MONITORING HUMAN EXPOSURE TO ENVIRONMENTAL CARCINOGENS

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INTRODUCTION

One of the long range goal of research in chemical carcinogenesis is the identification of individuals at increased risk of cancer development. Cancer is a multistep, multistage process in which many factors effect ultimate risk. The initiating event in the process of chemical carcinogenesis is the binding of the reactive electrophilic species of the carcinogen to nucleophilic sites in DNA. The extent of this reaction is influenced by a number of factors including metabolism to the active species or less toxic metabolites, detoxification of reactive intermediates and repair of adducts once formed. Thus, individuals with the same exposure may be at very different risk for cancer development because of differences in these processes due to genetic susceptibility. Suggestive evidence for this genetic susceptibility has come from epidemiologic studies demonstrating a higher proportion of individuals with a specific phenotype (e.g. poor metabolizers of debrisoquin or lacking specific glutathione transferase activity) among cancer cases than controls (Ayesh et al. 1984, Seidegard et al. 1986).

By measuring levels of carcinogen-DNA adducts in humans we may be able to obtain information directly related to that individuals risk for cancer development. Measurement of carcinogen-DNA adducts in the target tissue, termed biologically effective dose, is believed to be a more relevant arker of exposure than measurement of the chemical itself either in the environment or in body fluids (Perera and Weinstein 1982). Such assays take into account individual differences in absorption and metabolism of carcinogens as well as
repair of adducts.

Methods are now available for the sensitive detection and quantitation of carcinogen-DNA adducts which do not depend upon radiolabeled carcinogens. These methods are essential for the measurement of adducts in humans with exposure to environmental and occupational carcinogens. Immunologic methods for measurement of DNA adducts have utilized monoclonal and polyclonal antibodies recognizing a number of specific carcinogen-DNA adducts (Poirier 1984, Santella 1988). Antibodies can be developed against either the carcinogen-nucleoside adduct covalently coupled to carrier protein or the modified DNA electrostatically complexed to methylated bovine serum albumin. These antibodies can be used in highly sensitive competitive enzyme-linked immunosorbent assays (ELISA) with color- or fluorescence-endpoint detection. Since femtomole (10^{-15}) sensitivities are readily attainable, DNA adduct

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levels in the range of $1/10^8$ nucleotides can be measured. With monoadduct-specific antibodies, higher sensitivities may be obtainable if large amounts of DNA are available and the adduct is isolated by various chromatographic procedures before quantitation in the ELISA. A major advantage of immunologic methods for adduct detection in humans is that once a sensitive and specific method has been developed it can easily be applied to the large number of samples that are collected in epidemiologic studies. However, before an immunoassay can be developed the structure of the adduct of interest must be known and it must be possible to systhesize the adduct for development of the antibody. Table 1 lists some of the available polyclonal and monoclonal antisera recognizing carcinogen-DNA adducts. In addition, we have recently developed monoclonal antibodies to 8-oxoguanosine, 4-aminobiphenyl-guanosine and 7-hydroxyethylguanosine (unpublished studies). A number of the antibodies listed in Table 1, including those recognizing alkylation, aflatoxin, benzo(a)pyrene diol epoxide, cisplatinum and 8-methoxypsoralen-DNA adducts, have been applied to adduct detection in humans (reviewed in Farmer et al. 1987, Santella 1988).

An alternate method for adduct detection utilizes [^{32}P] postlabeling of adducts after enzymatic digestion of the DNA to 3'monophosphates (Randerath et al. 1981). Very small amounts of DNA are required (1-50ug) and prior knowledge of the identity of the adducts, essential for the immunologic approach, is not necessary. Four dimensional thin layer chromatography of the labeled nucleoside bisphosphates, followed by autoradiography allows separation of the normal nucleotides and visualization of adduct spots. Quantitative data is obtained by counting areas of the chromatogram containing adducts. Adducts cannot be identified but because of the limitations of the thin layer chromatography system, must result from the binding of bulky hydrophobic carcinogens. While the alkylated adducts would be lost with the normal nucleotides in the standard assay, HPLC methods have been developed for their quantitation (Reddy et al. 1984, Wilson et al. 1988). Several methods have also been developed to give high sensitivity (up to a reported 1 adduct/10^{9-10}nucleotides) including butanol extraction of adducts (Gupta and Earley 1988) and nuclease P1 digestion of normal nucleotides (Reddy and Randerath 1986) before labeling to enrich the sample in adducts. The advantages of the method, including high sensitivity, small sample size and ability to detect a broad range of adducts, make it ideal for studying individuals with exposure to complex mixtures. The major disadvantages are the utilization of [^{32}P], the complexity of the assay and the inability to measure some adducts by standard procedures. The method has been applied to the detection of adducts in placental (Everson et al. 1986, Everson et al. 1988) and lung (Phillips et al. 1988a) DNA of smokers and nonsmokers and white blood cell DNA of roofers (Herberts et al. in press) and foundry workers (Phillips et al. 1988b).

