells (Vogelstein *et al.,* 1992). Stabilization of p53 protein and posttranslational modifications facilitate transcription-dependent and independent activation of a complex set of cellular responses (i.e., growth arrest and apoptosis) that allow adaptation to the initiating stresses (i.e., genotoxic damage, hypoxia, cytokines). Failure of these adaptive responses gives p53 a key role in carcinogenesis (Hall *et al.,* 1996).

MATERIALS AND METHODS

Study area. Mexico City extends over 2000 km^2 and is located in an elevated valley, 2250 m above sea level; the city has a mild tropical climate all year long (Jauregui, 1992). Sunshine, light winds, temperature inversions, poor ventilation, and mountains forming a closed basin, twenty million residents, four million cars, urban leakage of liquefied petroleum gas, and intense industrial activity contribute to make Mexico City an ideal environment in which complex photochemical reactions produce oxidant chemicals and other toxic compounds. Ozone concentrations in the city have been exceeding the U.S. Environmental Protection Agency National Ambient Air Quality Standard NAAQS for O_3 0.08 ppm as 1 h maximal concentration, not to be exceeded more than three times per year, on 71% of days in 1986 and 98% in 1992, with values as high as 0.48 ppm (Blake *et al.,* 1995). The marked increase in the number of daily ozone exceedences in Mexico City initially started in the fall of 1986, when the atmospheric air went from highly reductive to oxidative, coinciding with the introduction of a new gasoline with a tetraethyl lead concentration of 0.64 ml/gal (Garcia-Gutierrez *et al.,* 1991). In 1989, a new change in the reactivity of the emitted volatile organic compounds (VOC's) occurred following the introduction of methyl-*t*-butyl ether (MTBE) to Mexico City gasolines (Bravo *et al.,* 1991). Ozone is produced when sunlight triggers chemical reactions involving reactive hydrocarbons and nitrogen oxides. As a result of wind transport of the mass pollutants emitted in the industrial north and central parts of Mexico City, the maximal concentrations of O_3 precursors appear downwind of the emission zones, toward the southern part of the urban area, southwest and southeast Mexico City (Garcia-Gutierrez *et al.,* 1991; Bravo *et al.,* 1988, 1991). The southern part of metropolitan Mexico City is exposed to significant levels of photochemical smog (Garcia-Gutierrez *et al.,* 1991; Bravo *et al.,* 1988), formaldehyde and acetaldehyde (Bravo *et al.,* 1991, Baez *et al.,* 1995), airborne particulates (Bravo *et al.,* 1989; Villalobos-Pietrini *et al.,* 1995), polycyclic aromatic hydrocarbons (Bravo *et al.,* 1970), and alkane hydrocarbons (propane, isobutane, and *n*-butane) (Blake *et al.,* 1995).

Pollutant monitoring methodology. Atmospheric pollutants and meteorological conditions were monitored at the Pedregal air-monitoring station, located in SWMMC downwind of the major diurnal emissions in metropolitan Mexico City and 5 km or less from the SWMMC volunteers' residence and work places. Ozone was monitored using a Beckman 950 chemiluminescence analyzer with a calibration routine in accordance with US EPA procedures. Data on particulate matter <10 μm (PM10), SO_2, NO_2, NO, temperature, relative humidity, wind speed, and rain events were reviewed. For O_3 exposures we examined three measures: the maximal concentrations, the number of hours equal to or above the National Ambient Air Quality Standards (US-NAAQS), and the time of occurrence of pollutant peaks. Pollutant data available for SWMMC were reviewed for the past 10 years (1986–1996), including the sampling months. The data from Isla Mujeres—the control site—were obtained from the Capitanía del Puerto, where similar analytical procedures were used.

Study population. This project, which included a total of 66 male volunteers, was approved by the Instituto Nacional de Pediatria Review Board for Human Studies and informed written consent was obtained from all subjects. The volunteers were studied in November 1995, and comprised two groups: 12 control subjects exposed to minimal environmental pollution as permanent residents of Isla Mujeres, and 54 permanent residents in SWMMC. All participants in the study were healthy nonsmokers, with a past negative history of smoking or passive exposure to tobacco smoke. All volunteers were subjected to the collection of a detailed clinical history. The outdoor daylight exposure history relates to the last 5 years prior to the study. The control group (Group 1) included 12 subjects, age 23.9 ± 4.5 years (range 19–34 years), with 11.2 ± 1.4 h/day outdoor daylight exposure. These subjects were permanent residents in the small island, had never been to a large city (including Mexico City), and had no known exposures to local sources of air pollutants or toxic substances. Exposed SWMMC permanent residents were divided in two groups according to their place of enrollment: Group 2A (n = 12) enrolled at the Instituto Nacional de Pediatria, age 30.6 ± 3.9 years (range 24–35 years), with a

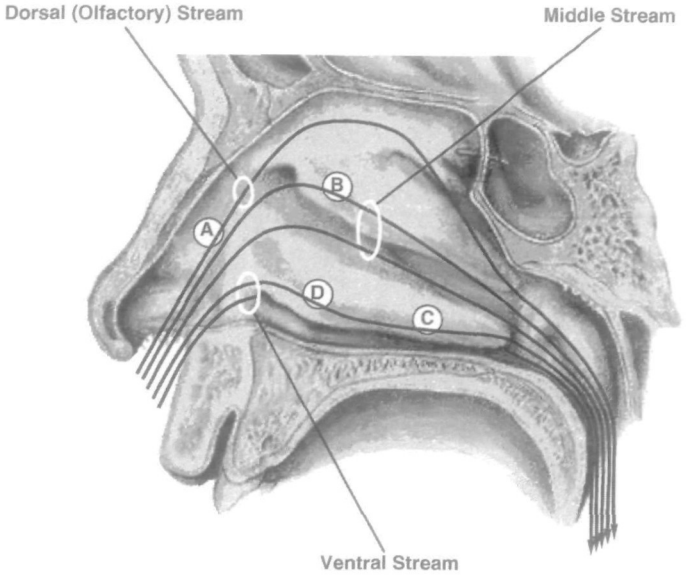


FIG. 1. Location of sites of biopsies in a diagrammatic midsagittal section of the nose, with the nasal septum removed to display the turbinates. A, antrum; B, anterior middle turbinate; C, posterior inferior turbinate; D, anterior inferior turbinate.

