after they heat up) by adding sexual induction, and the consequent production of drought-resistant zygospores, to their heat-shock repertoire.

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Detection of Smoking-Related Covalent DNA Adducts in Human Placenta

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The presence of covalent DNA chemical addition products (adducts) in human term placentas was investigated by recently developed immunologic and ^{32}P-postlabeling assays. DNA from placental specimens of smokers showed a small but not statistically significant increase in adduct levels when tested by antibodies to DNA modified with a benzo[*a*]pyrene dihydrodiol epoxide (BPDE-I), the ultimate carcinogenic derivative of benzo[*a*]pyrene. The postlabeling assay detected several modified nucleotides, one of which (adduct 1) strongly related to maternal smoking during pregnancy. This adduct was present in placental tissue from 16 of 17 smokers, but only 3 of 14 nonsmokers. Among smokers, levels of adduct 1 in general were only weakly related to questionnaire and biochemical measures of the intensity of smoking exposures, which suggests modulation by individual susceptibility factors. The adduct seemed to be derived from an aromatic carcinogen, but it may not result from several of the most intensely studied polycyclic aromatic hydrocarbons or aromatic amines in tobacco smoke. The data show the association of cigarette smoking with covalent damage to human DNA in vivo.

CIGARETTE SMOKING IS THE MAJOR single known cause of cancer mortality in the United States, and tobacco's contribution to all cancer deaths is estimated to be 30 percent (*1, 2*). Tobacco smoke contains numerous substances that cause cancer in animal bioassays, but it is not clear which of the several thousand components of this complex mixture are responsible for human carcinogenesis (*1, 2*). In animals and humans, chemical carcinogenesis is a multistage process consisting of mechanistically discrete events that may occur over a considerable portion of the lifespan of an individual (*3*). The initial event (initiation) is thought to involve DNA damage, as evidenced by the ability of the majority of chemical carcinogens to give rise to covalent chemical addition products (adducts) in the DNA of experimental animals (*4*), but little is known about the nature of DNA adducts formed in vivo in humans. In this study the presence of DNA adducts formed in placentas of women who smoked during pregnancy was investigated with complementary immunologic (*5*) and postlabeling (*6*) assays.

Smoking induces placental mono-oxygenase activity, alters placental morphology, and seems to increase the risk of malignant and benign gestational trophoblastic disease (*1, 7*). In addition to responding to smoking, placental tissue was appropriate for these studies because a large amount of fresh tissue was available from healthy subjects, which facilitated replicate analyses of each specimen by multiple assays. Women who donated placental specimens also provided multiple blood specimens and interview data concerning smoking and other exposures. These samples and data allowed a study of relations among measures of smoking exposure and the formation of adducts; they also provide materials for further studies of the effect of metabolism or DNA repair on these relations as well as for correlations with clinical end points such as birth weight.

Healthy pregnant volunteers receiving care from the obstetrical service of the University of North Carolina Memorial Hospital in Chapel Hill were selected on the basis of their smoking history. Informed consent and a maternal blood specimen were obtained during a clinic visit at about the 32nd week of pregnancy. A second specimen of maternal blood, a sample of cord blood, and the placenta were obtained at delivery. Blood specimens were analyzed for three biochemical markers of smoking exposure—cotinine, thiocyanate, and carboxyhemoglobin (*8*). Subjects were interviewed within a few days after delivery. Smoking data were collected separately for each trimester and the last 2 weeks of pregnancy; 17 of the 31 subjects reported active smoking during pregnancy and were classified as smokers. Shortly after delivery, placentas were frozen at −80°C. Laboratory personnel conducting the assays had no knowledge of the exposure status of the subjects. DNA was analyzed for adducts by a competitive enzyme-linked immunosorbant assay (ELISA) with antibodies to DNA modified with 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-benzo-[*a*]-pyrene (BPDE-I) (*5*), and by the ^{32}P-postlabeling assay (Fig. 1).

