Cytopathology of the Nasal Mucosa in Chronic Exposure to Diesel Engine Emission: A Five-Year Survey of Swiss Customs Officers

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The simple and cheap technique of nasal cytology was used to assess possible adverse effects of chronic exposure to diesel engine emission (DEE) on respiratory mucous membranes. Brush cytology probes were taken from the noses of 194 male, nonsmoking customs officers twice a year (January and July) over a period of 5 years. The study group of 136 officers was solely occupied with clearing of diesel trucks (8.4 hr/day, 42 hr/week). Measured DEE concentrations varied between 31 and 60 µg/m³ and of benzo[a]pyrene concentrations were between 10 and 15 ng/m³. The control group of 58 officers worked only in the office. Over the 5-year period, similar results were obtained in summer and winter. In contrast to those not exposed to DEE, those who were had clear goblet cell hyperplasia with increased metaplastic and dysplastic epithelia and an increase in leukocytes. We found no evidence of progression of the cytopathologic changes. The findings may be described as a chronic inflammation of the nasal mucous membrane in the presence of chronic DEE exposure (chemical-induced rhinitis). Additionally, the findings of metaplastic and dysplastic nasal epithelia in the exposed subjects may indicate a genotoxic effect of chronic DEE exposure in humans. Key words: chemical-induced rhinitis, diesel engine emission, genotoxic air pollution, nasal cytology. Environ Health Perspect 111:925–929 (2003). doi:10.1289/ehp.4401 available via http://dx.doi.org/ [Online 1 November 2002]

Since 1972, the north–south transit traffic through Switzerland of diesel heavy-goods vehicles has increased by a factor of almost five (1). Diesel engine emissions (DEEs) have increasingly aroused major concern about their potential health effects as air pollutants. DEE contains diverse, potentially toxic materials in the form of mucous membrane-irritating DEE as a human lung carcinogen (10). Its role in urinary bladder carcinogenesis is rather suggestive (11).

Because the detectable health hazards of DEE in humans may have a long latency, the use of biomarkers for the early detection of relevant exposures has become increasingly important, particularly in epidemiologic investigations.

The nose is important for cleansing inhaled air and for modifying respiration and is an accessible source for investigation of exposure to airborne contaminants. The mucus layer is important in conditioning the inhaled air and provides a sticky surface for the entrapment of inhaled particles and gases. Because humans are nose breathers, the nasal cavity is the initial site of injury induced by inhaled irritants (12–14), a common site for particle deposition (15–20), and a site for the absorption of potentially noxious gases and vapors (21–23).

As site of first contact with inhaled toxins within the nasal cavity, it is the epithelium that deserves particular attention concerning the possible effects of air pollutants. An easy in vivo approach studying possible changes of the nasal epithelium is the brush biopsy (24–28), which aids data collection for the assessment of human risks from air pollutants. With this cytologic technique, we have found a significantly higher frequency of squamous cell metaplasia and dysplasia of the nasal epithelia in cigarette-smoking office workers compared with their nonsmoking colleagues (28). To evaluate whether the nasal mucosa responds similarly to DEE, we employed the same method among customs officers occupied with the customs clearance of heavy-duty vehicles. The results of this group were compared with those of their colleagues working only in the office.

Materials and Methods

Subjects and health assessment. Brush cytology nasal probes were taken from 194 male, nonsmoking customs officers twice a year (January and July) over a period of 5 years. The study group of 136 officers (age, 42.5 ± 8.10 years, mean ± SD) was occupied solely with the clearance of heavy-goods vehicles with diesel engines (8.4 hr/day; 42 hr/week).

The control group of 58 officers (age, 50.6 ± 7.36 years) worked in offices without air conditioning in a 100-year-old building located off the main roads at the Lake of Lugano.