daylight outdoor exposure of 4.3 ± 0.6 h and a SWMMC residency time of 26.8 ± 7.6 years. These volunteers had sedentary work and no outdoor physical activity. Group 2B (n = 42) enrolled at a security corporation, age 25.8 ± 4.2 years (range 19–35 years), with a daylight outdoor exposure of 11.6 ± 1.2 h and a SWMMC residency of 23.2 ± 7.4 years. Control Group 1 and exposed Group 2B subjects had similar levels of physical activity, including routine outdoor exercise; they stayed outdoors from 0600 to 1800 h, with only brief intermittent periods indoors (<1 h during the day). Six days per week, from 0800 to 1400 h, the subjects exercised moderately outdoors and had intermittent light exercise the remaining time outdoors.

Clinical history. Clinical data were obtained for each subject with respect to age, location and duration in domicile, daily outdoor time, level and time of the day of physical activity, occupational history, respiratory and ear–nose–throat (ENT) symptoms, including epistaxis, rhinorrea, quantity and quality of nasal mucus, nasal dryness, nasal crusting, nasal obstruction, cough, thoracic pain, difficulty breathing, and recent (previous 3 months) acute respiratory illness.

Nasal biopsies. Samples of nasal epithelium were obtained by the ENT physician using a disposable plastic curette (Rhino-Probe, ASI, Arlington, TX), under direct visual inspection. Four biopsies were obtained from each of 51 subjects, including the 12 controls and 39 exposed SWMMC volunteers from the left nasal passageway (Fig. 1) as follows: (A) dorsal nasal antrum, (B) ventral medial margin of the anterior middle turbinate, (C) medial margin of the posterior region of the inferior turbinate, and (D) medial margin of the anterior region of the inferior turbinate. Biopsy A sampled squamous epithelium from the antrum, while biopsies B, C, and D targeted areas normally covered with ciliated respiratory epithelium and goblet cells. In addition, one biopsy from site C was taken in 15 SWMMC exposed group 2B individuals; this biopsy was targeted to sample gray–whitish abnormal patches over the posterior inferior turbinate and therefore the biopsies were taken from either nasal passage. Biopsies from the 66 volunteers were immediately fixed in 10% neutral buffered Formalin, embedded in paraffin, sectioned at 5 μm, and stained with (a) hematoxylin and eosin (HE) and (b) Alcian blue at pH 2.5 and periodic acid–Schiff (AB-PAS). Sections were examined and photographed using bright-field light microscopy. We evaluated the following histopathological parameters: basal cell hyperplasia, squamous metaplasia, goblet cell hyperplasia, dysplasia, neovascularization, and epithelial polymorphonuclear cell (PMN) infiltration. The nature of nasal lesions was recorded for each of the 4 regions sampled.

Immunohistochemistry. Control and exposed biopsies were stained using previously published procedures for p53 tumor suppression gene products (Wolf *et al.,* 1995; Shi *et al.,* 1995) with the streptoavidin-biotinylated–immunoperoxidase method. Briefly, 5-μm paraffin sections were mounted on silanized glass slides, dried overnight, deparaffinized in xylene, and rehydrated through graded alcohols. Endogenous peroxidase activity was blocked by immersing the sections for 5 min in absolute methanol with 3% hydrogen peroxide; nonspecific binding was inactivated for 10 min with 5% skim milk. The sections were next incubated overnight at 4°C, with p53 (Ab-2) pantropic monoclonal antibody Pab 1801 (Oncogene Science, Inc., Uniondale, NY) and p53 monoclonal antibody CM_1 (Novacastra Laboratories, Newcastle University, UK), both at a dilution of 1:750. Human lung squamous cell carcinomas with a known strong reaction to the antibodies served as a positive controls and consecutive sections of each nasal biopsy without the primary antibody were included as a negative controls. An intense brown color within the nucleus was considered positive for accumulation of p53 protein. All sections were jointly scrutinized by two pathologists (L. Calderon-Garciduenas and K. Morgan).

Statistics. Power calculations indicated that a sample size of 10 subjects in each of the three study groups (controls and exposed groups 2A and 2B) was sufficient to demonstrate an effect with an α of 0.05 and a power of 0.8. Results are expressed as means ± SD. Fisher's exact test was used to compare the incidence of epistaxis, nasal crusting, nasal obstruction, dryness, and rhinorrea between exposed groups 2A and 2B. Kendall's τ b was used to assess the strength of association among neovascularization, epistaxis, accumulation of p53 protein, and dysplasia.

RESULTS

Air Quality Data

SWMCC residents have been exposed daily to high O_3 concentrations, the main criterion pollutant for the area. SWMMC maximal O_3 concentrations, average number of hours per day above the NAAQS, and hours above the NAAQS per month for the years 1995–1996 are presented in Table 1. In the past 10 years, O_3 concentrations above the US EPA NAAQS for this gas have been recorded in SWMMC every day, all year long, an average of 3 ± 1.0 h/day (Garcia-Gutierrez *et al.,* 1991). The number of hours SWMMC residents have been exposed to O_3 above the NAAQS since 1985 are as follows: 40, 30, 740, 959, 1224, 1403, 1561, 1395, 1146, 1061, 1249, and 1080 h/year for the years 1985 through 1996, respectively. Ozone maximal peaks are usually recorded between 1300 and 1500 h (Fig. 2).

