Role of Biomarkers in Identifying and Understanding Environmentally Induced Disease

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Establishing associations between environmental agents and disease presents challenges to both epidemiologists and toxicologists, particularly in cases of complex gene-environment interactions and when there is a long latency between exposure and disease. Biologic markers, physiological signals that reflect exposure, early cellular response, or inherent or acquired susceptibilities, provide a new strategy for resolving some of these problems. Biomarker research assumes that toxicant-induced diseases are progressive and that injury proceeds from entry of the toxicant into target cells, which induces subcellular biochemical events, to cell- and organ-level events that eventually induce irreversible or persistent organism dysfunction. The epidemiologic value of a biomarker lies in its ability to predict backward toward exposure and forward toward risk of clinical outcome, which is largely unknown. Research in mechanistic toxicology will advance the range of useful biomarkers in epidemiology and clinical medicine.

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Chronic diseases such as cancer, heart disease, neurological disorders, and respiratory afflictions have, with the exception of the acquired immunodeficiency syndrome, replaced infectious diseases as the principal causes of deaths and illness in developed countries today. Because patterns and rates of these chronic diseases vary between and within countries, it is believed that a significant portion are related to environmental factors and, as such, may be preventable. Genetics, host factors, and environmental factors, broadly conceived, are major determinants of health. Environmentally induced disease may be generally defined to include all conditions that are acquired and are not solely the consequence of heritable genetic mutations. These categories may be difficult to distinguish in all cases because environmental exposures, such as radiation, can cause mutations in the germ cell that may be transmitted to offspring and because “not all that is familial is inherited” (1).

Environmentally induced diseases may be considered as adverse events reflecting the consequences of physical, chemical, biological, or physical exposures in individuals and populations. In any given population, the extent of such disease can be assessed relative to the normal range of phenotypic responses defined genetically. Because of the multiplicity of causes and diversity of types of chronic diseases, it is difficult to identify specific etiologic factors and apportion relative causality or risk. Moreover, many chronic diseases have a long latency between induction and clinically detectable expression. These characteristics compound the problems of understanding the links between environmental factors and disease.

For instance, with breast cancer, both acquired and hereditary genetic factors clearly play a pivotal role in current patterns of this disease. Inherited genetic factors are more important for younger women because hereditary factors account for about a third of breast cancer cases in women aged 20–29 years, but only 1% of cases in women >80 years (2). A recent population study suggests that about 6 women per 1000 with breast cancer carry an inherited breast cancer-predisposing mutation in a series of codons on chromosome 17 (3). Since only a small number of all cases of breast cancer in the population reflect inherited traits (1, 4), most cases must be the product of gene–environment interactions that induce mutations or other events in carcinogenesis. Wolff et al. found that women whose serum concentrations of the DDT metabolite DDE were in the highest percentile had more than a fourfold excess risk of breast cancer compared with those in the bottom 10% of exposure (5).\textsuperscript{4} It has recently been hypothesized that, rather than being due to lifetime exposure to endogenous estrogens, a substantial proportion of breast cancer cases could be due to exposure to compounds such as DDE or other lipophilic xeno-estrogens, other environmental hormones, or other environmental carcinogens that affect pathways of hormonal metabolism (6). This hypothesis suggests that biomarkers of exposure and other gene–environmental interactions should be studied so that environmental factors that influence gene

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\textsuperscript{4} Nonstandard abbreviations: DDT, 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane; DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene; ALA, 5-aminolevulinic acid; PB, blood lead; PCBs, polychlorinated biphenyls; EROD, ethoxyresorufin-O-demethylase; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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expression can be identified and, when appropriate, recommended for avoidance.

