Significance of Individual Sensitivity to Chemicals: Elucidation of Host Susceptibility by Use of Biomarkers in Environmental Health Research

Mark R. Cullen⁠�and Carrie A. Redlich

Biomarker research has become the predominant theme for study of human dose–host-response relations to environmental chemicals. Increasing interest has been focused on identifying markers for host susceptibility, with mixed results. Efforts to identify markers for host variability in carcinogenic risk, on the basis of theoretical knowledge of carcinogen metabolism, have been disappointing. New work in the area of acquired risk modifiers, such as nutritional status, is theoretically attractive, but results have been limited. Impressions of achievement have been made in the area of immunological variability, which may elucidate the molecular basis of as well as provide practical biomarkers for several diseases. The problem of multiple chemical sensitivities, on the other hand, has proved refractory to biomarker research, reflecting inadequate knowledge of the mechanism and inappropriate application of biomarker methods.

Indexing Terms: epidemiology/relative risk/carcinogens

The incorporation of biological markers into environmental health research ~15 years ago marked a turning point for our discipline, driven by the growing frustration of occupational and environmental epidemiologists with the limitations of population studies that depend on traditional external methods for classifying exposure and on limited methodology for assessing health outcomes, typically relatively crude and late measurements of effects such as mortality, cancer incidence, or field tests such as spirometry or questionnaires. Only very late effects with very high relative risks related to exposure could be deciphered in the face of inevitable misclassification and the inherent biases of observational research. The growing explosion of advances in biotechnology emerging from toxicological experiments and from many other medical disciplines unrelated to environmental health provided the opportunity for revolutionary approaches (1).

At first, the major focus of interest was on the biomarkers of exposure, borrowing concepts and techniques from pharmacology, which in the previous decade had begun to move from external estimates of dose to measurements of internal, biological, and ultimately target-site, i.e., specific-receptor dose. Thus, one could reduce misclassification based on exposure estimate and gain simultaneous new insights into mechanisms. Concerted efforts to measure carcinogens at the molecular level, e.g., DNA adducts, occupied a substantial fraction of early biomarker work.

In parallel, earlier, more quantitative, and more sensitive endpoints for etiological studies were sought. Again, with resources from advancing technologies in cytogenetics, pathology, and immunology, extensive studies were conducted on such markers as lymphocyte sister-chromatid exchanges, micronuclei in epithelial tissue, and other changes deemed likely to represent genome damage. Data on confirmed carcinogens such as ionizing radiation and tobacco have demonstrated the theoretical basis for such efforts and documented the potential for meaningful dose–response modeling in the future. However, these types of endpoints are still too nonspecific for application to new hazards of uncertain human carcinogenic potential. Recent work focusing on more specific early-effect markers such as certain oncogenes or tumor suppressor genes has substantial promise as demonstrated by recent work on aflatoxin (2) and on early lung cancer in asbestos workers (3). As with the molecular markers of exposure, such studies also enhanced mechanistic insight. Perhaps more important, some of these efforts may lead to important practical benefits such as earlier diagnostic tests.

It was only a matter of time before the same approach was turned towards the third and in many ways most perplexing element of the dose–host-response relation, the host factor. Although it is obvious that the nonhomogeneity of human populations renders precarious the establishment of a single dose–response curve for a population, the lack of simple strategies for classifying subjects by underlying risk allowed no choice but the usual assumption of a single curve, in essence introducing yet another form of misclassification limiting etiological research. The theory of biomarker research and the advances in cellular and molecular biology have led to an upsurge in interest in host susceptibility factors (Table 1), composing the focus of our discussion. The aim is to elucidate some of the special issues that have arisen in this area from both research and practical perspectives.

Pharmacogenetic Model: Theoretical Basis and Potential Limitations

Given the fundamental role of metabolism in toxicological research and the accomplishments of pharmacologists in deciphering host variability in drug metabolism, initial biomarker research into host factors has been directed at the identification of interindividual differences in metabolic pathways. A wide range of
enzymes known or assumed to be important toxicologically have been explored, demonstrating substantial differences in levels of activity within the population, such as N-acetyltransferase, several cytochromes P-450, and glutathione transferase. Each of these has a potential role in the activation or detoxification of one or more potent carcinogens or other chemical exposures; theoretically, variation in expression could account for differing host susceptibilities to the effects of the carcinogens. As the genetic loci of these and other metabolic enzymes have been determined, the identification of polymorphisms and therefore the ability for distinguishing genotypic as well as phenotypic differences in the population have become possible. The results of this work have yet to explain consistent differences in cancer risk that theory might predict, although newer methods will allow study of a wider array of polymorphisms. Part of the problem appears to be identifying adequate populations of individuals with known exposures to carcinogens of interest and established health outcomes, such as cancer. However, even where study groups have been available, differences in risk between high- and low-risk genotypes are typically threefold or less (4, 5).

