

Induction of Aryl Hydrocarbon Hydroxylase in Human Pulmonary Alveolar Macrophages by Cigarette Smoking

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ABSTRACT Pulmonary alveolar macrophages were obtained from healthy volunteers by saline pulmonary lavage, and aryl hydrocarbon hydroxylase was measured in the cells. Enzyme activity was low in cells from five nonsmokers with a mean of 0.008 ± 0.004 U/ 10^6 cells. Cells obtained from nine cigarette smokers contained higher enzyme levels, with a mean of 0.095 ± 0.024 U/ 10^6 cells. A former cigarette smoker was lavaged on five occasions. Enzyme activity during two lavages 4 mo apart were 0.010 and 0.009 U/ 10^6 cells, respectively. 1 wk after smoking was resumed, the enzyme activity rose slightly to 0.013, and reached 0.041 U/ 10^6 cells by 1 mo. Upon cessation of smoking, the enzyme activity returned to control levels by the next lavage, 2 mo later. These data indicate that aryl hydrocarbon hydroxylase may be induced in pulmonary alveolar macrophages of subjects chronically exposed to cigarette smoke.

INTRODUCTION

Cigarette smoking has been linked epidemiologically to lung cancer (1, 2), but the mechanisms by which smoking and cancer are related have not been delineated. Cigarette smoke and constituent chemicals such as benzo[α]pyrene (BP)¹ can induce tumors in a dose-related manner in experimental animals (3, 4).

Aryl hydrocarbon hydroxylase (AHH) is an inducible enzyme system found in the endoplasmic reticulum of many tissues in the body. Liver and lung contain large amounts, while lesser amounts are found in the intestine, thyroid, adrenal, testis, muscle, placenta, skin, and leuko-

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¹Abbreviations used in this paper: AHH, aryl hydrocarbon hydroxylase; BP, benzo[α]pyrene; MC, 3-methylcholanthrene; PAM, pulmonary alveolar macrophages.

cytes (5-13). A function of this enzyme system is to transform carcinogenic polycyclic hydrocarbons such as 3,4-BP or 3-methylcholanthrene (MC) to metabolites with altered carcinogenic properties (14-16).

Because of the widespread occurrence of polycyclic hydrocarbons in the environment, a mechanism which can modify the carcinogenicity of these agents in the respiratory tract, a major portal of entry, would be of importance. In order to examine AHH in the respiratory tract of man, we selected pulmonary alveolar macrophages (PAMs) because relatively homogeneous populations of these cells may be obtained by saline pulmonary lavage. Because PAMs reside within the lung alveoli, they are heavily exposed to cigarette smoke in the smoker.

The present study reveals that levels of AHH are higher in PAMs from smokers than from nonsmokers. Induction of AHH in PAMs obtained serially from a volunteer occurred between 1 and 4 wk after resumption of cigarette smoking.

METHODS

Preparation of pulmonary alveolar macrophages. Pulmonary alveolar macrophages were obtained from 14 healthy volunteers, ranging in age from 18 to 48 yr. Nine volunteers were cigarette smokers who consumed from 10 to 40 cigarettes/day for a minimum of 2 yr. Before acceptance in the study, informed consent was obtained in writing. The volunteers were anaesthetized by breathing a mist of 4% lidocaine hydrochloride (Xylocaine®, Astra Pharmaceutical Products, Inc., Worcester, Mass.), generated by an ultrasonic nebulizer. Viscous Xylocaine® was applied to one of the nares, and an Olympus 5-B2 fiberoptic bronchoscope (Olympus Corporation of America, New Hyde Park, N. Y.) was passed transnasally into a dependent segmental bronchus. With the volunteer in an upright position, 250 ml of sterile normal saline was instilled in 50-ml portions and recovered by suction.

The lavage suspension, which contained surfactant and pulmonary cells (80-97% of which were PAMs), was pooled and centrifuged in the cold at 180 *g* for 10 min in an angle head rotor in the Beckman J-21 refrigerated cen-

TABLE I
Recovery of Macrophages from Smokers and Nonsmokers

	PAMs recovered $\times 10^6$	Recovery volume <i>ml</i> *
Nonsmokers ($n = 5$)	14.9 ± 3.4	170 ± 14
Smokers ($n = 9$)	61.3 ± 9.5 †	163 ± 8

* All subjects received 250 ml lavage fluid.

† Difference between nonsmokers and smokers significant, with $P < 0.001$.

trifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Cells were washed twice in normal saline, then suspended in Hanks's balanced salt solution.

Cell counts were made in a hemocytometer, and the suspension was adjusted to contain 10^7 cells/ml. Slides were prepared for differential cell counts by staining with Wright-Giemsa. Cell diameters were measured using a calibrated ocular micrometer at a magnification of 1000. At least 100 cells were measured from each lavage studied.