Biomarkers: Concepts and Definitions

Over the past decade, the concept of biomarkers has been developed to assess the relationship between environmental exposures and subsequent disease in individuals and groups (7). Better characterization of exposure increases the sensitivity of studies on environmental health outcomes. Thus, the practical goal of biomarker research and application is to prevent disease by reducing exposures to hazardous agents through the early identification of exposure and response. This strategy assumes that toxicity progresses from early subcellular events to severe disease, and that each step in the progression increases the likelihood of the next step.

Biomarkers, defined as signaling events in biological systems, can sometimes be identified at the molecular or subcellular level (8). Biomarkers can be conceptualized, as shown in Fig. 1 (9), and defined in three operational classes: exposure, effect (or response), and susceptibility. Here we review some problems in the validation of biomarkers of exposure in the study of environmental health, discuss some limits of the developing field, and identify some important research opportunities in this growing area.

Within the three broad classes of markers, there is considerable flexibility of definition. The same signal can be used as a marker of exposure, effect, or susceptibility, depending on the purpose and design of an epidemiological or toxicological study. For example, the enzyme δ-aminolevulinic acid dehydrase (ALAD) has been widely used as a biomarker of exposure to lead (9, 10). ALAD inhibition is also an effect or response to lead on heme biosynthesis, and its significance depends on its role in the mechanism of cell or organ toxicity [e.g., cellular anoxia, anemia, altered hepatic drug metabolism, or central nervous system (CNS) toxicity] (11, 12). ALAD may also be a marker of susceptibility, because it is heterozygously expressed in human populations and differing alleles for the ALAD gene may confer differing sensitivity to lead (13, 14).

The identification of early and sensitive biomarkers of exposure allows the development of strategies to prevent cell damage that results in persistent or irreversible injury. The use of erythrocyte protoporphyrin (EP) concentrations to indicate blood lead concentrations (PbB) illustrates this concept. In the 1970s, EP constituted a valuable and reliable biomarker of exposure to PbB > 30 μg/dL because elevations in EP could reliably indicate such exposure (9). By 1985, public health consensus was that PbB ≥ 20 μg/dL was toxic, making EP useless as a biomarker because it could not be detected at these lower lead concentrations. Other indicators of cellular perturbation uniquely associated with these lower exposures to lead are therefore needed.

Biomarkers of Exposure

Exposure assessment is one of the most difficult tasks in environmental and occupational epidemiology. Fundamental to environmental health investigations is the determination of some relationship between exposure to a given toxicant and the dose required to elicit a particular health effect. The ability to assess dose accurately varies according to the type of study. In highly controlled studies, exposure is precisely limited although, even in such studies, absorbed dose at the target tissue varies with individual differences, nutritional factors, and genetic susceptibility. In epidemiologic studies, dose is often inferred from questionnaires or reconstructed from historic records. In both of these types of studies, measurement of biomarkers of exposure substantially improves the assessment of exposure.

In environmental health research, we often require complex exposure information: what exposures occurred in the past; what was or is the duration and rate of exposure; what are the internal doses, or exposures at critical target sites; and what factors may alter the internal distribution of absorbed exposures. Standard methods of exposure assessment in epidemiologic studies include querying subjects by questionnaire, monitoring the environment and (or) food and water, modeling environmental pathways and routes of exposures from sources to human receptors, personal monitoring, and total exposure monitoring (15, 16). However, these methods may not provide accurate information on the actual absorbed dose for an individual or group, because of the important unmeasured variables of personal behavior and those genetic and other factors that modulate absorption and metabolism. Nevertheless, these standard exposure assessment methods remain essential for two reasons: first, to validate biomarkers of exposures and, second, to estimate likely dose rates and

Fig. 1. Simplified flow chart of classes of biological markers (boxes). Solid lines indicate progression, if it occurs, to the next class of marker. Dashed lines indicate that individual susceptibility influences the rates of progression, as do other variables described in the text. Biological markers represent a continuum of changes, and the classification of change may not always be distinct. Source: Committee on Biological Markers of the National Research Council, 1987.
to obtain other information not measurable with biomarkers.