Several other methods have been developed for quantiation of DNA adducts but have not been as extensively applied to human adduct detection as have immunoassays and postlabeling. They include synchronous fluorescence spectroscopy (Harris et al. 1985) and gas chromatography/ mass spectroscopy (Weston et al. 1989).

While DNA is believed to be the critical target, chemical carcinogens also bind to RNA and proteins. Quantitation of protein adducts on either hemoglobin or albumin, has been used as an alternate marker of exposure to environmental carcinogens. Large amounts of protein can be obtained from blood samples (6mg albumin and 140mg hemoglobin/ml of whole blood). This can be contrasted with the 500-700ug of DNA normally obtained from 30-35ml of blood. Thus, much smaller amounts of blood are required for protein adduct measurement making these methods more generally applicable to routine occupational monitoring. In addition, a number of studies have demonstrated a correlation between DNA and protein adduct levels suggesting that it is an appropriate surrogate for DNA adduct measurement (Neumann 1984). No repair occurs on protein thus, chronic low levels of exposure may be measurable. Red blood cells have an average lifespan of 4 months while albumin has a half life of 21 days indicating that only recent exposure will be detectable.

Table 1. Antisera Recognizing Carcinogen-DNA Adducts

Adduct	References
acetylaminodimethyldipyridoimidazole (gluP3)-guanosine	(Hebert et al, 1985)
Acetylaminofluorene-DNA	(Ball et al. 1987, Leng et al. 1978, Sage et al. 1979)
Acetylaminofluoreneguanosine	(Baan et al. 1985, Guigues and Leng 1979, Poirier et al. 1977, Van der Laken et al. 1982)
Aflatoxin B_1-DNA	(Haugen et al. 1981, Hertzog et al. 1982, Hsieh et al 1988)
4-Aminobiphenylguanosine	(Roberts et al. 1988)
Aminofluoreneguanosine	(Poirier et al. 1983, Rio and Leng 1980)
Aminopyrene-DNA	(Hsieh et al. 1985)
Benzo(a)pyrene diol epoxide-DNA	(Poirier et al. 1980, Santella et al. 1984, Slor et al. 1981, van Schooten et al. 1987)
O^6-Butylguanosine	(Muller and Rajewsky 1981, Rajewsky et al. 1980)
Cisdiammine dichloroplatinum	(Fichtinger-Schepman et al. 1985, Malfoy et al. 1981, Mustonen et al. 1987, Poirier et al. 1982, Sundquist et al. 1987)
cyclic 1,N^2-propanoguanosine	(Foiles et al. 1986)
Ethenoadenine	(Eberle et al. 1989, Young and Santella 1988)
Ethenocytidine	(Eberle et al. 1989, Young and Santella 1988)
O^6Ethylguanosine	(Muller and Rajewsky 1980, Rajewsky et al. 1980, Van der Laken et al. 1982)
O^4Ethylthymidine	(Muller and Rajewsky 1981, Rajewsky et al. 1980)
Melphalan-DNA	(Tilby et al. 1987)
8-Methoxypsoralen-DNA	(Santella et al. 1985, Zarebska et al. 1984)
O^6Methylguanosine	(Muller and Rajewsky 1981, Wild et al. 1983)
N^6-Methyladenosine	(Munns et al. 1977)
7-Methylguanosine	(Degan et al. 1988, Meridith and Erlanger 1979, Munns et al. 1977)
iro-7-Methylguanosine*	(Stein et al. 1989)
O^2Methylthymidine	(Strickland and Boyle 1984)
4-Nitroquinoline-N-oxide-DNA	(Morita et al. 1988)
Trimethylangelicine-DNA	(Miolo et al. 1989)
Thymine dimer	(Ley 1983, Strickland and Boyle 1981, Wani et al. 1984)
Thymine glycol	(Leadon and Hanawalt 1983)

*iro, imidazole ring opened

Most data collected to date on protein adduct levels in humans has utilized GC/MS methods. Several different approaches have been used to increase the sensitivity of these measurements. For several aromatic amines including 4-aminobiphenyl, globin adducts are measured after cleavage of the amine from the protein followed by isolation and derivatizion (Bryant et al. 1987). Similarly, for benzo(a)pyrene adducts, acid treatment cleaves the adduct releasing BP tetrols, which can then be quantitated (Weston et al. 1989). In contrast, ethylene oxide globin adducts are measured by cleavage of the N terminal modified valine by an Edman degradation reaction (Tornqvist et al. 1986). All of these methods allow enrichment of the adduct to be measured by separation from the bulk of the nonmodified protein. Elevated levels of 4-aminobiphenyl-globin adducts (Bryant et al. 1987) and hydroxyethylvaline (Tornqvist et al. 1986) have been measured in smokers compared to nonsmokers.