AHH assay. AHH was determined by a modification of the method of Nebert and Gelboin (17). The cells were centrifuged and resuspended in cold 50 mM Tris-Cl, pH 7.5, with 3 mM $MgCl_2$ (Tris-Mg) so as to contain 2-3 million cells/ml. 2-ml portions of the cells were homogenized in the cold by 20 strokes of a glass tissue grinder and placed into duplicate tubes, each containing 1.0 ml of homogenate. Blank tubes contained Tris-Mg without cells. To each tube was added 1.0 mg of NADPH in a volume of 0.1 ml Tris-Mg, followed by 25 μg of BP in a volume of 5 μl of acetone. The tubes were mixed and incubated at 37°C in the dark for 30 min. The reaction was stopped by addition of 4 ml of 25% acetone in hexane. The tubes were mixed for 1 min on a vortex mixer and centrifuged 5 min at 500 g . The upper phase of each tube was transferred to another tube containing 0.5 ml of 1 N NaOH. The tubes were mixed for 1 min, then centrifuged for 5 min at 1000 g to separate the phases. The fluorescence of the lower (aqueous) phase was measured in an Aminco-Bowman spectrofluorometer (American Instrument Co., Inc., Silver Spring, Md.), with excitation of 396 nm and emission at 522 nm. Quinine sulfate was employed as working standard (excitation at 352 nm, emission at 452 nm) after comparison with authentic 3-hydroxybenzo[α]pyrene, a gift of Dr. H. V. Gelboin, National Institutes of Health. A unit of enzyme activity is defined as fluorescence produced equivalent to a picomole per minute of 3-hydroxybenzo[α]pyrene. Values were corrected for extraction efficiency of 90%.

RESULTS

The recovery of lavage fluid was the same from smokers and nonsmokers, although the number of macrophages in the lavage fluid from smokers was four times that of nonsmokers (Table I). The cell pellets of smoker PAMs were brown, while the packed cells of nonsmokers were white. Microscopically, most smoker PAMs contained abundant reddish-brown cytoplasmic inclusions. In contrast, few PAMs from nonsmokers had a noticeable quantity of this material. The size of mononuclear cells

was similar for PAMs obtained from smokers and nonsmokers (Fig. 1); however, the material lavaged from smokers often contained a few large multinucleated cells (comprising less than 1% of cells present) ranging from 40 to 120 μm in diameter.

AHH activity was 11 times greater in smoker PAMs than in nonsmoker PAMs (Fig. 2). There was no overlap of values; every smoker had levels above that of the highest nonsmoker. These data indicate that the mixture of agents in cigarette smoke, which includes polycyclic hydrocarbons, is a potent inducer of AHH in pulmonary alveolar macrophages.

In order to determine AHH induction in an individual, a nonsmoking volunteer was lavaged on five different occasions. Lavages were performed before, during, and after a period of smoking (Fig. 3). Two control observations made almost 4 mo apart showed AHH values in close agreement. After the second lavage, the volunteer began smoking 10-15 cigarettes/day for a month. After 1 wk, an upward trend in AHH activity was noted. By the next lavage, performed after 1 mo of smoking, AHH had reached 400% of the control level. Enzyme activity returned to control range by the next lavage 2 mo after cessation of smoking. The increase in AHH

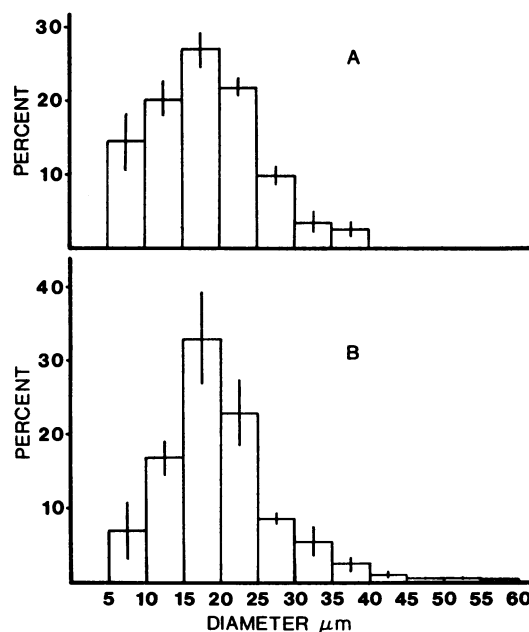


FIGURE 1 Size distribution of cells recovered from nonsmokers and smokers. (A) values for four nonsmokers, mean cell size = $17.5 \mu m$; (B) values for four smokers, mean cell size = $19.7 \mu m$. Each bar represents the mean of the percent of cells within a 5- μm size range. The vertical lines indicate the standard error of the mean. The cell size was determined with a calibrated ocular micrometer after the cells were fixed and stained with Wright-Giemsa.