All customs officers underwent an internal medical examination every 2 years. There were no abnormal findings of clinical examination among the customs officers we examined cytologically. An abnormal finding was a reason for exclusion. Immediately before the cytologic swab was taken, the temperature of each subject was taken using a Thermoscan probe (Braun GmbH, Kronberg, Germany) in the auditory canal. All volunteers were able before the cytologic examination. Because we examined the customs officers in the workplace, we assumed that they felt well enough to work (were not ill) on the day of examination and were in correspondingly good general physical condition.

The ear, nose, and throat status of each officer was established. In addition to inspecting the nasal cavities using a headlamp and nose speculum, we also inspected the auditory canals and eardrums, the mouth, the ephysynx, and the larynx. Finally, the neck was palpated for abnormal lymph nodes. Any abnormal finding in the ear, nose, or throat constituted a criterion for exclusion.

We conducted a standard skin-prick test (21 solutions; Allergomed, Reinbeck, Germany) on each customs officer. Subjects showing a seasonal/perennial sensitivity with positive skin tests were also excluded from our investigation.

The social status of the test and control groups was comparable in terms of income, education, and standard of living. Over 70% of the persons examined had not changed residences for more than 12 years, and none complained of annoying smells or residential toxics.

As the smell threshold for the vast majority of residential toxics is generally 10–20 times
lower than the lowest level of the time-weighted average (TWA) values, we assumed that no residential toxins were present at clinically relevant levels. Spot measurements by the Chemical Section in Suva’s Occupational Safety Department have also confirmed that the measured values of xylol, toluene, formaldehyde, ozone, and mineral fibers were well below the TWA according to the American Conference of Governmental Industrial Hygienists. The values of these toxicants measured at the workplace of the control group volunteers were well below the TWA.

**Cytologic examination.** For the cytologic examination, nasal epithelial cells were swabbed from both sides of the middle third of the inferior nasal concha by the translational and rotational movement of a small nylon brush (as commonly used in bronchoscopy). The cell swabs were taken using a head mirror and a nasal speculum to prevent unintentional contact with the squamous epithelium lining the nasal vestibule.

After removal, the cells were transferred to a microscope slide, fixed immediately, and stained using the Papanicolaou method. All slides were examined at the Institute of Pathology, Cantonal Hospital Lucerne, by one trained cytopathologist blinded as to the origin of the sample.

The light-microscope evaluation of the cytologic slides was done at a magnification of ×280. We examined 25 fields and counted 500 cells from representative sectors in each sample. Each case showing metaplastic and dysplastic cells was peer reviewed by a second cytopathologist. The diagnosis of squamous cell metaplasia was established when the cell contained a central nucleus and a wide cytoplasm stained orange or light red. Dysplastic squamous and columnar epithelial cells showed pleomorphism with anisokaryosis, enlarged and coarsely structured nuclei, and prominent nucleoli (Figure 1).

DEE soot measurements were taken regularly at the workplace of the exposed study group using a coulometric method. The mean ambient air pollutants (other than DEE) in Chiasso, where the study was performed in July and January of 1995 and 1999, are presented in Table 1.

**Statistical analysis.** We used the Mann-Whitney U test or the Student t-test to determine the significance of differences in the results of the two groups. We used contingency tables and chi-squared analysis to compare each of the cytologic features between the two groups. The significance level was set at p < 0.05.

**Results**

**Cell counts.** The quantitative cell distribution of the nasal swabs varied to some extent between January and July in each of the 5 years, but remained similar for these months throughout the 5-year test period. Therefore, the winter and summer results are shown separately in Figure 2A and B for 1995 and in Figure 2C and D for 1999, thus representing the whole test period.

The ratio between epithelia and leukocytes deviated from the normal in the DEE-exposed customs officers throughout the test period. Normally, the ratio of nasal mucous swabs is 80% ± 5 epithelial cells to 20% ± 5 leukocytes (29). In the nonexposed subjects, this ratio was always within normal limits (Table 2). However, in the DEE-exposed subjects, the mean ratios of epithelial cells to leukocytes during the study period was 50% to 50%. This difference was highly significant (p < 0.01).