The antibodies used in the ELISA cross-react with several other polycyclic aromatic hydrocarbons containing a diol-epoxide region similar to BPDE-I (*9*), so that inhibition of antibody binding may have resulted from adducts other than BPDE-I derivatives. In the ELISA, human placental DNA competitively inhibited antibody binding to an extent equivalent to BPDE-I adduct levels ranging from nondetectable to 5.8 fmol

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of adduct per microgram of DNA with the monoclonal antibody (*5*) and from nondetectable to 2.1 fmol of adduct per microgram of DNA with the polyclonal antibody (*10*) (Table 1). Limits of detection were estimated to be about 0.06 fmol of adduct per microgram of DNA (one adduct in 5×10^7 normal nucleotides) but depended on the cross-reactivity of the antibody for adducts actually present (*9*). Although the mean adduct level was about 50 percent higher for smokers than nonsmokers as measured by either antibody, differences between smokers and nonsmokers were not statistically significant (Table 1) (*11*).

The ^{32}P-postlabeling assay revealed the presence of several chromatographically distinct adducts in human placental DNA (Fig. 1). One adduct was observed most frequently and with the highest autoradiographic intensity (adduct 1 in Fig. 1). Its presence in placental DNA was strongly related to maternal smoking: only 3 of the 14 nonsmokers tested showed evidence of this spot at a low intensity (*12*), whereas the adduct was present in all but 1 of the 17 smokers tested (Wilcoxon rank sum test, $P < 0.0001$) (Fig. 2). The single exception was a woman who reported she started smoking an average of four cigarettes per day during the second trimester of pregnancy; her serum cotinine value (*9*) during the last trimester was 145 and at delivery was 80, however, suggesting at least moderate cigarette consumption. In contrast, adduct 1 was found in the DNA of a woman who reported she started to smoke four to seven cigarettes per day during the last trimester of pregnancy and had lower cotinine and thiocyanate values. The highest adduct value estimated (*12*) for a nonsmoker was 1.5 (Fig. 2); this woman reported the heaviest exposure to passive smoking among nonsmokers in the study, averaging more than 16 hours per day throughout pregnancy. Of the two other nonsmokers showing adduct levels of 0.5 (Fig. 2), one was exposed to passive smoking less than 1 hour per week and one was not aware of exposure to passive smoking.

Since the procedure used was selective for nucleotides substituted with aromatic carcinogens (*6*), adduct 1 was presumably a derivative of an aromatic carcinogen. In an attempt to identify adduct 1, human placental DNA samples were mixed with test DNA preparations containing known aromatic carcinogen–DNA adducts and then subjected to the ^{32}P-postlabeling assay. The test DNA's were isolated from livers or lungs of mice 24 hours after a single injection with the compound of interest (*6*). In these experiments the adduct did not seem to be derived from several aromatic genotoxic chemicals in cigarette smoke: benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a,h*]anthracene, pyrene, chrysene, fluoranthene, benzo[*g,h,i*]perylene, 4-aminobiphenyl, 2-naphthylamine, or the isomeric methyl or ethyl derivatives of aniline. It is possible that this adduct was specifically formed in human or placental tissue from one of the chemicals tested, or resulted from a chemical not tested, such as one of the carcinogenic nicotine-derived tobacco-specific *N*-nitrosamines [recently reviewed by Hoffmann and Hecht (*2*)]. Recent experiments have demonstrated the formation of DNA adducts when mouse skin was painted with cigarette smoke condensate (*13*). One of these adducts appeared to cochromatograph with adduct 1. Isolation of subfractions of condensate yielding this adduct may provide materials to facilitate its identification.

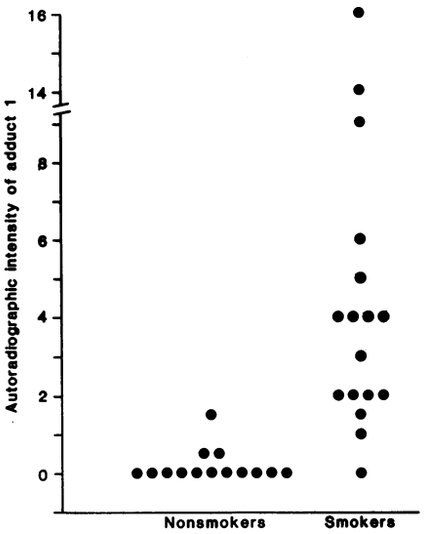

Fig. 2. Autoradiographic intensity of adduct 1 in relation to smoking status during pregnancy. For estimation of intensity values, see (*12*).