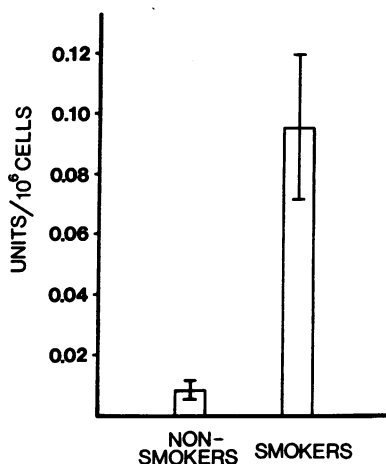


FIGURE 2 Aryl hydrocarbon hydroxylase activity in alveolar macrophages from nonsmokers ($n=5$) and smokers ($n=9$). A unit of enzyme activity is fluorescence produced equivalent to a picomole of 3-hydroxybenzo[α]pyrene per minute. The vertical lines indicate standard error of the mean, $P < 0.001$.

activity occurred without appearance of brown pigment or morphological changes (by light microscope) in the macrophages, suggesting that the presence of pigment is unrelated to induction of AHH.

DISCUSSION

The present data indicate that human PAMs can respond to substances in cigarette smoke by increasing their capacity to metabolize polycyclic hydrocarbons. The location of these cells in the lung alveoli provides maximum opportunity for exposure to the particles in cigarette smoke. Phagocytosis of particulates by macrophages provides a means whereby polycyclic hydrocarbons adsorbed to smoke particles may be ingested and retained within cells for eventual elimination. Prior studies demonstrated that lysosomal hydrolases were increased up to sixfold in macrophages from smokers, roughly paralleling the increase in cytoplasmic inclusions in smoker PAMs (18). The present study shows that microsomal enzymes were also increased, although there was no concomitant appearance of cytoplasmic inclusions.

The capacity of lung cells to induce microsomal enzymes would seem to be advantageous in view of reports that prior administration of AHH-inducing agents to laboratory animals prevented tumorigenesis by BP (19) or 7,12-dimethylbenz[α]anthracene (20).

Recent evidence, however, suggests that induction of AHH may be disadvantageous, in that the intermediate epoxides of polycyclic hydrocarbons are generally more carcinogenic than the parent compounds. Inhibition of epoxide formation therefore may be desirable. Because inhibition of AHH results in less epoxide formation,

and induced AHH levels lead to rapid conversion to noncarcinogenic metabolites, then either inhibition or induction of the enzyme results in lower rates of tumor formation (14, 19-21).

Recently it was reported that a microsomal fraction prepared from PAMs obtained from rabbits by lavage had a low level of AHH activity as compared with microsomal fraction prepared from whole lung (22). Reid, Glick, and Krishna (23) reported that AHH in rabbit PAMs was not induced by three daily injections of MC. In contrast, we found that AHH was not inducible in cultured PAMs, but AHH was induced in rabbit PAMs in vivo by feeding β -naphthoflavone for 3½ wk (unpublished results). These observations and the present report suggest that induction of AHH in PAMs occurs over an extended period of time. A likely explanation is that PAMs are not induced in situ but the apparent induction reflects influx of macrophages which had been previously induced while in marrow or lung interstitial spaces. The course of induction parallels the turnover rate of PAMs in the alveoli (24), if a lag period of 3-4 days for induction in the precursor cells is assumed.

It is not known if AHH induction in man is related to risk of developing lung cancer. Since both propensity to developing lung cancer and AHH induction are probably genetically determined (1, 25, 26), it is tempting to speculate a correlation of these heritable traits. Kouri, Salerno, and Whitmire (27) found that strains of mice in which AHH could be induced were more susceptible to tumors after exposure to MC, but strains in which the enzyme was relatively noninducible were resistant to MC-induced tumors. Yet no correlation was found with susceptibility to tumors induced by BP or 7,12-dimethylbenz[α]anthracene. Further studies are needed to determine whether any correlation of AHH and cancer exists, particularly in man. To find such a relationship would be a step toward identification of high-

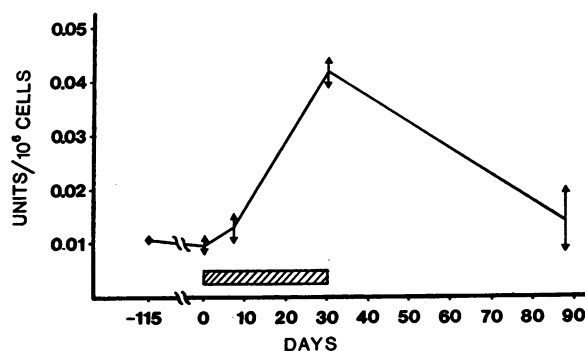


FIGURE 3 Response of macrophages in an individual to cigarette smoking. The shaded bar indicates duration of smoking. The vertical lines indicate the range of duplicate determinations at each time period.

risk individuals. Such persons may then be counselled appropriately about their exposure to polycyclic hydrocarbons and other chemical carcinogens in the environment.

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