In theory the pharmacogenetic model could explain some of the interindividual differences in response to directly acting toxic chemicals as well. Polymorphisms and (or) acquired differences in enzyme function might be a likely basis for differential responses to metals, solvents, and cholinesterase-inhibiting pesticides, but consistent correlations between human enzyme concentrations and host differences in toxicity remain to be demonstrated. Recently, for example, our own group attempted to explain differential hemato- and spermatoxicity in a cross-sectional study of men heavily exposed to ethylene glycol ethers, the toxicity of which is mediated via a metabolic product (oxyacetic acid) of the ethers oxidized by alcohol dehydrogenase. Neither measured enzyme concentrations nor ethanol consumption histories correlated with the toxic effects hypothesized (6).

This approach yielded noteworthy successes such as the reasonably high predictive value of the deficiency of glucose-6-phosphate dehydrogenase or other glycolytic enzymes in developing hemolysis caused by potent nitrogenous oxidant compounds. These effects were well established by clinical pharmacologists long before the current fashion in our own discipline but, without attempting to dampen enthusiasm for this heuristically attractive line of research, it is worth recognizing that, at least for now, neither measurement of enzyme concentrations themselves nor identification of major polymorphisms has proved more valuable in environmental health research than in clinical pharmacology, where only a trivial portion of adverse effects or interindividual difference in clinical effects can be traced to measurable enzymatic or metabolic differences. Most likely, the interindividual differences are not as great, or the consequences of any differences in one system are offset by redundancy elsewhere. Possibly our in vitro assays are not accurate reflections of true responses in vivo.

Finally, even if certain carcinogens and direct-acting toxins could be shown to affect human risk as a direct function of a measurable enzyme system, the ramifications for disease control might still be disappointing. Only when a small segment of the population has a marked excess risk could such knowledge be used to exclude people from work or comparable host-based control strategies from either an ethical or a public health perspective. An ongoing search for such ultra-high-risk individuals, e.g., people affected with xeroderma pigmentosa, who constitutively express certain oncogenes or harbor defective suppressor genes closely associated with important cancer types, may be fruitful but probably relevant to only a small segment of potentially exposed populations. On the other hand, there would be little practical benefit from recognition of any risk factor that is too widespread in the population or, conversely, of a factor that markedly protects a small minority. Factors associated with small differential risk, however theoretically or mechanistically important, also offer little practical use. In all of these situations, the primary control option must be reduction of exposure to accommodate the more sensitive fractions of the population.

There has been increasing attention to certain modifiable host factors of risk for cancer or direct effects, such as nutritional factors. Examples include antioxidants, such as β-carotene and selenium, and retinoids, which are important in cell differentiation. Extensive clinical trials are ongoing in human populations exposed to important toxins such as tobacco and asbestos to determine whether the impacts of these hazards can be modified by dietary changes or nutritional supplementation (7). Although theoretically promising on the basis of experimental data and observational studies, results remain preliminary.

**Idiosyncratic Responses to Chemicals**

If host factors modify the carcinogenic and direct toxic effects of many chemical hazards, their role appears far greater in that subset of health effects that are more clearly “idiosyncratic”: effects seen at a low dose among a small fraction of individuals but only at far higher doses or not at all among most people. For such effects, which are becoming increasingly impor-
tant in occupational and environmental health practice, efforts to define the basis for host variability are crucial in providing the links between exposure dose and response and are central to developing control strategies. This area has received far less attention to date than carcinogenesis, although historically the epidemiology and toxicology of these disorders have lagged behind cancer and direct organ toxicities.

Idiosyncratic responses to chemicals fall into two broad groupings. First are those that, on the basis of clinical characteristics and descriptive epidemiology, seem to be of immune origin. By immune we mean antigen-specific responses of lymphocytes and associated leukocytes related to one or more of the well-characterized major groups of immune responses, e.g., immediate hypersensitivity, classical delayed-type hypersensitivity. Important diseases include some asthma caused by high- and low-molecular-mass agents, extrinsic allergic alveolitis, chronic beryllium disease, allergic contact dermatitis, and possibly a few others such as hard metal pneumoconiosis.

The second category includes idiosyncratic reactions to chemicals that are not clinically related to a well-described pattern of immunoreactivity. There is no widely accepted nomenclature for describing this group, although occupational and environmental health service workers recognize the increasing frequency of health complaints reported in association with levels of chemical exposures far below those with established toxic effects. The most vexatious syndrome within the group has been named “multiple chemical sensitivities” (MCS), although other patterns, such as solvent-triggered headaches and classical chemical phobias, are probably otherwise mechanistically explained. Each of the clinical problems in this group is poorly understood toxicologically. It is their idiosyncrasy, however, not our lack of a coherent theory of mechanism, that distinguishes these problems from other toxicologically perplexing environmental disorders such as “sick building syndrome,” which typically affects large fractions of exposed occupants and predictably remits when ventilation is improved.

