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Use and misuse of biomarker tests in “Environmental conditions”

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AIDS	Acquired immunodeficiency syndrome
ANA	Antinuclear antibody
IL	Interleukin
IL-2R	Interleukin-2 receptor
NK	Natural killer
sIL-2R	Soluble interleukin-2 receptor

In the diagnostic evaluation of suspected building-related illness, one generally must thoroughly evaluate the following: (1) workplace or office industrial hygiene measures; (2) material safety data sheets on products or agents used in that particular building; (3) medical records of all persons who claim to have building-related illness; (4) the type and incidence of symptoms, which is usually evaluated by conducting epidemiologic surveys; and (5) current health status of allegedly affected persons, which is evaluated by obtaining a history and performing a physical examination and routine laboratory tests. Depending on the findings gained in these diagnostic evaluations, one may also want to perform psychiatric or neuropsychologic evaluations, a personality test evaluation, and certain laboratory tests, such as complete allergy testing by in vitro or in vivo (skin) tests, selected skin testing with a suspected environmental or occupational allergen, or, where indicated, challenge testing with the suspected offending allergen. These measures would appear to be reasonable when performed by teams of investigators who are selected depending on the situation. Among these investigators would be internists, allergists, pulmonologists, toxicologists, environmental engineers, industrial hygienists, psychologists, and others. Nonetheless, there has been a recent tendency to suspect, without objective evidence and on the basis of belief only, that chemicals or related agents existing in trace amounts in special environmental settings such as buildings, offices, or factories may damage the immune system in humans. Suppression of the immune system would theoretically result in an increased incidence of infections or a decrease in immune tumor surveillance mechanisms. Activation of the immune system by a putative offending environmental agent could theoretically result in autoantibody formation and autoimmune disease or some other form of hypersensitivity, such as IgE-mediated extrinsic asthma, rhinitis, or generalized urticaria. At present there is no basis in fact to substantiate these claims in humans other than anecdotal reports. In spite of this lack of evidence, many persons who have allegedly been exposed to such chemicals are being referred to laboratories or laboratory-based physicians for assessment of possible "immunotoxic" affects on cellular components of the immune system. Immunotoxicology might be defined as a discipline concerned with the selective effect of xenobiotic substances on the cellular and humoral components of the immune system. This concept holds that exposure to such substances at dose levels below those inducing severe generalized organ toxicity can result in the aforementioned immune deficiency, autoimmune, and hypersensitivity phenomena. In this article I will attempt to outline clinical conditions under which biomarker tests to detect immune system "damage" might be performed. The lack of a rationale for performance of such tests in patients with alleged environmental illness, multiple chemical sensitivity, or building-related illness will also be discussed.

Biomarker assays of immune cell populations and subpopulations require the use of automated methodology and are expensive. Biomarker assays depend on the production of monoclonal antibodies against a variety of CD differentiation antigens on the surface of T cells, B cells, natural killer (NK) cells, and various T cell phenotypic subsets, and on the technique of flow cytometry. This technique can be used to analyze many types of particles, including viruses, organelles, liposomes, bacteria, fungi, large immune complexes, cell aggregates, and other small multicellular organisms.¹ The most useful clinical application of flow cytometry is in the identification and characterization of immune cell subpopulations. Because levels of activated T cells are thought to be an indicator of the dynamics of immune function, levels of such activated cells can be determined by flow cytometry simply by counting absolute or relative numbers of different activated lymphocyte types in the blood. The surface expression of several antigens appearing on the cell surface only after mitogenic stimulation can also be assessed. For example, the interleukin 2 (IL-2) receptor is expressed on stimulated human T cells, and it has been determined that all proliferating cells bear the so-called transferrin receptor and human IL-2 receptor.^{2 3}

B Cell and T Cell Phenotypic Subset Analysis by Flow Cytometry

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Lymphocytes have a mean transit time of 30 minutes, and approximately 500×10^9 lymphocytes migrate through the vascular system each day.⁴ Quantitation of peripheral blood lymphocytes has been performed in a wide variety of human disease states, but there are serious questions as to whether the values obtained provide any useful information. When used in a clinical context, these assays are also difficult to interpret because of many confounding variables, among which are a wide range of normal values, varying normal ranges among different laboratories, and age- and time-related differences.⁵ Other variables such as concurrent viral infections, cigarette smoking, and influence of drugs, stress, and associated disease states can all affect the quantity and function of lymphocyte cell populations.^{6, 7} The extent of these variations can easily obscure alterations that might otherwise be of diagnostic help. For example, the number of CD4 helper T cells can vary by 50% or more, depending on the time of day that a blood sample is drawn. It should also be remembered that the lymphocyte composition of the blood differs from that of most body organs and tissues. Peripheral lymphocyte numbers do not necessarily reflect the pathologic changes occurring in specific organs. In addition, the peripheral blood contains only approximately 2% of the total lymphocyte pool in the adult human body, and small alterations in lymphocyte numbers within lymphoid organs can cause major alterations in peripheral blood numbers. Thus it is obvious that one cannot make clinical evaluations based on results of isolated in vitro assessment of quantity and function of immune system cellular components without considering all of these variables and without knowledge of the complete case history and physical examination. This is especially true when a test subject with alleged environmental injury "environmental illness," "multiple chemical sensitivity," or building-related immune system dysfunction has no symptoms characteristic of immune deficiency or no abnormal signs on physical examination, as often is the case. If attention is not paid to the aforementioned variables, patients can be misdiagnosed as having various forms of "immune dysregulation," "immune dysfunction," "low NK cell syndrome," or even "chemical acquired immunodeficiency syndrome (AIDS)" in the absence of evidence of overt clinical disease by history and physical examination on the sole basis of minor deviations in quantitative cellular and functional assays of lymphoid cell populations.

