Application of Biologic Markers to Studies of Environmental Risks in Children and the Developing Fetus

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Young children and the developing fetus may be more susceptible to effects of environmental toxicants than adults due to differential exposure patterns and developmental immaturities. Biologic markers offer the potential of quantitative dosimeters of biologic dose and/or indices of biologic effect associated with fetal/childhood exposures. They can facilitate evaluation of interindividual variability in response and the magnitude of age-related susceptibilities. Thus far, biologic markers have not been widely used in developmental epidemiology of environmental exposures. Research by our group and others has seen elevations in biologic markers in samples from children and fetal tissue associated with a spectrum of environmental exposures, including tobacco smoke (active and passive), ambient pollution, and dietary contaminants. Studies also suggest that biologic markers can provide powerful dosimeters for investigating reproductive effects. Validation of biologic markers offering the greatest promise for developmental epidemiology is needed. — Environ Health Perspect 103(Suppl 6):105–110 (1995)

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Introduction

There is evidence that young children and the developing fetus may be more susceptible than adults to the adverse effects of many environmental carcinogens and other toxicants. Their increased susceptibility can arise from differential exposure patterns and/or immaturities in physiological development. Infants and young children have higher breathing rates, ingest more drinking water, and consume more calories of food per unit body weight than do adults (*1,2*). As a result, they can have significantly greater intakes per unit body weight of carcinogens and other contaminants in food, water, and air (*1,3*). Physiologic immaturities can also lead to greater absorption, retention, or increased target organ sensitivity, depending on the toxicant (*4,1,5*). For certain carcinogens, risk of cancer has been shown to increase if exposure begins *in utero* or infancy rather than in adulthood (*6,7*). The increased susceptibility is due presumably to the increased rate of cell proliferation, differing metabolic capabilities during early development, and the long future life during which cancers initiated in childhood can develop (*8,6,9–11*).

While increased susceptibility of the young is of concern, age-related differences in response to environmental toxicants have not been well characterized. Epidemiologic studies quantifying effects of environmental exposures during infancy and childhood are limited (*3*) and have been hampered by uncertainties regarding the extent and timing of exposure. Human data are currently available for only a few toxicants, most notably lead (*12–14*). Exposures in carcinogenicity bioassays typically occur after maturation of the test animals is largely completed (*15*). In the case of developmental bioassays, in which exposure begins *in utero* or during infancy, extrapolation of results from animals to humans can be compromised by interspecies differences in developmental patterns and growth rates (*3*).

The use of biologic markers in molecular epidemiologic studies of fetal and childhood exposures can circumvent some of these limitations. To date, biologic markers have not been widely used in developmental epidemiology of environmental exposures (*16,17*); however, they offer the potential to provide quantitative dosimeters of biologic dose and/or indices of biologic effect associated with fetal and childhood exposures to environmental carcinogens and other toxicants. Further, they can facilitate quantification of differences in the magnitude of response in the young relative to that in adults. The following review provides an overview of biologic markers used in human studies. Most have involved adult populations with a variety of environmental exposures. The term environmental is broadly defined to include lifestyle (cigarette smoke), occupation, and ambient pollution. Collectively, they demonstrate the sensitivity of a number of biologic markers to environmental toxicants and support their applicability to developmental studies. The review concludes with examples from our group and others of research incorporating biologic markers into studies of environmental exposures during fetal development and early childhood.

Overview of Biologic Markers*

Molecular epidemiology bridges from basic research in molecular biology to studies of disease causation in humans by combining laboratory measurements of internal dose, biologically effective dose, biologic effect, and susceptibility with epidemiologic methodologies (*18–20*). Research has shown

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marked elevations in biologic markers associated with a spectrum of exposures and has demonstrated the ability of multiple biologic markers to detect genetic and molecular damage in humans. Case–control studies in patient populations also suggest that specific biologic markers may indicate heightened risk of disease.

Internal dose refers to the measurement of the amount of a toxicant or its metabolite present in cells, tissues, or body fluids. Examples of internal dosimeters include DDT and PCBs in serum and adipose tissue from environmental contamination, plasma, or salivary cotinine from cigarette smoking, urinary aflatoxin indicative of dietary exposure, and lead from various exposures. Examples from our group include the findings of increased urinary 1-hydroxypyrene levels associated with ambient exposures to polycyclic aromatic hydrocarbons (PAH) in Finnish foundry workers and dermal exposures in coal-tar-treated psoriasis patients (*21,22*). Internal dose markers take into account individual differences in absorption or bioaccumulation of the compound in question and have the advantage of being comparatively easy to monitor. However, they do not provide data about interactions of the compound with critical cellular targets.