A limited number of antibodies have been developed against protein adducts (Table 2). Only the antisera recognizing aflatoxin and BP adducts have been applied to human adduct detection. Data on aflatoxin have been published (Gan et al. 1988, Wild et al. 1990) and our initial studies in humans for BP adducts are summarized below.

Table 2. Antisera Recognizing Carcinogen-Protein Adducts

Acetaldehyde-protein	(Israel et al. 1986)
Acetaminophen-protein	(Roberts et al. 1987)
Aflatoxin B_1-albumin	(Gan et al. 1988, Wild et al. 1990)
Benzo(a)pyrene diol epoxide-protein	(Santella et al. 1986)
Ethylene oxide-hemoglobin	(Wraith et al. 1988)

MEASUREMENT OF EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

Antibodies to Benzo(a)pyrene Diol Epoxide-DNA

Benzo(a)pyrene (BP), a polycyclic aromatic hydrocarbon (PAH), is a ubiquitous environmental pollutant found in cigarette smoke, various foods and in all products of combustion. BP is metabolized *in vivo* to benzo(a)pyrene diol epoxide (BPDE-I) which reacts with the N2 position of guanine to form a covalent DNA adduct (Jeffrey et al. 1977). We have developed both polyclonal and monoclonal antisera recognizing BPDE-I-DNA. These antisera have been used to quantitate adducts in biological samples obtained from humans with various occupational or environmental exposure to PAHs. When first developed, these antibodies were found to be highly specific for the modified DNA not recognizing BP itself nor nonmodified DNA. In addition, there was no crossreactivity with several other carcinogen modified DNAs, including acetylaminofluorene and 8-methoxypsoralen-DNA. More recently, the reactive diol epoxides of several other PAHs were synthesized. These were used to modify calf thymus DNA *in vitro* for testing antibody crossreactivity. Both the monoclonal and polyclonal antisera were found to crossreact with structurally related diol epoxide adducts of several other PAHs including chrysene and benz(a)anthracene (Santella et al. 1987). Polyclonal antisera #29, obtained from animals immunized with BPDE-I-DNA, recognizes DNA modified by chrysene-1,2-diol-3,4-epoxide more efficiently (50% inhibition at 18 fmol) than it recognizes BPDE-I-DNA (50% inhibition at 30 fmol). This antibody also reacts with DNA modified by benz(a)anthracene-8,9-diol-10,11-epoxide (50% inhibition at 42 fmol) and by 3,4-diol-1,2-epoxide (50% inhibition at 114 fmol). Humans are exposed to BP in complex mixtures containing a number of other PAHs. Multiple PAH adducts may be present but at such low levels they cannot be

identified. Thus, the appropriate standard to use cannot be determined and absolute quantitation of adducts is not possible. BPDE-I-DNA is used as the standard since it was the antigen originally used for antibody development and measured values are expressed as femtomole equivalents of BP adducts which would cause a similar inhibition in the assay. Since a number of PAHs in addition to BP are carcinogenic, the ELISA provides a biologically relevant general index of DNA binding by this class of compounds.

We have also recently determined that polyclonal antisera #29 detects adducts more efficiently in highly modified DNA (1.2 adducts/100 nucleotides) than in low modified DNA (1.5/10^5) (Santella et al. 1988). This efficiency also varied with the type of ELISA used. With the color endpoint ELISA there was a 2.5 fold difference between the high and low modified DNA samples but with the fluorescence endpoint ELISA the difference was 10 fold (Figure 1). This antisera was obtained from animals immunized with highly modified DNA. Clustering of adducts or some unique determinents present on highly modified DNA may be responsible for the higher sensitivity with these samples. In our original studies, we utilized highly modified DNA in the standard curve which resulted in an underestimation of adduct levels. Similar preferential reactivity with highly modified DNA has been seen with other antisera against BPDE-I-DNA (van Schooten et al. 1987) and melphalan-DNA (Tilby et al. 1987). In contrast, antibodies recognizing 8-MOP-DNA have similar crossreactivity with adducts in high and low modified samples (Yang et al. 1987). These results demonstrate the importance of thorough characterization of antisera before application to human samples and the utilization of appropriate standards for analyzing biological samples. For adduct detection in humans with antibody #29, we currently use a low modified standard with fluorescence endpoint detection.