NO_2 concentrations in SWMMC do not exceed the annual arithmetic mean of 0.053 ppm, while SO_2 levels exceed the 24 h primary standard of 0.14 ppm in the winter months (Bravo *et al.,* 1990). PM10 is usually below 150 $\mu g/m^3$ in 24 h, except when the meteorological conditions are unfavorable (i.e., weak winds, dry weather) (Fig. 3). Formaldehyde and acetaldehyde levels in SWMMC are reported to be in the range of 5.9 to 110 and 2 to 66.7 ppbv, respectively; maximal peaks are recorded between 0800 and 1000 h for acetaldehyde and 1000 to 1200 h for formaldehyde (Bravo *et al.,* 1991; Baez *et al.,* 1995) (Fig. 3). Formaldehyde concentrations are higher on sunny days, to

TABLE 1

Number of Hours with Ozone Concentrations >0.12 ppm per Month, Average Number of Hours per Day with O_3 > 0.12 ppm and Maximal O_3 Peaks for the Years 1995–1996 Monitored in Southwest Metropolitan Mexico City

Month	h/month	h/day (average)	Max O_3 peak (ppm)
1995			
January	125	4.03	0.238
February	123	4.34	0.240
March	134	4.32	0.266
April	104	3.46	0.272
May	175	5.64	0.250
June	106	3.53	0.320
July	72	2.32	0.349
August	83	2.67	0.274
September	77	2.56	0.286
October	63	2.1	0.260
November	106	3.53[a]	0.251
December	81	2.7	0.299
	Total: 1249		
1996			
January	131	4.22	0.281
February	106	3.65	0.288
March	72	2.32	0.225
April	90	3	0.226
May	67	2.16	0.232
June	67	2.23	0.261
July	91	2.93	0.243
August	76	2.48	0.245
September	102	3.51	0.279
October	99	2.60	0.323
November	84	2.80	0.253
December	95	3.16	0.248
	Total: 1080		

[a] Sampling month.

coincide with atmospheric stability and heavy smog conditions. These SWMMC outdoor formaldehyde and acetaldehyde values are considered among the highest reported in urban air around the world (Baez *et al.*, 1995). Benzo(*a*)pyrene and benzo(*k*)fluoranthene concentrations measured in Mexico City in 1968 (Bravo *et al.*, 1970) were in the range of 9.7 to 89.5 and 5.6 to 34.5 $\mu g/m^3$, respectively. Recent SWMMC monitoring data of these polycyclic aromatic hydrocarbons are not available in the literature. The control environment on Isla Mujeres was sampled in November 1995 with atmospheric and meteorological conditions average for the season: 28°C, relative humidity 90%, wind speed 13–15 km/h, $O_3 < 0.010$ ppm, and PM10 < 7 $\mu g/m^3$.

Clinical Data

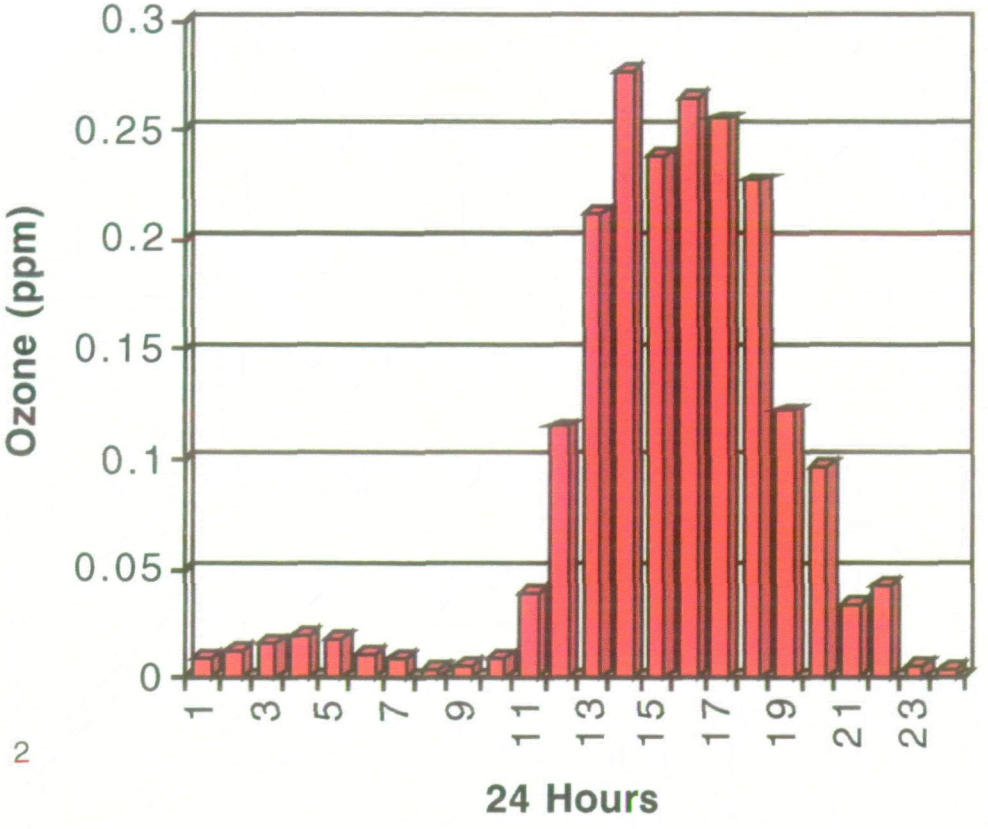
Volunteers in the control group reported no nasal or respiratory symptoms. Clinical data from subjects in groups 2A and 2B exposed to SWMMC are summarized in Table 2. Exposed volunteers in group 2A ($n = 12$) complained of intermittent increased mucus secretion (11/12), nasal crusting (3/12), nasal dryness (7/12), nasal obstruction (5/12), rhinorrea, epistaxis, and cough (4/12), and chest pain (3/12). Cough and chest pain were usually associated with outdoor exposure. Subjects in group 2B ($n = 42$) had more clinical symptomatology: intermittent increased nasal mucus (40/42), epistaxis (33/42), nasal crusting (30/42), nasal obstruction (26/42), rhinorrea (24/42), and chest pain and cough (18/42). Epistaxis was the most striking clinical finding in this group and it ranged from a few droplets of blood detected while the subjects cleaned their nose to moderate epistaxis that required noninvasive medical treatment (iced packs, nasal vasoconstrictors). Subjects with episodes of moderate epistaxis generally bled in association with physical activity outdoors in the early afternoon. There was a significant difference in epistaxis ($p = 0.008$), and nasal crusting ($p = 0.0007$) between subjects in groups 2A and 2B (Table 2).