Biologically effective dose reflects the amount of toxicant that has interacted with cellular macromolecules at a target site or with an established surrogate. Examples include levels of specific carcinogens bound to genetic material (carcinogen–DNA adducts) or an established surrogate (carcinogen–protein adducts). This class of markers is more mechanistically relevant to disease than internal dose, since it takes into account differences in metabolism (activation vs detoxification) of the chemical in question as well as the extent of repair of DNA adducts. The biological basis for measuring DNA adducts derives from extensive experimental data supporting their role in the initiation and possibly the progression of cancer. Adducts formed between DNA and PAHs as well as other carcinogens have been correlated with carcinogenic potency in experimental studies and are therefore considered to be a relevant indicator of the effective dose and potential risk of carcinogens. There is evidence that protein adducts can serve as surrogates for DNA adducts.

Numerous studies have shown PAH–DNA and other carcinogen–DNA adducts in peripheral white blood cells (WBC) of workers to be associated with occupational exposures, including foundry workers, roofers, coke oven workers, and aluminum plant workers (*23–27*). Carcinogen–DNA adduct levels in WBC have also been associated with ambient air pollution exposures in Poland (*28*). Dietary exposures to aflatoxin have been correlated to urinary excretion of aflatoxin–DNA adducts (aflatoxin-N^7-guanine) in populations studied in China and West Africa (*29,30*). Some, but not all, studies have seen increases in carcinogen–DNA adducts in WBC of cigarette smokers compared to that in nonsmokers (*31–35*). In lung tissue, a linear relationship between DNA adduct levels and daily or lifetime cigarette consumption was seen (*36*). Adducts formed between 4-aminobiphenyl and hemoglobin (4-ABP-Hb) are also significantly elevated in smokers compared to nonsmokers (*37,38*). In a case–control study of lung cancer by our group, a significant association was seen between lung cancer risk and PAH–DNA adduct formation among current smokers after controlling for the number of cigarettes smoked per day (*32*). These findings suggest that adducts are not only an environmentally relevant dosimeter, but that they may also indicate heightened risk of cancer.

Biologic effect markers reflect irreversible damage resulting from a toxic interaction, either at the target or an analogous site, which is known or believed to be pathogenically linked to disease. A wide variety of biologic markers fall into this category, including gene mutations at the *hprt* and glycophorin A (GPA) loci, alterations in oncogenes and tumor suppressor genes, DNA single-strand breaks, unscheduled DNA synthesis, sister chromatid exchanges (SCE), chromosomal aberrations (CA), and micronuclei. None of these markers is chemical- or exposure-specific, and other factors (lifestyle and environmental) that affect these end points can act as confounding variables in a molecular epidemiologic study.

Our research has found increases in *hprt* gene mutations in lymphocytes associated with occupational exposures to PAHs among Finnish foundry workers. Consistent with experimental data, the frequency of mutation was significantly correlated with levels of PAH–DNA adducts (*39*). In another study by our group, ambient air pollution in Poland was found to be significantly related to SCE and CA, including gaps (*28*). Further, PAH–DNA adducts were significantly correlated with chromosomal mutation, linking molecular dose with a genetic effect of air pollution. Research on liver cancer patients in China and Southern Africa has implicated AFB_1 as an etiologic agent and indicated a possible mechanism by which the carcinogen may be exerting its effect (*40*). A total of 26 liver tumors were obtained from patients living in areas characterized by high exposure to AFB_1 and by high prevalence of liver cancer (*41,42*). Eleven (43%) of the tumors exhibited a specific mutation (G to T) at codon 249 of the tumor suppressor gene, *p53*. This signal mutation is produced by AFB_1 when administered to experimental animals.