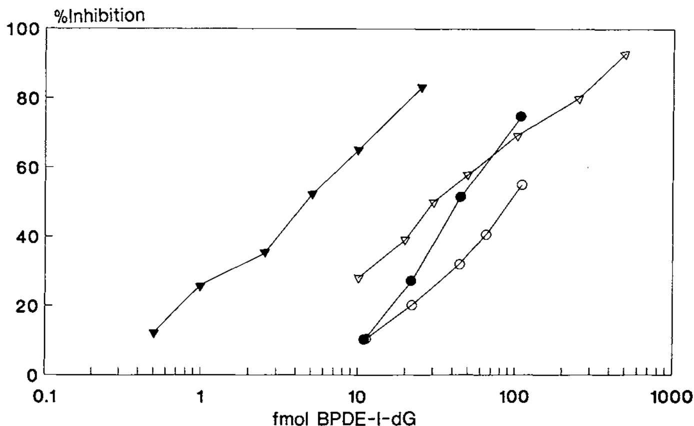

Figure 1. Competitive inhibition of antisera #29 binding to BPDE-I-DNA by high modified DNA (1.2adducts/100 nucleotides) and low modified DNA (1.5/105 nucleotides). Plates were coated with highly modified DNA and antisera diluted (1:30,000 for the color assay and 1:1,600,000 for the fluorescence assay) and mixed with the competitor before addition to the well. Incubation with the secondary antibody, goat anti-rabbit IgG-alkaline phosphatase was followed by the substrate, p-nitrophenylphosphate in the color assay and 4-methylumbelliferyl phosphate in the fluorescence assay. Details of the procedure have been published (Santella et al. 1988). High (▽) and low (○) modified DNA in the color assay and high (▼) and low (●) modified DNA in the fluorescence assay.

Antibodies recognizing PAH-DNA adducts have been used in several studies to quantitate adduct levels in humans. Since target tissue (eg, lung, liver) is not available on a routine basis from healthy individuals, white blood cells have been utilized in a number of biomonitoring studies as a surrogate. In collaboration with K. Hemminki, Institute of Occupational Health, Finland, adduct levels were measured in white blood cell DNA from foundry workers and controls (Perera et al. 1988). Workers were classified into high, medium exposure to low exposure to BP based on air monitoring data and an industrial hygenist's evaluation of the job description (Table 3). A dose response relationship was found between adduct levels and exposure. Adducts in these samples were also analyzed by two other laboratories by [^{32}P] postlabeling (Phillips et al. 1988). While adduct levels were lower in the postlabeling assay, there was a good correlation between the immunoassay and postlabeling data. A set of nine foundry workers were sampled after just returning from vacation and again three months later. Adduct levels after vacation were slightly elevated compared to controls (0.39adducts/10^7nucleotide for workers and 0.22/10^7 for controls) but increased dramatically after returning to thework environment. These results suggest a rather rapid repair of adducts over the one month vacation followed by a rise after reexposure.

Adduct levels were also quantitated in white blood cells of Polish coke oven workers, a local control group living near the plant and a rural control group from an unpolluted region of Poland (Table 3). Adducts in local controls were only slightly lower (1.3/10^7 nucleotides) than in coke oven workers (1.5/10^7) but rural controls were comparable to the Finish foundry worker controls. Similar data was obtained by the postlabeling assay (Hemminki et al. in press). Although the levels of BP are about 10-fold higher in the cokery than in the neighboring residential areas, there is only a modest difference in adduct levels between workers and local controls. It is likely that inhalation is only one component of the exposure; ingestion of PAHs in food and skin absorption may also be important.

Table 3. PAH-DNA Adducts In White Blood Cells of Foundry and Coke Oven Workers and Cigarette Smokers and Nonsmokers

	N	mean aducts/ 10^7 nucleotides
Foundry		
High exposure (>0.2ug BP/m^3)[a]	4	5.0
Medium exposure (0.05-0.2ug BP/m^3)	13	2.2
Low exposure (<0.05ug BP/m^3)	18	0.80
Control (<0.01ug BP/m^3)	10	0.22
At work	9	3.08
After vacation	9	0.39
Coke Oven (0.1-50ug BP/m^3)	41	1.5
Local Control (0.015-0.057ug BP/m^3)	15	1.3
Rural Control (<0.01ug BP/m^3)	13	0.23
Smokers		0.15
Nonsmokers		0.12

Data from (Perera et al. 1988, Perera et al. 1987) and unpublished data.
[a] Estimated air levels of BP.