Nasal endoscopy revealed patches of macroscopically abnormal nasal mucosa characterized by irregular areas of thinner, depressed, opaque, whitish–gray, sharply demarcated regions on the middle and inferior turbinates. In the less-exposed group 2A 4/12 (33%) subjects exhibited small abnormal mucosal areas, while for group 2B in 30/42 (71%) the abnormal areas were more extensive. The antral squamous epithelium in exposed subjects extended backward significantly, with group 2B subjects having the most posterior projection of the squamous epithelium into the area normally lined by transitional epithelium.

Histopathology

Control biopsies. Antral biopsies (A) showed nonkeratinizing squamous epithelium with a well-defined basal layer and normal cell orientation and maturation. The majority of antral biopsies also contained regions of transitional respiratory epithelium, characterized by microvillous cells or ciliated cells with short cilia and scattered goblet cells. Biopsies from the middle (B) and inferior turbinates (C, D) exhibited an unremarkable tall pseudostratified ciliated epithelium with numerous goblet cells. The majority of the control biopsies in the four anatomical sites showed small numbers of scattered intraepithelial neutrophils. No submucosal neovascularization was noted.

Exposed biopsies. Antral biopsies exhibited basal cell hyperplasia in 62% of the examined samples (Table 3). Some of these samples displayed a significant elongation of the rete ridges, which appeared club-shaped and showed small, budlike extensions. Slight intercellular edema was also noticed in a few cases. The majority of dysplasias in the subjects with four biopsies were antral in location (12/39) (Table 3). Loss of normal polarity, atypia, and mild to moderate pleomorphism of squamous cells was observed. Neoformation of small capillar-



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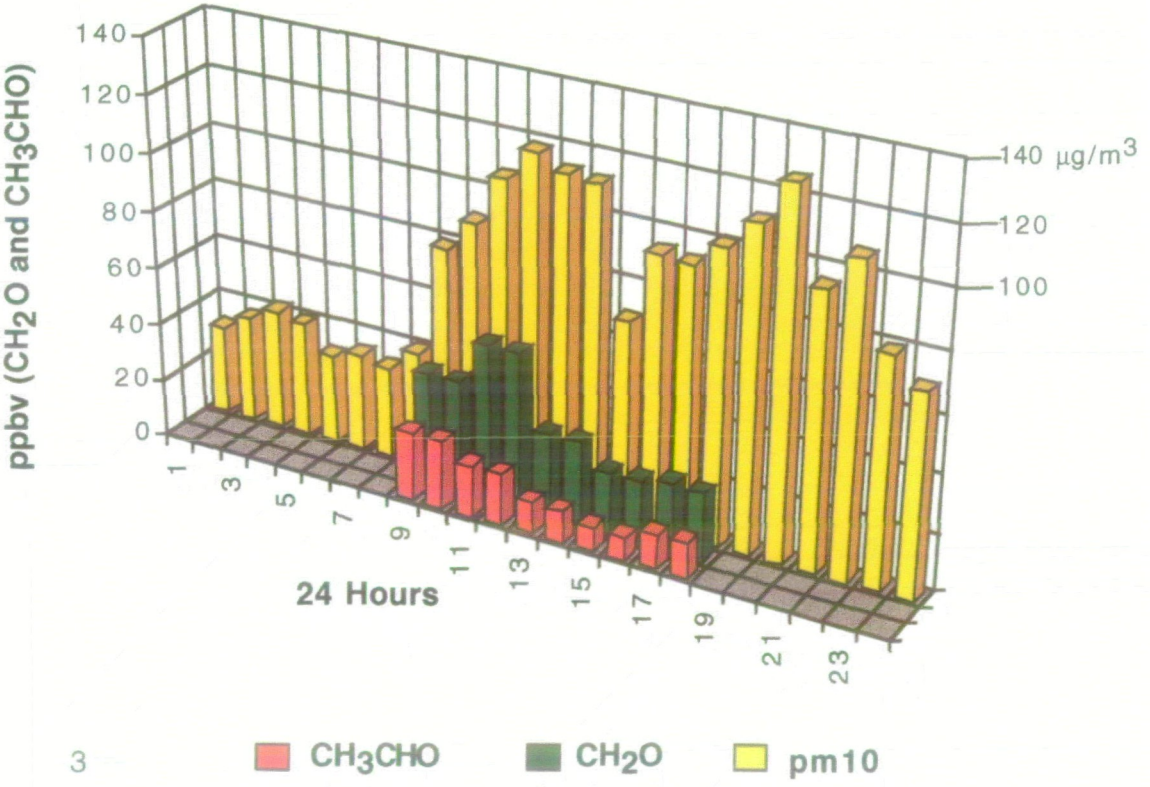

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FIG. 2. Typical 24-h ozone concentrations (ppm) in southwest Mexico City (October 21,1995).

FIG. 3. PM10 ($\mu g/m^3$), formaldehyde parts per million per volume (ppbv), and acetaldehyde (ppbv) average values in a typical average day in Southwest Metropolitan Mexico City (October 21, 1995).

TABLE 2
Clinical Respiratory Symptomatology in Control and Exposed SWMMC Subjects (Groups 1 and 2)

	Control group 1	Exposed group 2A	Exposed group 2B
No. of subjects	12	12	42
Age ± SD	24 ± 4.52	30 ± 3.91	26 ± 4.23
No. h outdoors	>10 h	<6 h	>10 h
Nasal dryness	0%	58%	69%
Nasal crusting	0%	25%	71%*
Nasal obstruction	0%	42%	62%
Rhinorrea	0%	33%	57%
Nasal mucus	0%	92%	95%
Epistaxis	0%	33%	79%**
Chest pain	0%	25%	43%
Cough	0%	33%	43%

* $p = 0.0007$.
** $p = 0.008$.

ies impinging upon the basement membrane were commonly seen in this anatomical location (Figs. 4A and 4B).