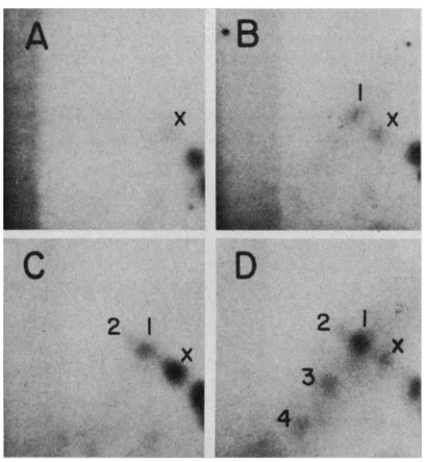

Fig. 1. Autoradiograms of placental DNA adduct maps obtained by the ^{32}P-postlabeling assay. Radioactivity (^{32}P) was incorporated into mononucleotides in enzymatic digests of placental DNA, and evidence for the presence of adducts was provided by the appearance of extra spots on autoradiograms of thin-layer chromatograms of ^{32}P-labeled DNA digests (*6*). Placental DNA (4 μg) was digested to deoxyribonucleoside 3′-monophosphates in 10 μl of 20 m*M* sodium succinate, 10 m*M* $CaCl_2$, *p*H 6.0, containing 0.054 unit of micrococcal endonuclease and 0.45 μg of spleen exonuclease per microliter, at 38°C for 3½ hours. The labeling reaction led to the formation of deoxyribonucleoside 3′,5′-bisphosphates of adducts and normal nucleotides labeled at the 5′ location; it was conducted at *p*H 9.5 in a total volume of 15 μl containing digest equivalent to 800 μ*M* DNA-phosphate, 1.65 μ*M* [γ-^{32}P] adenosine triphosphate (9120 Ci/mmol), and 0.12 unit of polynucleotide kinase per microliter. Incubation was at 38°C for 45 minutes. Two-dimensional DNA-adduct chromatography was carried out on polyethyleneimine-cellulose thin layers (*6*), except that labeled normal nucleotides were removed by an initial development with 1*M* sodium phosphate (*p*H 6.8) and that solvents for the resolution of adducts were 3.6*M* lithium formate, 6.4*M* urea (*p*H 3.5) (from bottom to top) and 0.6*M* LiCl, 0.37*M* tris-HCl, 6.4*M* urea (*p*H 8.0) (from left to right). Residual radioactive background was reduced by a final development (from left to right) with 1.7*M* sodium phosphate (*p*H 6.0). Intensifying screen-enhanced autoradiography was performed at -80°C for 4.5 days. Adduct quantitites were estimated from the intensity of their autoradiographic image (*12*). (A) DNA from a nonsmoker; (B) DNA from a woman smoking an average of 20 cigarettes per day during pregnancy, adduct 1 = 6 count/min; (C) DNA from a woman smoking an average of 19 cigarettes per day during pregnancy, adduct 1 = 9 count/min; a second adduct (2) was also present; (D) DNA from a woman smoking an average of 25 cigarettes per day during pregnancy, adduct 1 = 14 count/min; three additional adducts (2 to 4) were also present. Furthermore, the spot marked X appeared exceptionally strong, suggesting that an adduct (*5*) cochromatographed with background spot X in this sample.

Because adduct 1 was associated with maternal smoking, data for active smokers were examined for quantitative relations between questionnaire and biochemical indices of smoking exposure and adduct intensity (Table 1). Among the 17 smokers there was a weak positive association between the intensity of adduct 1 and the number of cigarettes women reported smoking during the three trimesters and the last 2 weeks of pregnancy; none of these reached statistical significance. Values for the biochemical markers of smoking exposure in maternal and cord blood also were not strongly associated with the intensity of adduct 1. An exception was the strong association between adduct 1 levels and maternal carboxyhemoglobin values obtained about week 32 of pregnancy (Table 1); this association was not observed for carboxyhemoglobin values obtained at delivery ($\tau_B = 0.30$, $P = 0.24$ for maternal; and $\tau_B = 0.10$, $P = 0.76$ for cord blood). The weak correlations between

Table 1. Assay results comparing nonsmokers and smokers and, for smokers only, correlations between levels of adduct 1 in the postlabeling assay and results of other assays.