In contrast to these results, several studies on adducts in smokers and nonsmokers have not detected significant differences between these populations. White blood cell DNA adducts were not significantly different in smokers compared to nonsmokers (Table 3) (Perera et al. 1987). Mean adduct levels in placental DNA were higher than in white blood cells but again no difference was seen between smokers ($1.9/10^7$) and nonsmokers ($1.2/10^7$) (Everson et al. 1986, Everson et al. 1988). These results probably reflect the ubiquitous exposure of the general population to PAHs from a number of sources including air pollution and, more importantly, diet. Another factor effecting adduct levels appears to be season of blood collection. We recently found higher adduct levels in blood samples collected during the late summer and early fall than in those collected during the other two seasons (Perera et al. 1989). This seasonal effect is consistent with observations of a peak in aryl hydrocabon hydroxylase (AHH) inducibility during this period (Paigen et al. 1981). While this initial data are from seasonal analysis of samples collected throughout the year, current studies involving the collection of repeat samples from the same individual should provide more conclusive evidence of a seasonal effect.

PAH Exposure in Coal Tar Treated Psoriasis Patients

Topical application of 2-5% crude coal tar followed by skin irradiation with UVB (Goeckerman theraphy) is used as an effective therapy for psoraisis, a hyperproliferative disease of the skin. Coal tars, including pharmaceutical-grade preparations, are complex mixtures of PAHs and well established mutagens and animal carcinogens (IARC 1985). A number of studies have confirmed that occupational exposure to coal tar results in elevated risk of cancer (IARC 1985). Isolated case reports have also suggested that therapeutic coal tar treatment can produce squamous and basal cell carcinomas (reviewed in Bickers 1981).

PAHs are known to be absorbed through the skin after topical application as demonstrated by elevated levels of several PAHs in blood (Storer et al. 1984). Urinary excretion of 1-hydroxypyrene, a fluorescent metabolite of pyrene, has also been used as a marker of internal dose of coal tar in treated psoriasis patients (Clonfero et al. 1989, Jongeneelen et al. 1985). Elevated levels of urinary mutagens in coal tar treated patients have also been found with the Salmonella mutagenesis assay (Clonfero et al. 1989, Wheeler et al. 1981). Recently, elevated levels of mutagens and PAHs in urine as well as sister chromatid exchange (SCE) and chromosomal aberrations in peripheral blood lymphocytes related to exposure level were seen in treated patients (Sato et al. 1989).

We have carried out a small pilot study on blood samples from coal tar treated psoriasis patients and controls with a panel of biomarkers for exposure to genotoxic agents as a model system for skin exposure in the occupational setting. The markers utilized include SCE and micronuclei (MN) in pheripheral lymphocytes, white blood cell PAH-DNA adducts measured by ELISA and [^{32}P] postlabeling and serum antibodies to BPDE-I-DNA adducts. Blood samples were obtained from 22 coal tar treated psoriasis patients and 5 controls. DNA was isolated from white blood cells and PAH-diol epoxide DNA adducts were determined by competitive ELISA. Only one of five controls had a detectable value while 13/22 patients were positive. The mean value (Table 4) for patients was $1.70/10^7$ compared to $0.79/10^7$ for controls. The control value is higher than seen in previous studies on nonoccupationally exposed smokers and nonsmokers ($0.15-0.23/10^7$). This was due to the very high adduct level, comparable to that of foundry workers, seen in the one positive control ($3.3/10^7$), a nurse on the dermatology floor. However, her occupational exposure to coal tar should be minimal and it is not clear what exposures resulted in the high adduct levels. Adducts in this sample were also high by postlabeling. Mean values by [^{32}P] postlabeling are also given in Table 4 and are slightly lower than the ELISA values.

Antibodies recognizing PAH-DNA adducts have been reported in the sera of humans with occupational or environmental exposures (Harris et al. 1985, Weston et al. 1987). To determine if the presence of serum antibodies to carcinogen adducts could serve as a marker of exposure, we determined the number of individuals with detectable levels of serum antibodies against BPDE-I-DNA as described (Harris et al.

