Reversibility of Bronchial Cell Atypia

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ABSTRACT

Various degrees of cellular atypia were induced in the bronchial epithelium of dogs by means of repeated submucous 20-methylcholanthrene injections. Thereafter, the 20-methylcholanthrene treatment was stopped, and the outcome of the bronchial cell atypias in individual dogs was studied using cytomorphological and cytochemical methods. The results suggest that the various degrees of 20-methylcholanthrene-induced cellular atypias, including those cytologically interpreted as malignant, may reflect reversible cellular alterations which disappear after removal of the carcinogen. Similar observations were made in a group of cigarette smokers who, after malignant appearing cells were found in the sputum material, stopped smoking or significantly reduced their cigarette consumption.

INTRODUCTION

During the last years, increasing evidence has been obtained that bronchial carcinoma may develop through a sequence of cellular events reflected by morphological and cytochemical alterations (1, 8-24, 26-29, 31). It was shown experimentally that, when normal bronchial epithelium in individual dogs is exposed to a carcinogen, e.g., 20-MC3, during a relatively long period, a replacement of normal bronchial cells by regular squamous metaplastic cells, cells with various degrees of atypia, and finally by cancer cells can be observed (10, 12, 13, 22). In humans similar cellular changes have been demonstrated, e.g., in smokers (15-17, 27).

In the present study, the reversibility of carcinogen-induced cell alterations was investigated. Individual dogs were exposed to submucous 20-MC injections at a well-defined locus in the bronchial tree until various degrees of bronchial cell atypia were found. Thereafter, the 20-MC treatment was stopped, and the character of the carcinogen-induced alterations was studied by means of cytomorphological and cytochemical methods. The findings in those dogs in which the 20-MC-induced atypia no longer could be compared with similar findings in patients in which the disappearance of a marked cellular atypia, morphologically equivalent to cancer cells, had been observed.

MATERIALS AND METHODS

Experimental Dog System. Six beagle dogs and 2 mongrel dogs were repeatedly treated with a suspension of 50 mg 20-MC in 1.5 ml sterile water. The 20-MC suspension was injected submucously once a week during a period of at least 130 weeks at the bifurcation of the apical and cardiac bronchi by means of a special needle through a bronchofiberscope. Cell sampling was performed once a week immediately prior to the 20-MC injections. After the cessation of 20-MC treatment, weekly cell sampling was continued for at least 50 weeks. Punch biopsies for histological investigation were performed at 4-week intervals. The experiments described above were performed at the Department of Surgery, Tokyo Medical College Hospital, Tokyo, Japan (13).

Clinical Material. The clinical material used herein is composed by archival Papanicolaou-stained sputum cytological slides from 9 patients examined at the Cytology Laboratory, Sabbatsberg Hospital, Stockholm, Sweden. In each of these patients, abnormal cells cytologically interpreted as malignant had been observed. Since no tumor could be found at further examinations, e.g., X-ray or bronchoscopy, no treatment was performed in these particular patients. During the following period, which lasted between 27 and 168 months, the patients were regularly controlled by means of various clinical examinations including sputum cytology.

Cytomorphology. The cytological criteria for grading of bronchial cell atypia used in the present study was based on a grading system for human bronchial epithelium developed at the Sabbatsberg Hospital (16, 17). This system is similar to that of the World Health Organization recommended nomenclature (25). Recent investigations performed by the present investigators, including cytological and histological examination (10-13, 22), showed that 20-MC-induced alterations in the bronchial epithelium of dogs are morphologically similar to those observed in patients and that a similar grading system could be used for the cytomorphological description of the carcinogen-induced cellular changes in the present model system. The features of both dogs and human bronchial cells exhibiting various degrees of morphological alterations are illustrated in Figs. 1 to 5 and described in the legends to the figures.

Clear-cut cancer cells and cells indicating cancer in situ have been described here in one context since those changes cannot be separated in cytological dog material (11).

Cytochemistry. Papanicolaou-stained smear preparations from dog brush material and human sputum material were examined cytomorphologically (Figs. 1 to 5) and mapped photographically in order to be able to identify the cytomorphologically evaluated cells for the following cytochemical analysis. The preparations were destained in acidic alcohol, fixed in 10% neutral formalin for at least 15 hr, hydrolyzed (5 N HCl, 20°C, 60 min), and Feulgen stained (7). The nuclear Feulgen-DNA content in individual cells was determined using a rapid-scanning microspectrophotometer (4-6). In order to compare cytophotometric measurements from smear preparations stained on different occasions, all DNA values were expressed in units of 2C, with 2C denoting the mean Feulgen-DNA value of normal diploid canine bronchial epithelial cells or human leukocytes (control cells) that were adventitiously present in all cell samples. The difficulties of measuring DNA values in leukocytes with very condensed chromatin were partially eliminated by "off-peak" measurements at a wavelength of 610 nm (7, 12). At least 100 regular squamous metaplastic cells, metaplastic cells with various

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2 To whom requests for reprints should be addressed.