Adduct level or measure of smoking exposure	Nonsmokers		Smokers		Correlation with adduct 1	
	N	Mean ± SEM	*N*	Mean ± SEM	π_β	*P*
ELISA assays						
Monoclonal antibody (fmol adduct/μg DNA)	14	0.96 ± 0.34	17	1.65 ± 0.46	0.42	0.02
Polyclonal antibody (fmol adduct/μg DNA)	14	0.37 ± 0.07	16	0.56 ± 0.14	−0.06	0.75
Intensity of adduct 1*	14	0.18 ± 0.11	17	4.7 ± 1.08†		
Cigarettes per day during third trimester of pregnancy	14	0 ± 0.00	17	16 ± 2.1	0.32	0.10
Cotinine (ng/ml)‡	12	0.17 ± 0.11	14	155 ± 18.8†	−0.05	0.82
Thiocyanate (μmol/liter)‡	13	44.6 ± 4.7	14	127 ± 9.4 †	0.25	0.22
Carboxyhemoglobin (%)‡	14	0.69 ± 0.13	17	4.9 ± 0.67†	0.50	0.007

*See (*12*) for scale. †Wilcoxon rank sum test comparing active smokers with nonsmokers, $P < 0.0001$. ‡Values for maternal plasma or blood obtained at about week 32 of pregnancy.

levels of adduct 1 and intensity of smoking exposure may indicate intervention by individual susceptibility factors such as differences in mono-oxygenase and other metabolic activity, or DNA repair.

In addition to adduct 1, several other adducts were detected (Fig. 1). Adduct 2 was present in five specimens, adduct 3 in four, adduct 4 in only one, and a heavy X spot suggesting adduct 5 in two. These spots were detected only in DNA from smokers. An additional adduct (number 6, not shown), which chromatographed to the left of adduct 1, was observed only in specimens from two nonsmokers. The single noteworthy exposure shared by these two subjects was the use of portable kerosene heaters during their pregnancy. Because 6 of 27 women without adduct 6 for whom questionnaire data were available also reported some exposure to kerosene stoves, this finding was not significant ($P = 0.07$, Fisher's). The presence of adducts in DNA specimens from nonsmokers indicates that the postlabeling assay may be able to detect DNA damage from exposures unrelated to smoking. The postlabeling assay appears particularly well suited for the analysis of DNA adducts of unknown chemical origin and structure and for detecting genotoxic components in complex environmental mixtures (*13*).

Inconsistencies between estimates for levels of adducts measured by the immunologic assays (which ranged as high as the immunologic equivalent of about 2 BPDE-I adducts in 10^6 nucleotides) and estimates of adduct 1 identified by postlabeling (which ranged up to 1.4 adducts per 10^8 nucleotides) may relate to differences in the chemical forms of adducts each assay measures, errors in quantitation by either of the procedures, or both of these factors. The ELISA assays would measure not only the BPDE-I adduct used to produce the antibodies but other cross-reacting materials as well (*9*). Materials or assay conditions causing degradation of antibody could also cause false positive values in a competitive ELISA assay. The possibility cannot be excluded that some material measured by immunologic assays may not be detected by the postlabeling assay. Likewise, especially because postlabeling analyses were run under carrier-free conditions (*12*), the absolute quantitation of adducts identified by this assay is uncertain. Since assays for all individuals were done under identical conditions, these difficulties should not affect interpretation of associations between adduct levels and exposure histories.