1985). No significant difference in positive titer was seen between patients and controls suggesting this assay may not be a marker of exposure. SCE and MN were analyzed in a subset of 17 of the patients and in the 5 controls. Mean SCE levels were higher for patients (9.43) than controls (7.74). No significant difference was seen in MN between patients (13.59) and controls (13.75). We are currently carrying out a larger scale study of patients and controls.

Table 4. Mean Assay Results For Coal Tar Treated Psoriasis Patients and Controls

Assay	Patients	Controls
ELISA		
Mean adducts/10^7nucleotide	1.70+/-3.98	0.79+/-1.43
Range	ND-19	ND-3.3
Number detectables/assayed	13/22	1/5
^{32}P postlabeling		
Mean adducts/10^8nucleotide	0.54+/-1.14	0.35+/-0.50
Range	<0.01-5.5	0.050-1.2
Number detectable/assayed	22	5
Serum Antibodies to BPDE-I-DNA		
Number with positive titer/assayed	13/22	4/5
Sister chromatid exchange		
Average number/metaphase	9.43+/-1.16	7.74+/-0.87
Range	8.30-11.46	6.74-8.72
Number assayed	17	5
Micronuclei		
Average number/1000cells	13.59+/-3.87	13.75+/-5.06
Range	8-21	8-20
Number assayed	17	5

Immunohistochemical Studies on Skin Biopsies

Antibodies to particular carcinogen-DNA adducts can also be utilized to investigate localization of adduct formation in specific cell or tissue types. Indirect immunofluorescence staining with primary antibodies followed by fluorescein isothiocyanate labeled secondary antibodies allows visualization of adducts. We obtained 4mm punch biopsies from several coal tar treated patients as well as from untreated volunteers. Sections were cut and stained with the polyclonal antisera used for quantitating PAH-DNA adducts in white blood cells. To enhance antibody binding, slides were first treated with RNase A, proteinase K and HCl. Staining of skin biopsy sections from coal tar treated psoriasis patients indicated specific nuclear staining in the stratum spinosum and granulosum of the epidermis (Figure 2). Staining was also scattered variably throughout the dermis with some localization to fibroblasts and vessels. No staining was visible in sections from an untreated control.

With the conventional immunofluorescence methods utilized here, quantitative adduct levels cannot be determined. However, they can be estimated by comparison to previous studies carried out in keratinocytes treated in culture with BP. In these studies, adduct levels in the range of $1/10^6$ gave detectable immunofluorescence (Poirier et al. 1982b). This is in the same range as found to result in detectable staining in immunofluorescence studies utilizing antibodies to 8-methoxypsoralen-DNA adducts (Yang et al. 1987).

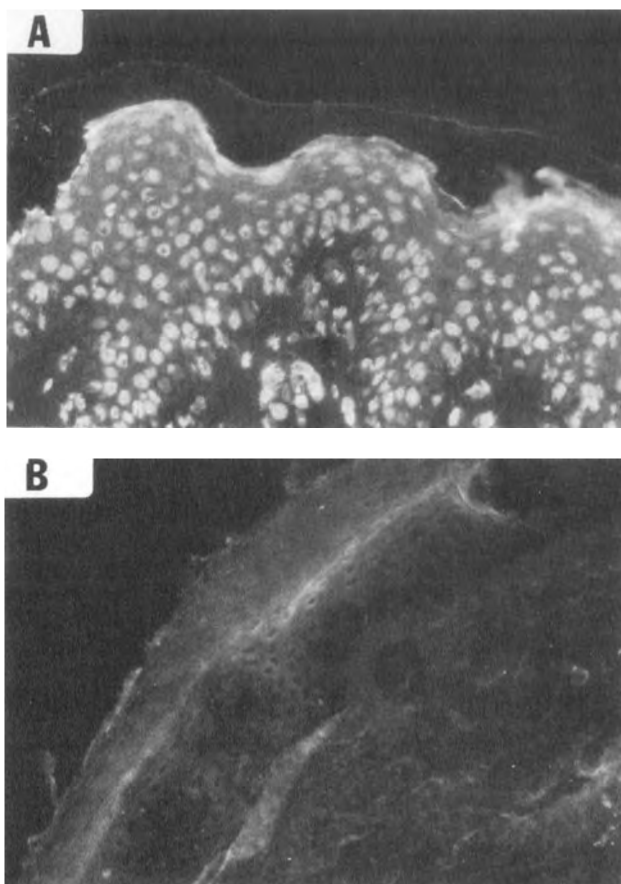

Figure 2. Indirect immunofluorescence staining of human skin biopsies from a patient treated topically with coal tar and a control. Slides were treated with RNase, proteinase K and 4N HCl before staining with antisera #29 (1:50 dilution) and goat anti-rabbit IgG antibody conjugated with fluorescein (1:30). Magnification was 220 fold. a) section from patient; b) section from control.