When biomarkers of exposure are not available, or if exposure is improperly assessed, misclassification of individuals or groups can occur. Studies based on inaccurate exposure assessment or on poorly designed exposure surrogates tend to be biased toward type 2 errors, leading to acceptance of the null hypothesis, that is, a finding of no effect when one actually does exist.

Significant advances have been made in developing and validating biomarkers of exposure. Validation in this context primarily entails backward extrapolation, from the biomarker to other indices of exposure. Several types of exposure biomarkers have been reported: DNA and protein adducts as biomarkers of exposure to occupational and environmental carcinogens (17, 18), neurotoxic esterase as a marker of organophosphate exposure (19), serum dioxin levels as biomarkers of adipose tissue uptake and storage (20), urinary small molecular weight proteins as markers of exposure to lead and cadmium (21–23), and lipid and serum organochlorine levels (24–25).

The interpretation of these signals as markers of effect or risk can only be made prospectively. At present, they have been shown through retrospective or concurrent validation to be useful in increasing and refining our knowledge of exposure. To the extent that we understand the role of these events in pathophysiology [e.g., microglobulins and kidney function (26)], we may be able to interpret these biomarkers as predictive of risk and use them to guide regulations and other risk reduction measures (such as removal of exposed workers).

Limits

Biomarkers are limited by the following constraints, some of which are amenable to change through research: lack of knowledge of the target organ system and mechanisms of toxic response, lack of access to relevant biological events, lack of knowledge of toxicokinetics, lack of sufficient sensitivity and specificity, and inherent or interindividual variability in the signal. These latter constraints lead to difficulties in determining baseline or normal levels of exposure.

Access. Ethical and practical considerations limit access to compartments in humans where many biological events occur. Particularly in the absence of disease, individuals may be reluctant to submit to invasive sampling. The following compartments are often measured for biomarkers: urine, hair, saliva, skin, exhaled air, seminal fluid and sperm, blood (plasma, serum, lipids, erythrocytes, lymphocytes, platelets), bone, subcutaneous fat biopsy, and cerebrospinal fluid. Some limits should be noted: less than quantitative 24-h urine collections may give misleading information on certain biomarkers if these are diurnally secreted; external contamination may compromise the validity of hair analyses; sperm count is known to vary with frequency of ejaculation; and the complexity of the blood compartment needs to be considered. Concentrations of biomarkers are often adjusted or normalized on a lipid or red cell basis for blood, or on the basis of creatinine for urine. However, if exposure is affecting these parameters—lead-reducing hematocrit, PCBs increasing circulating lipid levels, or mercury damaging the kidney—then “normalization” may obscure both exposure and effect markers.

The CNS is one of the least accessible systems for assessing exposure and response. Although magnetic resonance imaging and positron emission tomography allow real-time visualization of the brain, their use in toxicology and epidemiology is limited, as demonstrated by recent studies of solvent-exposed workers (27, 28). Biochemical signals of brain function, such as neurotransmitter metabolites, can be detected in the remote compartments of urine, saliva, plasma and blood, but interpretation is limited by the likelihood of significant processing and the contribution of other tissues (such as gut, liver, platelets, or adrenals) (18).

Toxicokinetics. Both the selection and interpretation of exposure biomarkers depends upon an adequate understanding of toxicokinetics. The distribution of lead varies between blood, soft tissue, and mineralized tissue (29). The half-life of lead in blood is about 30 days, and less than 5% of the total body burden of lead is found in blood at steady state. The half-life of lead in bone is between 5 and 30 years, and more than 90% of the total body burden of lead occurs in bone at steady state. Thus for cross-sectional studies of the sequelae of chronic lead exposure, measurement of lead in teeth or bone is appropriate, as shown by Needleman et al. (30), while for prospective studies, measurement of lead in blood is appropriate (10, 31). Only when we understand the relations among compartments is it appropriate to use measurements in one to infer concentrations in the other.