While postlabeling identified smoking-related DNA adducts, the immunologic assay may be responding to as yet unidentified environmental exposures. Among active smokers only, however, a significant association was observed between values for immunologic assays using the monoclonal antibody and levels of adduct 1 (Table 1), which suggests that the monoclonal antibody and postlabeling assays responded to high levels of a similar or related exposure. These relationships will be explored further as more data become available and would be clarified by comparisons with other assays for DNA adducts (*14*) or approaches to measurement of biologically effective exposure (*15*). The preliminary nature of clinical and other associations generated from the small database available for this report should be emphasized: larger and more comprehensive studies are needed to establish genetic and environmental determinants of the formation of DNA adducts in human tissues.

Two previous studies have reported the presence of BPDE-I DNA adducts in human white cells (*14*) or white cells and lung specimens (*16*) by enzymatic radioimmunoassays or ELISA. Shamsuddin *et al.* found similar frequencies of positive assays among specimens of white cells from roofers (7 of 28 specimens), foundry workers (7 of 20), and control volunteer laboratory personnel (2 of 9) (*14*). Except for noting that the controls with positive assays were smokers, the data presented did not indicate a clear relationship between exposures and assay results. Perera *et al.* reported that lung tissue from 5 of 27 patients contained significant levels of adducts, but their presence was not related to current smoking (*16*). The five subjects were, however, former or passive smokers. In contrast, our data directly link an environmental exposure and DNA adducts in human tissue.

Lu *et al.* recently reported that the presence of DNA adducts in the placenta of animals exposed to the environmental carcinogens safrole, 4-aminobiphenyl, benzo[*a*]pyrene, and dibenzo[*c*,*g*]carbazole predicted the presence of adducts in both fetal and maternal tissues including brain, liver, lung, heart, kidney, skin, intestine, and uterus (*17*). These and other data (*18*) suggest that adducts found in the placenta of smokers will also be found in other maternal and fetal tissues and may be associated with initiation of malignancy or other adverse effects in the mother or her progeny. The approach used here should contribute to a definition of chemical components of cigarette smoke as well as other environmental exposures that most severely damage human DNA.

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9. Monoclonal antibody 5D11 cross-reacts with DNA modified by *trans*-8,9-dihydroxy anti-10,11-epoxy-8,9,10,11-tetrahydrobenz[*a*]anthracene, *trans*-3,4-dihydroxy anti-1,2-epoxy-1,2,3,4-tetrahydrobenz[*a*]anthracene, and *trans*-1,2-dihydroxy anti-3,4-epoxy-

1,2,3,4-tetrahydrochrysene, but with 5-, 3-, and 20-fold less sensitivity (respectively) than with BPDE-I-modified DNA.
10. M. C. Poirier *et al.*, *Cancer Res.* **40**, 412 (1980).
11. Statistical analysis was conducted by nonparametric procedures including Fisher's exact test, Kendall τ_B rank correlations, and Wilcoxon rank sum tests with programs provided by SAS Institute, Cary, NC.
12. In all positive samples, the levels of adduct 1 were close to the analytical limit of the ^{32}P-postlabeling assay, and prolonged autoradiography for 3 to 6 days at −80°C with intensifying screens was required for its detection. An intensity scale for adduct 1 was derived by visual comparison of spot darkness on autoradiograms for different specimens assayed under identical conditions. Three or four replicate analyses of DNA were performed for each specimen. For intensities greater than 4 these estimates were equivalent to data (count/min) obtained by direct measurement of radioactivity, which provided estimates of absolute levels of adducts. For these measurements adduct and normal nucleotide spots and adjacent blank areas were cut from separate polyethyleneimine-cellulose chromatograms and assayed by Cerenkov counting (*6*). The strongly positive sample D (Fig. 1) exhibited 14 count/min after subtraction of the blank (counting efficiency 42 percent), the corresponding count rates for samples C and B (Fig. 1) were 9 and 6 count/min, respectively. Although low, these values for ^{32}P were replicable, with standard deviations for triplicate analyses averaging 30 to 40 percent. Count rates of spots <4 count/min could not be reproducibly determined by counting (relative standard deviations >50 percent). Since the assay was performed under conditions that resulted in a 10- to 20-fold greater labeling of adducts relative to normal nucleotides [E. Randerath *et al.*, *Carcinogenesis* **6**, 1117 (1985)], the DNA sample presented in Fig. 1, sample D, had 60 to 120 adducts in 10^{10} normal nucleotides, the approximate number of bases per mammalian genome. The estimated range of adduct 1 for the DNA specimens analyzed was 2 to 140 adducts per 10^{10} normal nucleotides.
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Human Monoclonal Antibodies to Pf 155, a Major Antigen of Malaria Parasite *Plasmodium falciparum*