Susceptibility markers measure individual differences that can modulate response to exposure. These include individual differences in micronutrient levels, DNA repair capacity, inherited mutations, and detoxification mechanisms. Examples include individual variability in "Phase I" and "Phase II" enzyme activities. The normal role of Phase I enzymes is to convert lipid-soluble xenobiotics to more water-soluble substances that can be excreted. However, some of the intermediates in this oxidative process are highly reactive electrophiles capable of binding to DNA. CYP1A1, a P450 enzyme with aryl hydrocarbon hydroxylase activity, catalyzes the oxidation of PAHs such as benzo[*a*]pyrene. This enzyme system is highly responsive to exposures to PAHs and other agents, and inducibility has been associated with higher risk of lung cancer in smokers (*43*). An MspI RFLP in the 3′ coding region of the *CYP1A1* gene (associated with a mutation in exon 7 of the gene) has been associated with lung cancer risk in Japan (*44,45*). Phase II enzymes conjugate the phase I metabolites with glucuronide, glutathione, or sulfate, resulting in less reactive, hydrophilic products for excretion. Glutathione-*S*-transferases (GST) are a family of multifunctional proteins that play an important role in the detoxification of PAHs and other xenobiotics through conjugation with glutathione (*46,47*). A polymorphism has been detected in the *GSTM1* gene, which has been shown to be a deletion of the entire gene locus. Published reports indicate that 30 to 60% of the population may be homozygous deleted for this gene (*48*). Smokers with low lymphocyte *GSTM1* activity are reported to be at higher risk for lung cancer (*49,50*). More recently, in a cancer case–control study in Japan, a relative risk of 1.87 was seen for the null genotype and

squamous cell carcinoma of the lung (*45*). A remarkably high relative risk of 9.1 for squamous cell carcinoma was seen with the combined CYP1A1 exon 7 mutation and GSTM1 null genotypes.

Application of Biologic Markers to Developmental Epidemiology

Thus far, biologic markers have not been used widely in studies of developmental effects associated with environmental exposures. Further, studies incorporating biologic markers generally have not related them to disease outcomes such as birth defects or childhood cancers in infants and children. Rather, biologic markers have primarily been relied on as dosimeters for fetal and childhood exposure. Research by our group and others has seen elevation in biologic markers in samples from children and in fetal tissue associated with a spectrum of environmental exposures including tobacco smoke (active and passive), dietary contaminants, and ambient pollution.

Since evidence suggests that children may be at heightened risk of cancer from environmental tobacco smoke (ETS) exposure during early childhood (*51*), we initiated a study using a panel of biologic markers to assess ETS exposure in mothers and their preschool-age children (2–5 years) (*52*). A number of markers, including serum cotinine (a metabolite of nicotine) and PAH–albumin adducts, were evaluated in peripheral blood samples from 87 African–American and Hispanic mother–child pairs. Children of smoking mothers had significantly higher levels of both cotinine and PAH–albumin than children of nonsmoking mothers. Their cotinine was significantly correlated with numbers of cigarettes smoked daily by the mother. Cotinine was also markedly elevated in children of nonsmoking mothers who were exposed to ETS from other household smoking. These results underscore the importance of programs aimed at smoking prevention among mothers and women of childbearing age.

Monitoring of PAH–DNA and other carcinogen–DNA adducts in placental tissue of smokers and nonsmokers has also demonstrated increases related to cigarette smoking (*53–56*). Hansen et al. (*55*) found carcinogen–DNA adducts in both placental tissue and umbilical cord DNA to be associated with smoking. Adduct levels in the placenta of both smokers and nonsmokers were higher than corresponding adduct levels in umbilical cord DNA.

Coghlin et al. (*57*) measured levels of 4-ABP-Hb adducts in paired maternal blood–fetal cord blood samples from smoking and nonsmoking women. Adduct levels in both maternal and fetal samples were significantly associated with cigarette smoke exposure. Fetal adduct levels were consistently lower than maternal levels.

A recently initiated investigation by our group is using a panel of biologic markers to assess effects of smoking, diet, and ambient air pollution on women and the developing fetus. Markers are being assayed in placental tissue samples and maternal and infant cord blood samples collected from 73 mother–newborn pairs residing in Kraków, Poland, an industrial city with severe ambient air pollution, and 90 mother–newborn pairs from a rural town in Poland with lower pollution levels. Preliminary results show CYP1A1 mRNA levels in placental tissue and PAH–DNA adduct levels in maternal WBC to be significantly increased in current smokers compared to nonsmokers (*58*). Adduct levels in maternal WBC were also significantly associated with ETS exposure. Dietary consumption of smoked and fried meats, cheese, and fish was a significant determinant of placental CYP1A1 mRNA levels, presumably as a result of PAHs formed during cooking. A dose response in both placental CYP1A1 mRNA levels and maternal PAH–DNA adduct levels with ambient pollution was apparent. Unlike the Coghlin et al. findings of lower adduct levels in fetal compared to maternal samples, in the current study levels of both serum cotinine and PAH–DNA adducts in infant cord blood samples were higher than those in the corresponding maternal blood samples. These findings suggest a reduced ability of the fetus relative to that of the mother to detoxify cigarette smoke constituents. They also point to the possibility of increased susceptibility of the developing fetus to PAH–DNA adduct formation. This is particularly striking, given evidence from experimental bioassays that transplacental exposure to PAHs is an order of magnitude or more lower than maternal exposure (*59,60*).