Biopsies from the turbinates were all abnormal with patchy shortening of cilia and deciliated areas, basal cell hyperplasia, and patchy goblet cell hyperplasia. Goblet cell hyperplasia was recorded in 9/12 subjects (75%) in exposed group 2A, but only in 9/42 (21%) in group 2B (Table 4). A clear increase in alcianophilic mucus was noted in relation to goblet cell hyperplasia. Basal cell hyperplasia, squamous metaplasia, and neovascularization were prominent in the highly exposed group 2B (Table 4). The inferior turbinate had the most extensive areas of squamous metaplasia and neovascularization and it was second to the antrum with respect to the frequency of dysplasias (Table 3). Dysplastic lesions arose in squamous metaplastic epithelium and were scored mild to moderate (Fig. 4D). Only two subjects (16%) from group 2A had dysplastic lesions, while 20/42 in group B had them (48%) (Table 4). There were no severe dysplasias or squamous cell carcinoma *in situ*. Mild to moderate polymorphonuclear (PMNs) intraepithelial infiltration characterized all exposed biopsies, regardless of anatomical site. There was an important association between the presence of submucosal neovascularization and dysplastic lesions (Kendall $\tau\, b = 0.4940$, $p < 0.0001$), epistaxis and neovascularization (Kendall $\tau\, b = 0.3528$, $p = 0.0064$), and epistaxis with dysplasia ($p = 0.0014$).

Immunohystochemistry

Nuclear accumulation of p53 protein was associated with dysplastic areas in all anatomical locations in the exposed individuals. $p53^+$ cells were generally distributed in a diffuse pattern throughout the layers of the epithelium (Fig. 4E); in a few cases, $p53^+$ cells were located above the basal layer and surrounded by negative p53 cells (Fig. 4C). Of the two subjects in group 2A with dysplastic lesions, only one had positive nuclear staining, while in group 2B of the 28 dysplastic lesions seen in 20 subjects, 25 had $p53^+$ accumulation (89%) (Table 4). Eighty-three percent of antral dysplastic lesions, 89% of the dysplastic lesions from area C in the subjects with 4 nasal biopsies, and 86% of posterior inferior turbinate biopsies in the subjects with only one biopsy had $p53^+$ accumulation (Table 3). The majority of the $p53^+$ biopsies were immunoreactive to the p53 gene product Pab 1801 (92%). There was a strong association between dysplasia and p53 accumulation (Kendall $\tau\, b = 0.4188$, $p = 0.0015$).

DISCUSSION

This study documents the presence of nasal lesions in permanent adult male residents in southwest metropolitan Mexico

TABLE 3
Major Clinical and Histopathological Findings in the Set of Four Nasal Biopsies Taken from Each Exposed Subject in Group 2A ($n = 12$) and Group 2B ($n = 27$), According with Anatomical Site

Bx area	#Bxs	Dysplasia	+p53 dysplasia	Neovascularization	Goblet cell hyperplasia	Squamous metaplasia	Basal cell hyperplasia
A, antral	39	12/39	10/12	17/39	1/39	NA[a]	24/39
		31%	83%	44%	3%		62%
B, ant middle	39	1/39	0	1/39	1/39	6/39	11/39
turbinate		3%	0%	3%	3%	15%	28%
C, Post inf	39	9/39	8/9	11/39	9/39	17/39	18/39
turbinate		23%	89%	28%	23%	44%	46%
D, Ant inf	39	3/39	2/3	3/39	7/39	11/39	13/39
turbinate		8%	67%	8%	18%	28%	33%
C, Post inf	15	7/15	6/7	9/15	0	14/15	7/14
turbinate[b]		47%	86%	60%		93%	50%

Note. Data include all nasal biopsy anatomical sites.
[a] NA, squamous metaplasia does not apply to the antral biopsies, since they normally have squamous epithelium.
[b] Histopathology findings correspond to the single biopsy taken from the posterior inferior turbinate in 15 exposed individuals included in Group 2B. Macroscopically abnormal grey-whitish patches in the mucosa of the posterior inferior turbinate were targeted by this biopsy.