Computer-assisted video microscopy systems or the use of biotin-streptavidin staining should further increase sensitivity. It may then be possible to utilize these methods for adduct detection in human samples from occupational or environmental exposures. Since adducts can in theory be visualized in single cells, the small amount of material obtained at biopsy could be utilized.

Detection of PAH-Protein Adducts

Measurement of carcinogen-protein adducts has been used as an alternate marker of exposure for several carcinogens. To determine PAH-protein adduct levels, we have utilized a monoclonal antibody, 8E11, developed from animals immunized with BPDE-I-G coupled to bovine serum albumin (Santella et al. 1984). This antibody recognizes BPDE-I modified dG, DNA, and protein as well as BPDE-I-tetrols. More recently, we have further characterized this antibody in terms of crossreactivity with

a number of other BP metabolites, other PAHs and other PAH diol epoxide modified DNAs. 8E11 crossreacts with a number of BP metabolites (Table 5), with higher sensitivity for the diols and weak recognition of phenols. There is some weak crossreactivity with other PAHs including pyrene, aminopyrene and nitropyrene. It also recognizes the diol epoxide adducts of several other PAHs. This antibody will thus recognize a number of PAHs and their metabolites with variable sensitivities.

Table 5. Competitive Inhibition of Antibody 8E11 Binding to BPDE-I-BSA

Competitors	femtomole causing 50% inhibition
BPDE-I-tetrol	350
BPDE-I-BSA digested	400
BPDE-I-BSA nondigested	1450
BP-7,8-diol	250
BP-9,10-diol	150
4-OH-BP	42700
5-OH-BP	$>1x10^5$
BP	6000
1-aminopyrene	70000
1-nitropyrene	16000
Dimetylbenz(a)anthracene	$>1x10^6$
1-OH-pyrene	3400
Pyrene	16200
BPDE-I-DNA	350
Chrysene diol epoxide-DNA	160
Benz(a)anthracene diol epoxide-DNA	1350

Plates were coated with 25ng BPDE-I-bovine serum albumin. Antibody 8E11 was used at a 1:30,000 dilution and mixed with competitor before addition to the plate. Goat anti-mouse IgG-alkaline phosphatase at a 1:500 dilution and p-nitrophenyl phosphate were used for antibody detection. Details of the assay have been published (Lee and Santella 1988).

Direct quantitation of adducts on intact protein cannot be carried out sensitively due to the low affinity of the antibody for the adduct in intact protein. This low sensitivity is probably due to burying of the adduct in hydrophobic regions of the protein. Others have suggested that release of tetrols with acid treatment is a sensitive method for determination of protein adducts (Shugart 1986, Weston et al. 1989). However, our initial studies in mice treated with radiolabeled BP indicated that only low levels of radioactivity could be released from globin by acid treatment (Wallin et al. 1987). For this reason, we used an alternate approach for measurement of protein adducts. Globin was enzymatically digested to peptides and amino acids before ELISA. When tested on protein modified *in vitro* with BPDE-I, a 3-4 fold increase in sensitivity resulted (Table 5). This assay was validated using globin isolated from animals treated with radiolabeled BP. The ELISA was able to detect 90-100% of the adducts measured by radioactivity (Lee and Santella 1988). These animal studies also indicated that adduct levels were about 10 fold higher in albumin than in globin. For this reason, our initial work on human samples has been with albumin isolated from workers occupationally exposed to PAHs. Albumin was isolated by Reactive blue 2-Sepharose CL-4B affinity chromatography and enzymatically digested with insoluble protease coupled to carboxymethyl cellulose which could be easily removed by centrifugation. Samples were then analyzed by competitive ELISA with antibody 8E11. Initial studies have been carried out on a small number of roofers

occupationally exposed during the removal of an old pitch roof and application of new hot asphalt. These studies were carried out in collaboration with Dr. Robin Herbert, Mt. Sinai Medical Center, NY. Seventy percent of the roofers samples were positive with a mean level of 5.4fmol/ug while 62% of the controls had detectible adduct levels (mean of 4.0fmol/ug). In this small number of subjects there was a trend but no significant difference between roofers and controls. However, we are continuing studies of PAH-albumin adducts in a larger sample.