Additionally, in the DEE-exposed subjects, we observed distinct deviations from the cytologic cell count obtained in the nonexposed subjects. In the exposed subjects, goblet cells increased up to 54% of all epithelial cells (Figure 3). In the control subjects, the goblet cells never exceeded 25% of all epithelia (Figure 2). This difference is also significant.

Moreover, in the exposed subjects we found a distinct increase in metaplastic squamous cells, partly associated with dysplasia, and in dysplastic columnar cells (Figure 1). Proportionally, the metaplastic squamous cells represented 12–19% of the epithelia in the exposed subjects, in contrast to only 5–7% in the nonexposed subjects (Figure 2). Dysplastic squamous and columnar cells were found exclusively in the exposed subjects.

The leukocyte counts revealed a significant increase of lymphocytes in the DEE-exposed subjects; 51–42% of all leukocytes were lymphocytes in exposed subjects compared with 20% of all leukocytes in the nonexposed subjects (Figure 2). We observed a slight but not significant increase in

![Figure 1. Nasal cytopathologic findings in nonsmoking customs officers exposed to DEE throughout the year (Papanicolaou stain). (A) Squamous cell metaplasia. (B) Cylinder cell dysplasia. (C) Squamous cell dysplasia with mitotic figure (arrow). The scale bar in (B) also applies to (A) and (C).](image-url)
Table 1. Mean values of the measurements of air pollutants in Chiasso, Ticino, the workplace of the DEE-exposed customs officers, in 1995 and 1999.

| Air pollutants (mean)       | January 1995 | July 1995 |
|----------------------------|--------------|-----------|
| Ozone (µg/m³)              | 2            | 71        |
| Hours > 120 µg/m³          | 0            | 123       |
| Nitrogen dioxide (µg/m³)   | 60           | 35        |
| Hours > 30 µg/m³           | 7            | 0         |
| Sulfur dioxide (µg/m³)     | 29           | 4         |
| Hours > 100 µg/m³          | 0            | 0         |
| PM₁₀ (µg/m³)               | 42           | 23        |
| VOCs (ppm)                 | 0.23         | 0.17      |
| PAHs (µg/m³)               | 51           | 13        |
| Humidity (%)               | 75           | 77        |

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; PM₁₀, particulate matter ≤ 10 µm aerodynamic diameter; VOCs, volatile organic compounds. Analisi della qualità dell’aria in Ticino 1995, 1999; Sezione Protezione Aria e Acqua, Divisione Ambiente, Dipartimento del Territorio, Bellinzona, Ticino, 1996, 2000.

Discussion

The significant goblet cell hyperplasia found in exposed subjects of our study, together with a clear increase of leukocytes (Figures 2 and 3), can be taken as an indication of a chronic state of irritation of the nasal mucosa with an inflammatory response (chemical-induced rhinitis) (30).

A state of chronic irritation of nasal mucosa is followed by hyperplasia of the goblet cell population (decrease of the ratio between columnar and goblet cells) (29). This is assumed a protective reaction leading to an increase of the mucus layer. Comparable cyto logic changes in the nasal mucosa have been described in workers exposed to nickel and chromate (31). In mice, exposure to DEE causes a clear inflammatory reaction, with goblet cell hyperplasia of the respiratory mucous membrane (32).

The significant increase of lymphocytes in the DEE-exposed subjects is another indication of the chronic state of local inflammation seen in other types of chronic rhinitis (29).

The finding of metaplastic squamous cells in DEE-exposed customs officers is concordant with the assumption of a chronic damage of the nasal epithelial lining. “Metaplasia” means that cells of one phenotype (e.g., columnar cells) are eliminated and replaced by differentiated cells of a different phenotype (e.g., squamous cells), very likely caused by a switch in the local stem-cell program. Metaplastic squamous epithelial cells are also

eosinophilic granulocytes particularly in January in the exposed subjects.

Ambient air measurements. The DEE soot measurements varied between 31 and 60 µg/m³. Benzo[a]pyrene concentrations were between 10 and 15 ng/m³. The measurements of the other ambient air pollutants are shown in Table 1.