Given the increasing importance of both types of idiosyncratic responses to low levels of environmental exposure, we will review progress on the search for biomarkers of host susceptibility in these conditions. Although the quantity of work has been limited, the results have been impressive, at least for the disorders of likely immune origin.

**Immunogenetics**

Although efforts to define important polymorphisms in genes regulating various metabolic and detoxification processes have yielded only modest evidence of host differences, the evidence is unequivocal that the extraordinary polymorphism of the genes regulates immune function (8). Long before the genetic basis for this was clear, efforts to transfuse blood and transplant human tissue provided evidence that interindividual differences were profound and had a hereditary basis.

We now recognize this diversity as being related to the major histocompatibility complex (MHC) proteins, originally designated human lymphocyte antigen (HLA) because of their presence on human lymphocytes. We now recognize two classes and several loci present on lymphocytes and the crucial antigen-presenting cells, such as macrophages, sufficiently polymorphic that only rare unrelated individuals are identical at all sites. In addition to these fixed loci, the variable gene regions, which are very diverse, recombine with each other to provide the huge range of specific receptors on lymphocytes and macrophages that can recognize and process foreign antigens. This extreme polymorphism explains why chemicals that are antigenic alone or conjugaed with tissues are recognized very differently by different individuals. Beyond this recognition stage, it is not yet clear whether interindividual differences in functions such as cellular responses to recognized antigens, signaling to attract collaborating cells and the actual function of those cells once recruited, will be a basis for substantial differences in host responses to chemicals.

The search for markers of idiosyncratic immune responses to chemicals has focused on antibody production and on antigen recognition. The most obvious examples are tests, many in use for longer than a decade, for specific antibodies to antigens, such as the radioallergosorbent test for IgE to high-molecular-mass proteins in, e.g., grains, animal danders, and bacterial enzymes. The total quantity of IgE and the number of specific antibodies to common antigens such as grasses, pollens, and insect products provide a crude but reasonable marker for the likelihood of response to other antigens. Because the underlying basis for this proclivity to atopy remains obscure, our markers for atopic risk are necessarily crude, possibly too crude to predict risk meaningfully for any individual.

On the other hand, research on host susceptibility for the chronic granulomatous response to beryllium has made remarkable progress. Almost 20 years ago, the ability of beryllium salts to stimulate proliferation of lymphocytes in vitro was demonstrated in sensitized patients (9). This observation was later extended to demonstrate the extraordinary reactivity of the CD4 (helper) T cells in bronchoalveolar lavage from diseased subjects (10). This in turn led to recognition of beryllium binding to MHC class II complexes on the surface of lung macrophages (11). A historic observation that almost all diseased subjects had a specific amino acid at one site (Glu-69) on the HLA-DRB1 sequence, a finding shown to be far less common in reference populations of exposed, healthy workers (12). Although it remains uncertain whether this particular allele is necessary or whether other possible sequences may confer risk, it seems likely that the idiosyncratic difference in response is conferred at least partially by a genetic polymorphism at this site.

Researchers are now investigating HLA polymorphisms in other T-lymphocyte-mediated environmental disorders. In particular, as evidence mounted that
asthma from isocyanates may be mediated in part by T cells (although probably distinct from classic delayed-type hypersensitivity), Bignon et al. (13) found clustering of particular MHC alleles in patients with toluene diisocyanate-induced asthma as well. Hopefully, our increasing knowledge about the genetic predisposition to a wide range of environmental diseases of immune origin will benefit both our understanding of dose–response behavior and our efforts at disease control.

MCS

No area of modern environmental medicine has proved more troublesome to investigators than MCS. Patients with this syndrome present a myriad of symptoms—typically central nervous system, respiratory, gastrointestinal, dermal, and systemic—that reportedly occur after even trivial exposures to very low concentrations of innumerable chemically unrelated substances that are all irritating, odoriferous, or central nervous system intoxicants (14). Many patients develop this constellation after an episode of more classic environmental disease, such as an overexposure to chlorine, pesticides, or solvents. Despite impressive complaints and associated life dysfunction, results for clinical tests of organ function are typically within normal limits. For this reason, many investigators and clinicians have concluded that the patients suffer only a psychological disturbance, with phobic responses to chemicals and a high degree of somatization of psychic distress. This interpretation is attractive to toxicologists who can find no biological basis for reactions to such diverse chemicals affecting so many organ systems at such low concentrations; i.e., no evident biological theory can explain the idiosyncratic basis of the syndrome. On the other hand, many patients show no psychiatric disturbance, and no psychological theory or treatment has been adequately established (15).