Quantitation of 8-methoxypsoralen and Aflatoxin-DNA Adducts

We have utilized two other antisera recognizing DNA adducts to monitor adducts in humans. 8-methoxypsoralen (8-MOP), a photoactivated drug, is used clinically for the treatment of psoriasis, a hyperproliferative disease of the skin. Patients take the drug orally followed by skin irradiation with UVA. The advantage of working with a clinically used drug, as opposed to environmental carcinogens such as BP where there is ubiquitous exposure, is that individuals with high, well defined exposures as well as control, unexposed individuals can be readily identified. Monoclonal antibodies recognizing both the thymine monoadducts and interstrand cross-linked adducts have been developed (Santella et al. 1985). The antisera has been used in indirect immunofluorescence studies to demonstrate adduct formation in the stratified squamous epithelium of the epidermis of patients immediately after treatment (Yang et al. 1989). No adducts were detectable by ELISA on DNA isolated from the white blood cells of these same patients. This was not surprising since 8-MOP must be photoactivated and the UV dose to the circulating cells is minimal with skin irradiation.

Antibodies have also been developed which recognize the stable imidazole ring opened form of the N7 aflatoxin-DNA adduct. This antibody was used in a pilot study to monitor adduct levels in liver samples obtained from hepatocellular cancer patients in Taiwan (Hsieh et al. 1988). Detectable adducts, some as high as $3/10^6$ nucleotides were found in a number of patients. We are currently expanding these studies to a larger sample size of tumor and nontumor liver tissue from Taiwan and autopsy liver tissue from the US. We hope these studies will provide information on the relationship between aflatoxin exposure, hepatitis carrier status and liver cancer incidence.

Determination of Multiple Adducts

Humans are usually exposed to complex mixtures of environmental carcinogens and multiple adducts may be formed. Because of the limited availability of DNA from white blood cells, we would like to be able to determine multiple adducts in the same sample. Initial work has involved quantitation of adducts in a DNA sample modified in vitro with BPDE-I and 8-MOP. This sample was assayed with a mixture of antibodies recognizing the two different adducts each at the appropriate final dilution for the ELISA. The samples were sequentially incubated first on BPDE-I-DNA coated plates then on 8-MOP-DNA coated plates. Each plate was then incubated with the appropriate alkaline phosphatase conjugate as in the standard assay. When compared to the standard assay for each antibody (Figure 3) no significant difference is seen between detection of adduct on the DNA containing a single adduct or two adducts. Therefore, it may be possible to mix a panel of antibodies recognizing a number of different carcinogen adducts and by sequential incubation on plates with the appropriate antigen coating, quantitate multiple adducts. Finally, since the ELISA does not destroy the adduct, the DNA can be recovered from the microwell and repurified.

These studies demonstrate that immunologic methods have sufficient sensitivity to monitor human exposure to environmental carcinogens. Immunoassays also have the advantage of ease of application to a large number of samples making them ideal for epidemiologic studies. While methods for the determination of DNA adducts in humans provides information about the biologically effective dose of a carcinogen, and can therefore be used as a marker of exposure, information about the relationship of these measurements to risk is unknown. Future epidemiologic studies are needed to provide this information.

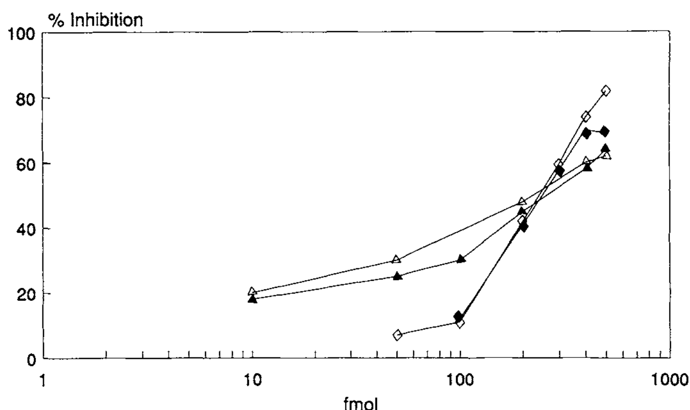

Figure 3. Multiple adduct analysis by competitive ELISA. The competitors were BPDE-I-DNA in the standard assay with antisera #29 (Δ) and 8-MOP-DNA in the standard assay with antibody 8G1 (◊). For the multiple adduct assay, DNA modified by BPDE-I and 8-MOP was mixed with antisera #29 and 8G1 and sequentially incubated on plates coated with BPDE-I-DNA (▲) and 8-MOP-DNA (♦).

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BIOLOGICAL REACTIVE INTERMEDIATES IV

Molecular and Cellular Effects and Their Impact on Human Health

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