In a series of studies, Wild et al. (*61,62*) have investigated dietary exposure to aflatoxin B_1 by measuring AFB_1–albumin adducts in blood samples from residents of Gambia. Umbilical cord blood and maternal blood were collected from 30 mother–newborn pairs, and blood was also collected from 323 children (ages 3–8). Adducts were present in 97% of maternal sera, 70% of umbilical cord sera, and over 95% of children's sera, indicating widespread dietary exposure to aflatoxin in this region and transplacental transfer of the carcinogen during pregnancy. Cord adduct levels were highly correlated with, but 10-fold lower than, maternal levels. Interestingly, children who were positive for the hepatitis B virus (HBV) surface antigen had significantly higher adduct levels than did children who had never been infected or who had markers of past infection. These data suggest that HBV infection can influence aflatoxin metabolism.

Polycyclic aromatic hydrocarbons are present in urban and industrial air pollution as well as in cigarette smoke and diet. PAH–DNA adduct levels have been assessed in human fetal tissues and placentas from 15 spontaneous abortions collected from nonsmoking women (*63*). Adducts were detected in 42% of fetal lung specimens, 27% of fetal liver specimens, and 43% of placentas. In the cases in which adducts were present in both placenta and fetal tissue, fetal tissue levels were not appreciably lower than the corresponding placental values. These results demonstrate that tissues of the developing human fetus are targets for DNA damage from ubiquitous exposures like that from PAHs.

A study using biologic markers to monitor exposure from an industrial waste site in Belgium found levels of SCEs (including high frequency cells) to be significantly higher in blood samples from a small number of children living near the site than in samples from matched controls (*64*). Air contamination by a mixture of genotoxics emitted from the site were presumed responsible for these cytogenetic effects.

In addition to using biologic markers as dosimeters of fetal and childhood exposure, several studies have shown associations between specific biologic markers and reproductive effects. A highly significant association was found between decreased birth weight and the level of smoking-related adducts in placental tissue collected from only 30 smoking mothers (*53*). By contrast, no association was seen between decreased birth weight and either intensity of smoking exposure assessed by questionnaire data or biochemical measures of smoking exposure (cotinine, thiocyanate, and carboxyhemoglobin). Several other studies have used umbilical cord blood serum cotinine levels from nonsmoking mothers as an internal dosimeter of fetal

ETS exposure. These studies found a significant inverse correlation between serum cotinine levels and birth weight (*65,66*).

Conclusion

This body of research has demonstrated that laboratory methods for detection of carcinogen–DNA adducts and other biologic markers of effect and susceptibility are adequately sensitive for studies of environmental exposures in human populations. Research on exposures during fetal development and childhood indicates that biologic markers can provide dosimeters of environmental exposures and tools for evaluating interindividual variability in response and age-related susceptibilities. However, incorporation of biologic markers into developmental epidemiology has been limited to only a few markers and to studies of small sample size. Validation of markers offering the greatest promise for developmental epidemiology is needed. Criteria for validation should include low-dose sensitivity and reproducibility of the assay as well as exposure specificity. Biologic markers can integrate exposure via multiple routes (inhalation, oral, dermal), multiple sources (ambient and indoor air, workplace air, cigarette smoke, diet, drinking water), and across all patterns of exposure (past, current, intermittent, continuous). This is an advantage, since risks can be assumed to be additive. However, it is also a disadvantage in that many environmental chemicals are ubiquitous in the environment and it is difficult to distinguish the effect of any particular exposure source. Markers vary greatly with respect to source specificity; for example, 4-aminobiphenyl-hemoglobin (4-ABP-Hb) has far fewer noncigarette-smoking-related background sources than ethylene oxide (EtO), PAHs, and *N*-nitroso compounds. The extent to which the marker will document specific time periods of exposure will depend upon the pharmacokinetics of the chemical and the persistence of the marker in the biologic sample assayed (itself a function of the turnover rate of the sample and repair processes). The feasibility of the marker should be determined. That is, how acceptable is it to the public, how cost-effective, and how stable in stored samples? Finally, because of their ability to provide information on gene–environment interactions within individuals, the potential for misuse of biomonitoring data, leading to discrimination related to employment or insurance, cannot be ignored (*20*). This review summarizes early and recent validation research. However, before applying methods in larger scale developmental epidemiologic studies, it is imperative that guidelines be developed to protect confidentiality and to guard against misuse of data.

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