Although many have tried to rationalize that this burgeoning problem is nontoxicological, still others have leaned on the domain of biomarker research to find the answer. Because major organ function appears to be preserved and exposures precipitating symptoms are typically low relative to industrial or even ambient environmental standards, researchers sought biomarkers of low-level cumulative exposure and subclinical effects. Examples of the former include detailed profiles of lipid-soluble toxins and their metabolites, such as 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane and 2,2′-bis(p-chlorophenyl)-1,1-dichloroethylene, or heavy metals that chelate in the hair matrix as evidence of "body burdens" of intoxicants. Thrasher et al. (16) proffered in vitro responses of immunocompetent cells to various environmental agents or the presence of antibodies of various classes to ambient chemicals or autoantibodies to demonstrate adverse chemical effects on health. Nor has the host factor component of the equation been forgotten. Many who are convinced that MCS is a biological disease reflecting a degeneration of cellular responses for effective handling of environmental chemicals have suggested that host susceptibil-

ity is associated with blood concentrations of antioxidants, metabolically important nutrients, and enzymes important in detoxification pathways. Subjects deficient in one or more of these may be at risk (17).

In our view, this use of biomarkers has been highly problematic. Many such investigations have relied on case series of affected individuals without reference to a larger normative population or appropriate control subjects. Because the presence of most of the xenobiotics of interest, e.g., metals, organochlorines, and solvents, is ubiquitous, their mere demonstration in affected subjects is of no value. So-called immunological tests, such as serum antibodies or patterns of leukocyte surface markers, remain of undetermined relation to human health, as are concentrations of most nutrients and antioxidant enzymes. Such tests may be used as biomarkers of MCS or similar idiosyncratic disorder only when clear distinction is shown between affected and unaffected people.

Despite these shortcomings, much may be learned here about both biomarkers and MCS. First, these missteps remind us of the proper architecture of etiological research: Only in relation to a defined health effect can markers of exposure and host modification be meaningfully compared. This does not mean that we must await a cogent biological explanation before beginning serious inquiry, but there must be some clear criteria for the health effect, even if purely clinical; markers of exposure and (or) host factors must be evaluated in groups of patients who rigorously meet these, compared with appropriate controls. Markers of effect can be of value only if they can be associated quantitatively with the effect itself, as with MCS, a clinically defined syndrome. The presence of antibodies, lymphocyte subpopulations, or any other finding remains meaningless in the absence of clear association with that syndrome. Biomarkers of exposure are meaningful only in relation to evidence associating levels of exposure with disease risk.

The current inability to apply biomarkers meaningfully to this problem reminds us that biomarkers are not a substitute for epidemiology and toxicology, but an adjunct. In the rubric of all environmental health research, it is only under the umbrella of a fundamental relation between external exposure and defined health effect that meaningful inquiry about dose–host-response relations can proceed. This fundamental relation may be drawn from traditional types of experimental or observational research. Biomarker research cannot meaningfully exist outside this framework and must always be related back to it for its application.

With MCS we remain at the earliest phase. We have begun to characterize some features of the affected population in relation to acute and chronic exposure experiences and certain demographic characteristics of people who meet definable research criteria. For example, there appears to be a female predominance, and it seems likely that individuals with long-standing exposures to chemical environments are at somewhat lower risk than white-collar workers and others who experi-
ence minimal chemical exposure in their routine lives (18). There may be some basis for the clinical impression that certain psychological profiles are overrepresented in these patients, compared with the population from which they come (19). Several attractive new theories might explain the peculiar patterns of idiosyncrasy, the diffuse and intense nature of symptoms after low-level exposure to many unrelated substances, and the paucity of physical and laboratory findings. Intriguing theories involve disruption of respiratory epithelial surfaces in the nose, with either transport of some chemical substances to the brain via the cribri-form plexus or, more likely, the development of malfunctioning olfactory–limbic hyperreactivity caused by a neuronal kindling response (20, 21).

The first step in confirming such theories must be identification of some measurable pathological consequence in MCS patients and controls. Then and only then may it be possible to correlate such changes with environmental exposure factors and (or) the specific host factors. Such differences, of course, may be difficult to identify and may be apparent only after provocation with exposure, as in classic allergic disorders. Such differences remain the sine qua non of effective applications of biomarkers to this problem. Attempts to study chemical effects on the pathways outside this paradigm may be interesting but are unlikely to shed light on this fascinating clinical syndrome.

Although probably only for the foolhardy or the intrepid, MCS is one of the major scientific challenges of environmental science and a remarkable testing ground for our fundamental methods—new and old—for establishing meaningful etiological relations between the environment and the host.

Supported primarily by grants from the National Institutes of Health. M.R.C. also provides consultation to corporations, unions, and government organizations, including the Aluminum Company of America and The International Chemical Workers.

References