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Pf 155, a protein of the human malaria parasite *Plasmodium falciparum*, is strongly immunogenic in humans and is believed to be a prime candidate for the preparation of a vaccine. Human monoclonal antibodies to Pf 155 were obtained by cloning B cells that had been prepared from an immune donor and transformed with Epstein-Barr virus. When examined by indirect immunofluorescence, these antibodies stained the surface of infected erythrocytes, free merozoites, segmented schizonts, and gametocytes. They bound to a major polypeptide with a relative molecular weight of 155K and to two minor ones (135K and 120K), all having high affinity for human glycophorin. The antibodies strongly inhibited merozoite reinvasion in vitro, suggesting that they might be appropriate reagents for therapeutic administration in vivo.

DUE TO THE RAPID RESURGENCE of malaria in many parts of the world and its enduring prevalence in tropical Africa, major efforts are being made to develop vaccines against the disease. Since the asexual erythrocytic stages of the parasite are the cause of the morbidity and mortality of malaria, interest has been focused on the identification and characterization of antigens expected to be instrumental in inducing protective immunity against these stages. Among the major candidates for a vaccine are antigens present on the merozoites or on the surface of infected erythrocytes. Antibodies to these antigens have been shown to mediate (or amplify) killing of the parasites, to block merozoite-erythrocyte interactions, or to block or reverse sequestration of parasitized erythrocytes, thereby preventing the parasites from escaping destruction in the spleen (*1–4*).

Of the four species of *Plasmodium* that cause malaria in humans, *P. falciparum* is the most malignant. Recently, we identified a *P. falciparum* antigen, Pf 155, which is deposited in the erythrocyte membrane at merozoite invasion. Pf 155 is a heat-stable polypeptide (molecular weight, 155K) associated with merozoites and present in the supernatants of *P. falciparum* cultures (*5*). By means of a modified immunofluorescence assay (IFA), Pf 155 is easily detected on erythrocytes infected with ring-stage parasites (*3, 5, 6*). Antibodies to Pf 155 efficiently inhibit merozoite reinvasion in *P. falciparum* cultures in vitro (*3, 6*). They are present in patients with acute *P. falciparum* infection as well as in clinically immune individuals (*3, 5, 6*). In residents of a holoendemic area of Africa (*7*), a good correlation was found between serum titers of antibodies to Pf 155 and acquisition of clinical *P. falciparum* immunity (*6*). Although the existence of an antigenic variation within or between *P. falciparum* strains has not been ruled out, some of the major antigenic structures of Pf 155 are present in all strains and clones investigated thus far (*5, 8*). Hence, Pf 155 appears to be a suitable candidate for a *P. falciparum* vaccine.

The use of rodent monoclonal antibodies (MAb's) has contributed to much of the progress in the characterization of *P. falciparum* antigens achieved thus far (*1*). However, in human disease, MAb's from B cells of malaria-immune donors should offer several advantages over mouse MAb's, including the possibility of assessing the antibody-forming potential of patients' B cells and a more direct identification of antigenic epitopes relevant for protection. In this report we describe the production of human MAb's specific for Pf 155. These antibodies, which efficiently inhibited merozoite reinvasion in vitro, were further used to establish the relation of Pf 155 to similar *P. falciparum* antigens (*9–11*).

Cell cultures producing human monoclonal antibodies were obtained by repeated cloning by limiting dilution early after immortalization of B lymphocytes transformed with Epstein-Barr virus (*12*). The B lymphocytes were from a donor hyperimmune to *P. falciparum*, who had a very high serum titer of antibodies to Pf 155 (1:15,000) as detected by IFA on glutaraldehyde-fixed and air-dried erythrocytes infected with *P. falciparum* (*3, 5*). This IFA procedure was also used to screen the cultures for production of antibodies to Pf 155. Twelve immu-

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