Figure 2. Differentiated counts of 500 cells/sample (mean percent) in nasal swabs among DEE-exposed (n = 136) and nonexposed (n = 58) customs officers. (A) January 1995. (B) July 1995. (C) January 1999. (D) July 1999. The p-value for each comparison of the two groups is shown above the bars.
found in individuals with vitamin A deficiency or after irradiation, or in individuals exposed to various inhalation noxes (e.g., wood dust, metal dusts, solvents) (24,30,31).

Dysplastic epithelia (columnar- and squamous-cell type; see “Materials and Methods,” Figure 1) were found exclusively in the DEE-exposed subjects. Dysplasia is recognized as a preneoplastic stepstone in the multistage development of carcinogenesis. The association between squamous metaplasia and dysplasia of the respiratory mucosa and tobacco smoking was demonstrated in up to 80% of smokers (33). As described in smokers, the present study obtained similar cytologic results in nonsmoking subjects with long occupational exposure to DEE.

Recently we showed that exposure to air contaminants (cigarette smoke) is clearly associated with squamous cell metaplasia and dysplasia of the nasal mucosa, with a significant correlation between the degree of metaplasia and the number of cigarettes smoked (28).

Interestingly, repeated measurements of DEE soot at the workplace of the study group found levels between one-third and one-sixth of the maximal tolerated value. In addition, the found levels between one-third and one-sixth of the maximal tolerated value. In addition, the results in nonsmoking subjects with long occupational exposure to DEE.

The extent to which genotoxic or nongenotoxic mechanisms are involved in the cell changes observed remains to be elucidated. Regarding the physicochemical complexity of DEE, we can assume that there are multiple combined noxious effects. For example, irritant vapors such as formaldehyde and acroleine, as well as soot particles, may lead to inflammation of the nasal mucosa. In a further toxicity study on rats, exposure of the nasal mucosa to acroleine, a component of DEE, at 0.25 mL acroleine/m³ for 6 hr induced basal cell hyperplasia and an increased mitotic rate (34). Occupational exposure to formaldehyde causes goblet cell hyperplasia and squamous cell metaplasia and dysplasia of the nasal mucosa (35,36). A chronic exposure to SO₂ led to goblet cell hyperplasia in experimental animals and caused a marked increase in the thickness of the mucus layer. This was followed by impairment of mucociliary clearance with increased risk of infections (37).

In our study, a progression of the cytopathologic findings was not observed within the study period; in particular, no evidence for a neoplastic transformation was detected (Figure 2).

In addition to its simplicity, the cytologic procedure used in this study is an inexpensive, noninvasive procedure requiring no anesthetics. The cytologic analysis of human nasal cavity cells could serve as a biomarker for the assessment of exposure to inhaled toxic substances (26–28,30).

The increasing numbers of vehicles with diesel engines on our roads makes the toxic potential of DEE a public health concern. Nasal brush cytology may be used as a simple and cheap method for the evaluation of the toxic effects of DEE on the nasal mucosa and as a biomarker in combination with epidemiologic surveys. Further biochemical, molecular, and DNA–adduct studies should be conducted on the brush cytology gathered material.

### Table 2. Relationship between epithelial cells and leukocytes in nasal swabs of exposed and nonexposed customs officers (in percent of all 500 counted cells/specimen).

|          | Exposed | Nonexposed |
|----------|---------|------------|
|          | January | January | July | July | January | January | July | July |
| Epithelia | 50      | 60       | 40   | 60   | 50      | 60       | 40   | 60   |
| Leukocytes | 30      | 15       | 25   | 20   | 30      | 15       | 25   | 20   |

### Figure 3. Cytologic findings from the nasal cavities of customs officers (Papanicolaou stain). (A) Normal ciliated columnar cells from the nasal cavity of a nonexposed subject. (B) Marked goblet cell hyperplasia in a DEE-exposed subject. The scale bar in (B) also applies to (A).

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