Usually our knowledge is less than complete. Although blood concentrations of organochlorines are often used to infer adipose tissue stores and past exposure (5, 20, 24), the data on human TCDD toxicokinetics, for instance, are actually quite limited, and estimated half-lives range from <1 year to >30 years (32).

Intercurrent events. Both normal and pathophysiological events may alter the meaning of exposure biomarkers through effects on toxicokinetics and metabolism. Pregnancy, lactation, and menopause have a significant impact on the long-term storage depots of adipose tissue and bone. Unless metabolic information is collected on these events, which may occur between exposure and measurement, it is possible to misinterpret biomarker signals. For instance, bone demineralization will mobilize lead from bone stores to blood such that women after menopause have higher blood lead concentrations compared with men of the same age (22, 33). This age-related trend is unlikely to be due to increased external exposures of postmenopausal women; however, without knowledge of the influence of altered mineral metabolism on lead toxicokinetics, this would be the most plausible interpretation of the data.

Women who have lactated for long periods have lower concentrations of lipophilic organochlorine compared with those who have not lactated (5). Weight loss or
gain can change body burdens of stable lipophilic chemicals such as PCBs, dioxins, and DDT metabolites. Thus the interpretation of measured concentrations of these chemicals, particularly in case-control studies, must take into account possible effects of cachexia in cancer, lactation, or weight gain induced by steroid pharmacotherapies.

Variability. Biomarkers may vary within and among individuals for several reasons: diurnal or other patterns of events; effects of diet or age; undetected exposures; genetic variation that may not be related to susceptibility. Undetected exposures to the same or a similarly acting toxinant may induce changes in the biomarker; if the cause is not identified, it may be incorrectly inferred that there is a "background" level of the event. Exposure to cigarette smoking may be universal in many societies; as suggested by the NHANES III monitoring of cotinine, a biomarker of nicotine exposure (34). Thus DNA or hemoglobin adducts detected in nonsmokers (17) may reflect this exposure rather than an endogenous rate of mutation.

There are genetic variants in some biomarkers. In addition to the ALAD heterozygosity mentioned previously, there is a range of responsiveness of the CYP450 IA1 enzymes to xenobiotic inducers. For example, human lymphocyte EROD activity varies several-fold in response to in vitro TCDD (35). In the absence of information on the state of biomarkers prior to exposure, it may be difficult to interpret them. In some cases, we can exclude genetic determinants between the marker and exposure. In a case report of TCDD intoxication, by studying family members it was possible to rule out hereditary porphyria as the cause of abnormal patterns of urinary porphyrins (36).

Research Needs and Goals

Biomarkers hold great promise in improving our techniques of exposure assessment. Inferences from external monitoring are limited by the range of human interactions with the external environment and by individual factors governing absorption and metabolism (16). Biomarkers can refine risk assessments by making available information that can be more closely related to internal dose and to concentrations at sites of toxic action. Biomarkers may assist in identifying persons at increased risk of toxicity because of increased exposure or sensitivity. Our response to this information must be considered in the broad context of public health goals to prevent disease while respecting the individual's rights to privacy and employment.

The most important need in biomarker research is validation. Validation of exposure markers is generally retrospective, through correlations of biomarkers with other information on exposure. For this purpose, the collection of comprehensive information on environmental concentrations, job and residential history, and personal monitoring will continue to be important. Biomarkers cannot substitute for careful exposure assessment by the standard methods of environmental and occupational epidemiology.

Only as we understand the toxicokinetics and mechanisms of action of specific toxants will we be able to identify new biomarkers that truly assist us in the prevention of environmental disease. Experimental research will thus remain an important component of public health research, because it permits the assessment of biomarkers in controlled experimental systems that can sometimes be validated in human studies. New techniques will facilitate the measurement of molecular biomarkers in tissue so that we can better determine the relations between markers of exposure and the health effects with which they are ultimately linked.

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