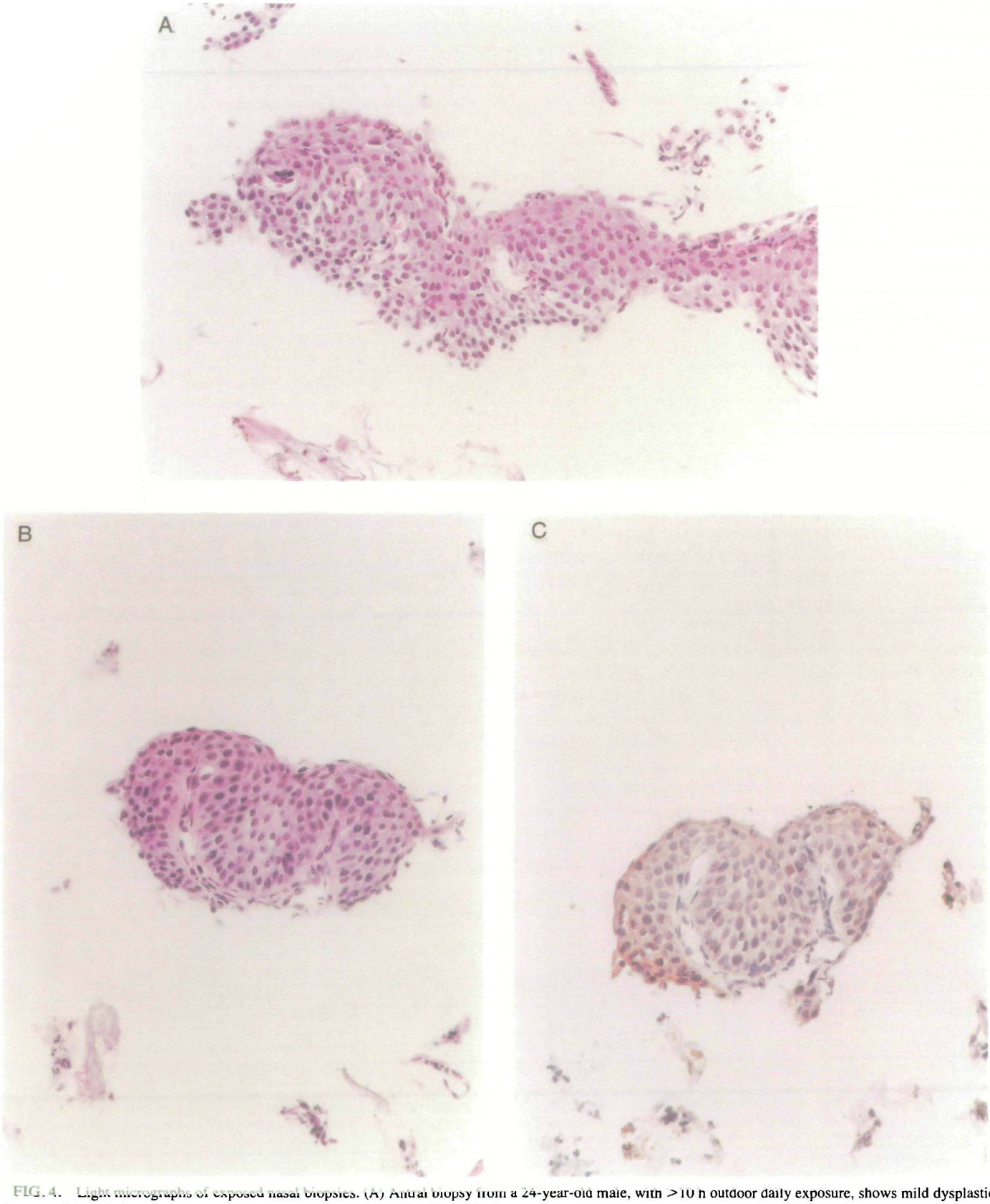

FIG. 4. Light micrographs of exposed nasal biopsies. (A) Antral biopsy from a 24-year-old male, with >10 h outdoor daily exposure, shows mild dysplastic changes with variation in nuclear size, neutrophilic epithelial infiltration and mild vascular proliferation (HE × 90). (B) Antral biopsy from a 19-year-old male, exposed group 2B, shows a hyperplastic squamous epithelium, with mild dysplastic changes (HE × 120). (C) Same biopsy as B, immunostained for p53 (Pab 1801), with intense nuclear staining in scattered cells in basal and suprabasal positions. (D) Posterior ventral turbinate biopsy from a 26-year-old male in group 2B shows a moderate dysplasia arising in a squamous metaplastic epithelium (HE × 120). (E) Same biopsy as D immunostained for p53 (Pab 1801) exhibits intensely stained nuclei throughout the full epithelial thickness (× 120).