3 The abbreviation used is: 20-MC, 20-methylcholanthrene.

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degrees of atypia, or malignant-appearing cells were measured at each observation point. On each slide preparation, at least 30 control cells were also measured.

Clear-cut cancer cells and cells indicating cancer in situ in human cytological material were analyzed separately because this distinction may be possible in human cytological material but not in cytological dog material (11).

RESULTS

Chart 1 illustrates the presence of squamous metaplastic cells without atypia; metaplastic cells with mild, moderate, and severe atypia; and malignant-appearing cells in 8 dogs during approximately 12 months following cessation of 20-MC treatment (see "Materials and Methods"). It can be seen from Chart 1 that the cellular atypia disappeared in the dogs exhibiting 20-MC induced moderately and severely atypical bronchial cells. After approximately 1 year, only nonatypical squamous metaplastic cells were observed. Among the dogs with 20-MC-induced malignant-appearing cells, a disappearance of cellular atypia was registered in 3 of 5 animals after the 20-MC treatment had been stopped and only nonatypical metaplastic cells were observed. In the other 2 dogs, the malignant-appearing lesions persisted, and one of these dogs died with invasive squamous bronchial carcinoma 1 year after cessation of 20-MC treatment.

Chart 2 shows the sputum cytological findings of 9 patients in which morphologically malignant cells were found in sputum and which thereafter were controlled by means of repeated sputum cytology. It is malignant-appearing cells disappeared from the sputum material and bronchial epithelial cells with less pronounced atypia.
were observed. Finally, only normal bronchial epithelial cells or metaplastic cells without atypia were found to be present in repeated sputum samples in all but one patient who showed mild to moderate cell atypia.

Charts 3 and 4 illustrate the DNA distribution patterns in dog (Chart 3) and human (Chart 4) bronchial cell populations morphologically graded as cancer cells, squamous metaplastic cells with various degrees of atypia, and metaplastic cells without atypia occurring during the described periods above when a disappearance of the cellular atypia was observed. It can be seen from the charts that, in both dog and human bronchial epithelium, the replacement of malignant-appearing cells by cells with less pronounced atypia or by nonatypical squamous metaplastic cells was paralleled by a disappearance of cells with highly increased DNA values and the occurrence of cells with DNA values corresponding with that found in diploid normal cells.

**DISCUSSION**

Cytological methods are of great importance in the routine diagnostic work in bronchial carcinoma. In addition, sputum cytology has been used for screening of high-risk groups and has also become a useful method in the diagnosis of cellular abnormalities in the bronchial tree in various groups exposed to air-borne carcinogens. However, bronchial cells exhibiting various degrees of atypia and also malignant-appearing cells have been found in the cytological specimens from patients without any clinically observable lesion. In this context, the question arises as to what extent the cytological findings are indicative for an irreversible malignant alteration or if even pronounced cellular atypia may be consistent with reversible bronchial lesions.

The aim of the present study was to investigate the reversibility of various degrees of bronchial cell atypia. For this purpose, cellular atypia was induced in the bronchus of individual dogs by means of 20-MC injections. After the 20-MC treatment had been stopped, the fate of the bronchial lesions was studied. The cellular changes observed in the experimental dog system was compared with cellular changes observed in cigarette smokers who, after malignant-appearing cells had been diagnosed cytologically in sputum specimens, had stopped smoking or significantly reduced the number of cigarettes per day.

The results show that in dogs all degrees of 20-MC-induced cellular atypia including the most pronounced abnormality, cytologically interpreted as malignant, may be reversible after removal of 20-MC. In all dogs exhibiting bronchial cell alterations graded as at most moderate, atypia disappeared after 20-
MC treatment was stopped. Among the 6 dogs with severe bronchial cell atypia or carcinoma cells, one dog showed persistence of pronounced cellular atypia and one dog showed development of a histologically demonstrable invasive carcinoma, whereas in 4 dogs disappearance of cellular atypia could be demonstrated. This reduction and disappearance of cell atypia was paralleled by a transition of DNA distribution patterns with highly increased and scattered cellular DNA amounts into DNA distribution patterns similar to those found in nonproliferating normal bronchial cells. Similar cytological and cytochemical changes were observed in patients exhibiting regression of very marked cellular atypia (cytologically diagnosed as malignant).