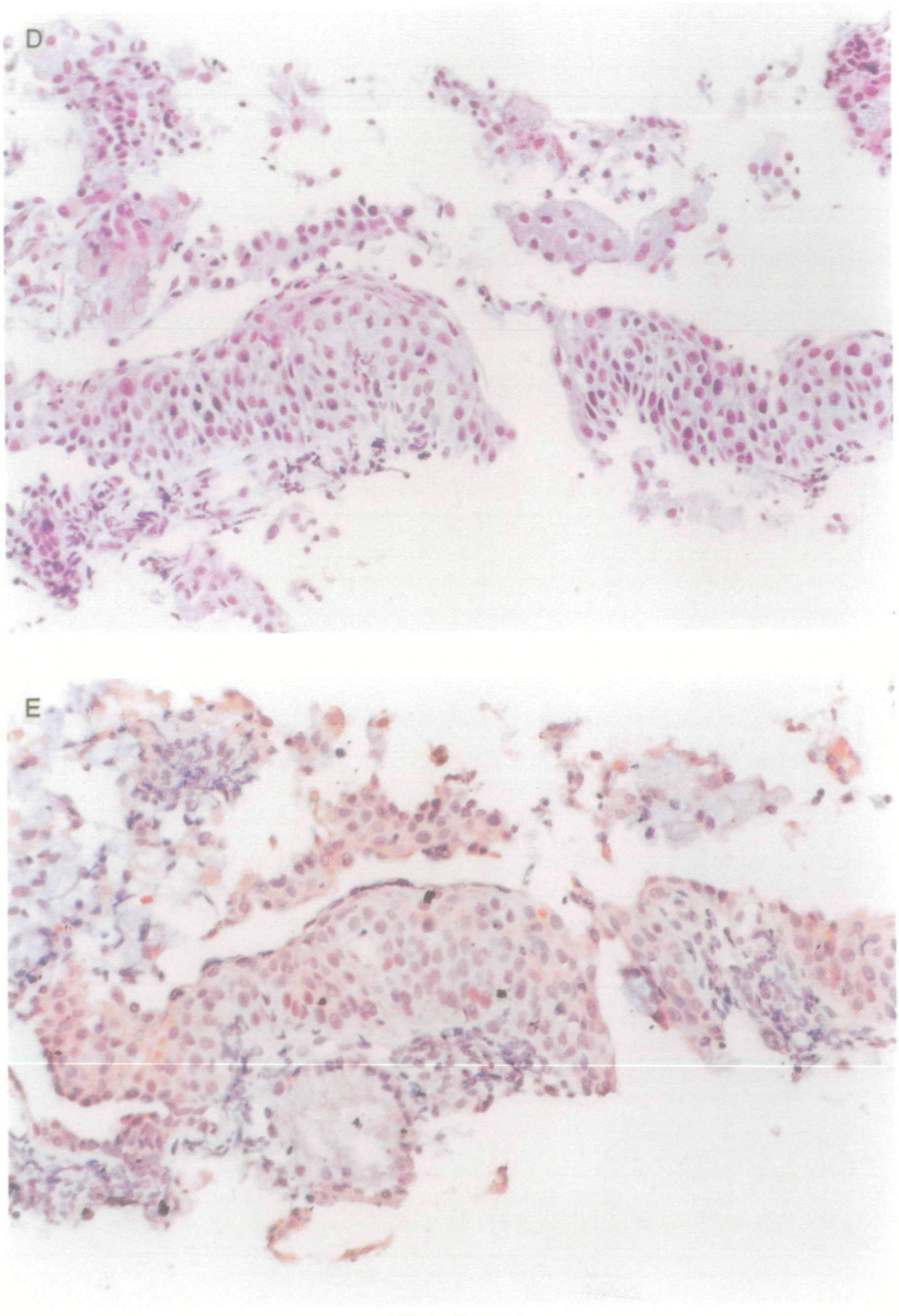

FIG. 4—*Continued*

City exposed sequentially to pollutants such as ozone, PM10 formaldehyde, and acetaldehyde, on daily basis, for more than 10 years. The profile of the SWMMC resident at risk for nasal dysplastic lesions includes an outdoor daily exposure of 10 h or more, clinical episodes of epistaxis, an abnormal nasal ENT exam with nasal crusting and extensive patches of whitish–gray mucosa on the inferior and middle turbinates, and a corresponding histopathological picture that includes squamous metaplasia replacing the normal ciliated respiratory epithelium. This metaplastic epithelium shows atypical and dysplastic areas significantly associated with p53 nuclear accumulation and neovascularization. The presence of macroscopically abnormal nasal mucosa patches and its association with atypical or dysplastic epithelium and vascular proliferation were previously noted in a SWMMC children's population (Calderon-Garciduenas *et al.,* 1997). Up to 35% of elementary school children ages 9–12, with outdoor exposures of >7 h/day, exhibited areas of nasal squamous metaplasia and dysplasia with statistically significantly higher nasal DNA damage—as evaluated by the single cell gel electrophoresis assay (SCGE)—when compared to the contralateral macroscopically normal site in the same child or to nasal biopsies of similarly exposed SWMMC children with identifiable respiratory epithelium or goblet cell hyperplasia. DNA strand breaks detected by the SCGE assay are quickly induced in nasal respiratory epithelium upon arrival of healthy, well-nourished young males to Mexico City, and lifelong resident adults and children have significant numbers of severely DNA-damaged nasal cells (Calderon-Garciduenas *et al.,* 1996). A children's population similar to the one examined for the SCGE assay (Calderon-Garciduenas *et al.,* 1997) showed a threefold increase in nasal cells of 8-hydroxydeoxyguanosine—detected by an immunoperoxidase method—when compared with matched children living in a low polluted area (Drs. R. Santella, Columbia University, and L. Calderón-Garcidueñas, personal communication). Oxidation of the C8 of guanine is a major mutagenic lesion, producing predominately G–T transversion mutations (Cheng *et al.,* 1992).

Clearly, the nasal epithelium in SWMMC residents is being subjected to several air pollutants with potentially different mechanisms of DNA damage. Ozone, the main pollutant in the area, is a highly reactive molecule that interacts with a wide variety of organic molecules, including unsaturated fatty acids, proteins, and nucleic acids to produce free radical intermediates. It initiates cascades of free radical reactions and causes extensive DNA damage as reflected by strand breaks, DNA interstrand crosslinks, and DNA protein crosslinks (Feron *et al.,* 1991; Victorin, 1992; Bascom *et al.,* 1996; Lee *et al.,* 1997). Formaldehyde—a major air pollutant in Mexico City—is a potent carcinogen in F344 rats (Wolf *et al.,* 1995). Rhinitis, squamous metaplasia, and hyperplasia of the nasal respiratory epithelium together with increases in cell proliferation precede the appearance of squamous cell carcinomas in the rat nasal passages (Morgan *et al.,* 1986). Interestingly, the tumor location correlates well with the anatomical regions where acute toxicity is demonstrated, and with areas of inhibition of nasal mucociliary function (Bermudez, 1991). Some particulate matter in ambient air is known to contain substances that exhibit carcinogenic activity in experimental animals (Hoffman *et al.,* 1977) and there is an association between particulate air pollution (PM10) and morbidity and mortality due to cardiopulmonary disease and lung cancer (Dockery *et al.,* 1993; Pope *et al.,* 1995). PM10 collected in Mexico City has shown a considerable mutagenic response in the *Salmonella*/microsome test (Villalobos-Pietrini *et al.,* 1995). Moreover, studies of clearance of particles in the human nose (Fry, *et al.,* 1973) have shown that the site of maximal deposition is at the junction between the ciliated and nonciliated epithelia. At least 45% of the retained particulate material (2–10 μm) is deposited in the anterior region of the nasal passages, the site of maximum deposition being 2–3 cm behind the tip of the nose, the area with the highest number of dysplastic lesions in the present study. Particles deposited on the unciliated region are retained for considerable periods of time. Particulate matter might play a role in the nasal lesions observed in the present study, since most people living in Mexico City have altered mucociliary clearance mechanisms, as evidenced clinically by the history of nasal crusting and dry rhinitis (Calderon-Garciduenas *et al.,* 1994, 1996, 1997) and pathologically by the areas of respiratory epithelial damage. Impairment of mucociliary clearance has the potential to increase the contact time between deposited particulate matter and the epithelial surface.

Tumor suppressor genes are vulnerable to critical DNA damage because they function as physiological barriers against clonal expansion or genomic mutability and are able to impede growth and metastasis of cells driven to uncontrolled proliferation by oncogenes (Harris, 1996). Nelson and Kastan (1994) suggested that agents which rapidly induce DNA strand breaks also trigger p53 protein elevation. Induction of increased normal p53 protein occurs physiologically as a response to genotoxins, hypoxic stress, oxidative damage from free radicals, and endogenous sources of DNA damage such as nitric oxide and accumulation of dATP caused by adenosine deaminase deficiency (Greenblatt *et al.,* 1994; Hansen, *et al.,* 1997; Levine, 1997). The biological activity of p53 indicates that the protein is involved in gene transcription, DNA synthesis and repair, programmed cell death, and genomic plasticity (Harris, 1996).