The data reported in the present investigation indicate that all degrees of cellular atypia as well as all degrees of DNA alterations observed in cytotological bronchial cell material may be consistent with reversible bronchial lesions. It occurs that neither cytomorphological methods nor DNA measurements allow to distinguish between cellular changes which still are reversible, e.g., disappear after removal of a carcinogen, and irreversible malignant cellular changes.

Earlier studies have shown that squamous bronchial carcinoma develops through a series of cellular events which seem to be correlated to progressive cytomorphological and cytochemical alterations (1, 8–24, 26–29, 31) similar to those demonstrated during the pathogenesis of cervical carcinoma (3). The process of malignant transformation appears to have a duration of 2 to 3 years in dogs with an expected life span of approximately 12 to 15 years (12, 13, 22). In cigarette smokers, squamous carcinoma is in general not observed until 2 to 3 decades after the smoking debut. Observations in dogs indicate that the cellular changes finally resulting in bronchial carcinoma can be subdivided into 3 periods. The first period seems to be relatively short and comprises cellular alterations similar to those occurring in normal growth-arrested cells (G0 cells) stimulated to proliferate (2, 12). The second period appears to be relatively time consuming and is characterized by progressive cytotological and cytochemical cellular alterations finally resembling those found in bronchial carcinoma cells but which still are reversible upon removal of the carcinogen (22, 30). The second period is followed by a period which begins as soon as the cellular alterations happen to result in irreversible malignant cells independent of the presence of the carcinogen (22). The probability that this last-mentioned final transformation will occur seems to be extremely low in cell populations exhibiting cytological changes herein defined as mild or moderate atypia. In contrast, in individuals with cell populations showing severe atypia and especially in those exhibiting malignant-appearing cells, irreversible malignant transformation must be expected in a significant number of cases.

In previous investigations, evidence has been obtained that the cytological and cytochemical alterations occurring in bronchial epithelium undergoing malignant transformation are in principal the same in 20-MC-exposed dogs and in cigarette smokers (1, 10, 13, 16, 17). The present data also indicate an interesting similarity between the cellular changes observed in the dog and human bronchial epithelium after interruption of 20-MC treatment or cigarette smoking (Figs. 1 to 5; Charts 3 and 4).

Together, these results suggest that carcinogen-induced bronchial carcinoma develops through a sequence of cellular alterations of which upon removal of alterations seem to exist during a relatively long time period, there should be a good possibility to detect bronchial lesions before irreversible malignant transformation takes place. Thus, the development of bronchial carcinoma may be avoided provided that the agent(s) inducing malignant transformation can be identified and eliminated.

REFERENCES

7. Cohen, A. H. Techniques for localized injections and topical applications of 20-MC treatment or cigarette smoking (Figs. 1 to 5; Charts 3 and 4).
Fig. 1. Squamous metaplastic cells without atypia in dog brush material (A) and human sputum material (B). There is good cell cohesion, and single metaplastic cells are rare. There is no notable variation of cell and nuclear size. The cell shape is polygonal, cuboidal, or spherical. The nuclei are round or slightly oval, the chromatin is finely granular, and prominent nucleoli are not present. Papanicolaou stain, x 706.
Fig. 2. Squamous metaplastic cells with mild atypia in dog brush material (A) and in human sputum material (B). There is a slight variation of cellular and nuclear size. In some cells, slight hyperchromasia is seen. Otherwise, the mildly atypical squamous metaplastic cells exhibit the same general character as do nonatypical squamous metaplastic cells (cf. Fig. 1). Papanicolaou stain, × 706.

Fig. 3. Squamous metaplastic cells with moderate atypia in dog brush material (A) and in human sputum material (B). The cells occur generally in clusters, but single moderately atypical cells may be seen. There is an increased variability of cellular and nuclear size and shape as compared to that observed in mildly atypical cells. Multinucleation and lobulation may occur, particularly in the dog cells, and hyperchromasia is more frequently observed and more pronounced than in mildly atypical cells. Papanicolaou stain, × 706.
Fig. 4. Squamous metaplastic cells with severe atypia in dog brush material (A) and in human sputum material (B). The cells occur generally in loose groups and clusters, but single cells are also frequent. Cellular and nuclear size and shape exhibit a significant variability. Multinucleation and lobulation (mainly in dog cells) as well as cells with coarse chromatin are common. Prominent nucleoli may be observed. Papanicolaou stain, x 706.

Fig. 5. Cancer cells in dog brush material (A) and in human sputum material (B). The cells occur generally in loose clusters of varying size and frequently as single cells. Cellular and nuclear size and shape exhibit pronounced variability. The chromatin is coarse, and prominent nucleoli are frequently observed (particularly in the dog cells). Papanicolaou stain, x 706.
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