An important finding in the present study was the accumulation of p53 protein in antral and turbinate dysplastic lesions. Bennett et al. (1993) have demonstrated, in early human bronchial neoplasia, that p53 protein accumulation starts in mild dysplasias and—of potential relevance to our findings—p53 accumulates just before invasion. p53 accumulation is seen in preneoplastic bronchial lesions and is not present in reactive or metaplastic epithelium (Rusch *et al.,* 1995). Composite immu-

TABLE 4
Major Histopathological Features in Nasal Biopsies from Control (n = 12) and Exposed Volunteers (n = 54)

Study groups	Basal cell hyperplasia	Goblet cell hyperplasia	Squamous metaplasia	Neovascularization	Dysplasia (%p53+)
Control	0/12	0/12	0/12	0/12	0/12
n = 12	0%	0%	0%	0%	0%
					(0%)
Exp 2A	11/12	9/12	6/12	5/12	2/12
n = 12	92%	75%	50%	42%	16%
					(50%)
Exp 2B	42/42	9/42	42/42	36/42	20/42
n = 42	100%	21%	100%	86%	48%
					(89%)

Note. Data are expressed as the percentage of subjects having the selected histopathological features.

nohistochemistry (IHC) data show progressive p53 protein accumulation: from 0% in normal bronchial mucosa, 7% in squamous metaplasias, 25% in mild dysplasias, 32% in moderate dysplasias, 69% in severe dysplasias, 70% in microinvasive carcinomas, and 76% in fully invasive carcinomas (Greenblatt *et al.,* 1994). According to Greenblatt *et al.* (1994) these results support a multistage model for squamous lung carcinomas where p53 is an important early target for mutations. The timing of p53 alterations in the oral cavity, oropharynx, larynx, and esophagus is very similar; p53 accumulation is reported at or before the stage of severe dysplasia (Greenblatt *et al.,* 1994). Missense mutations often increase the half-life and the amount of the p53 protein, allowing its detection by IHC; positive staining is considered a surrogate marker for gene mutations (Harris, 1996). The accumulation of p53 in dysplastic nasal lesions in the context of prolonged exposure to air pollutants and evidence of DNA damage raises the possibility that p53 mutations are already present and are providing the squamous cells with a selective advantage for clonal expansion. In this scenario, some of the dysplastic lesions in the heavily exposed individuals—including children (Calderon-Garciduenas *et al.,* 1997)—might progress in a few years to severe dysplasias and eventually to squamous cell carcinomas. Neovascularization was a striking finding associated with dysplastic lesions; recent studies have demonstrated that p53 mutations facilitate angiogenesis, by down-regulating the expression of angiogenesis inhibitors (i.e., thrombospondin-1) (Dameron *et al.,* 1994) and/or by inducing protein kinase C stimulation of vascular growth factors (Kieser *et al.,* 1994).

Investigations concerning environmental exposures face a myriad of challenges. In the respiratory tract, factors such as regional uptake patterns, cellular susceptibility to a given dose of a chemical, local clearance processes by the mucosa and the vasculature, airflow patterns and airflow diffusion, transformation, bioaccumulation, and bioavailability of the xenobiotics in the environment will affect the exposure an individual receives. Also crucial are the population heterogeneity and the interactions between biological variables such as inherited metabolic characteristics and synergistic effects of multiple environmental exposures (Semenza *et al.,* 1997; Morgan *et al.,* 1990). Semenza and Weasel (1997) remark that the first interaction between xenobiotics and organisms occurs on a molecular level prior to any clinical manifestation. Thus, if a target molecule of a xenobiotic is known, changes in that molecule can be used as a biomarker. p53 is a potential marker for early diagnosis of several human cancers that develop through multiple, morphologically defined stages, such as the dysplasia progression in the oral, esophageal, and bronchial mucosae. p53 can also be a biomarker of effect . The best-documented example of the value of p53 mutational spectrum analysis in identifying carcinogen-specific mutations is seen in skin carcinomas, in which the role of UV light is unquestioned (Greenblatt *et al.,* 1994). p53 has also been used as a susceptibility marker and as an intermediate biomarker for early chemoprevention studies (Greenblatt *et al.,* 1994; Semenza, *et al.,* 1997). One of the challenges in environmental exposures is to clarify their contribution to cancer causation and to identify high-risk groups and individuals for the purpose of prevention. Molecular epidemiology incorporates advances in the molecular biology and molecular genetics of cancer with epidemiology to understand the association between exposures, risk, and disease (Perera, 1995; Perera *et al.,* 1996; Bartsch *et al.,* 1996; Johnson *et al.,* 1997). The nasal lesions described in this paper open a series of important questions: Is p53 accumulation the result of a missense mutation in the p53 gene or in a gene downstream of p53? Or is it the result of stabilization of the protein by binding to cellular or viral proteins and DNA damage? Is the presence of p53 in the nasal dysplastic lesions followed by progression, basement membrane transgression, and squamous cell carcinomas, as is the case in oral, bronchial, and esophageal carcinomas? Is p53 in this setting a susceptibility marker? Is the nasal epithelium reflecting changes going on in the lower respiratory apparatus in the exposed populations? Could we intervene and modulate DNA damage through

chemoprevention? Carcinogenesis in humans is a complex and long process; the incidence of nasal neoplasms in Mexico City should be monitored and future studies should address the identification of risk groups among the exposed population.

Twenty million people in Mexico City alone and millions of people throughout the world are exposed to air pollutants, and all of the inhaled materials pass through their noses, the most-used portal of the human body. It should be no surprise that since the nasal passages are a common site for particle deposition and the absorption of many gases and vapors, they are indeed a prime site for toxicant-induced pathology (Proctor *et al.,* 1982; Leopold, 1994).

The nasal epithelium is a valuable sentinel of the human exposures to toxic or carcinogenic substances. As the first point of contact of the respiratory apparatus with airborne chemicals in the environment, the nose can be seen as the "window to the respiratory system," a window that is readily accessible, that can be easily monitored, and which once altered may be compromised in its ability to protect the lower respiratory tract from exposure to such pollutants (Proctor *et al.,* 1982; Proctor, 1995; Leopold, 1994).

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