Clinical Indications for B Cell, T Cell, and T Cell Phenotypic Subset Evaluation by Flow Cytometry

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There is no general agreement on precise indications for such biomarker lymphoid analysis, many of which are still in the experimental realm and have not yet been adequately evaluated by the clinical immunology community. Certain clinical and laboratory indications have been given, but these are by no means accepted by all groups. Some clinical indications are as follows: (1) Diagnosis and classification of immunodeficiency diseases; (2) follow-up of patients who have undergone immune reconstitution; (3) persistent lymphadenopathy and symptoms of recurrent viral fungal or protozoal infections that accompany immune deficiency or lead to a strong suspicion of AIDS; (4) determination of type and source of malignant lymphocytes in lymphocytic leukemia and lymphoma; (5) monitoring of cellular changes after bone marrow and other organ transplantation; (6) monitoring of patients undergoing immunosuppressive drug regimes; and (7) detection of changes in lymphocyte profiles in certain forms of carcinoma that may be of prognostic significance.

Among the laboratory indications that might be considered for B cell, T cell, and T cell phenotypic subset evaluation by flow cytometry are the following: (1) Marked increase or decrease in lymphocyte numbers; (2) significant quantitative immunoglobulin or immunoglobulin subclass abnormalities that vary markedly from the norm and are associated with symptoms of immunodeficiency; (3) antibodies to human immunodeficiency virus or detectable human immunodeficiency virus antigen; and (4) absence of delayed hypersensitivity skin responses (anergy).⁷

In cases that involve alleged exposure to trace amounts of environmental chemicals, either in the general environment, the workplace, or in so-called building-related illness, these tests would not routinely be indicated and it would be prudent to order them only when a specific clinical indication is present, such as physical signs or symptoms consistent with immune deficiency disease. There is no sound experimental evidence in humans that has revealed abnormal quantities or function of any specific cellular component of the immune system, including cell phenotypic subsets after these types of environmental exposure. In the few cases where exposure to environmental chemicals in trace amounts has been associated with slight abnormalities of lymphoid cell numbers or function, such effects have not been consistent and have had no meaningful value in terms of diagnosing a disease state or predicting a certain clinical course.

Cytokine quantitation has also been used by some investigators as a diagnostic aid in alleged environmentally induced immunoregulatory disorders. To date, however, there have been no properly controlled studies that definitively show altered production of cytokines in persons exposed to trace amounts of environmental chemicals. IL-2 production has been measured in many immunodeficiency, autoimmune, and related diseases and increases have been noted in a wide range of inflammatory and autoimmune conditions in humans.^{8 9} Impairment has also been noted in a variety of immune deficiency diseases, and plasma and urine IL-2 receptor levels are increased during inflammatory conditions such as allograft rejection.³ As in the case of cell quantitation by flow cytometry, levels of these cytokines vary widely, and random measurements in any given person neither support nor refute the presence of illness in themselves. The IL-2 receptor (IL-2R) and its β -chain component, referred to as the high-affinity receptor or soluble IL-2R (sIL-2R), have also been used by some investigators as a diagnostic aid for certain forms of alleged environmental injury. As in the case of T cell, B cell, and cytokine quantitation, there is no evidence for increased sIL-2R levels in persons with any type of alleged environmental illness. Many inflammatory conditions have been associated with increased sIL-2R. Among these conditions are a wide variety of infections, lymphoreticular malignancies, AIDS, autoimmune diseases, malignancies, and parasitic diseases.^{3 8 11} In some of these conditions, such as systemic lupus, rheumatoid arthritis, and acquired immunodeficiency diseases with secondary infections, very high serum levels of IL-2R and IL-2 have correlated well with disease activity.^{10 12}

The American Academy of Allergy and Immunology Training Program Directors group has discussed all aspects of the potential misuse of biomarker tests and has prepared a statement indicating that clinical evaluation should never be based on results of isolated in vitro assessment of quantity and function of immune system cellular components without consideration of all of the previously mentioned variables and without knowledge of the complete case history and physical examination in any person tested.¹³ The group further emphasizes that this is especially true when a test subject with any type of alleged environmental injury has no symptoms characteristic of any

immune deficiency or no abnormal signs on physical examination. The Training Program Directors and the Subcommittee on Immunotoxicology of the Committee on Biologic Markers of the National Research Council have proposed a tier-testing regimen that may be used to assess patients with known or suspected exposure to an immunotoxicant. When this tier-testing system is used, there should be adequate knowledge of the case history and some evidence that the environmental injury is at least associated with symptoms characteristic of immune deficiency, autoimmunity, or hypersensitivity or with some abnormal signs that suggest a disease state on physical examination. ^{1 5}

Tier-1 procedures, which may be applied to all persons exposed to immunotoxicants, include routine assessment of humoral immunity and quantitation of total numbers of T and B cells with surface analysis for CD3, CD4, CD8, and CD20 markers (i.e., markers of total T cells, helper and suppressor cells, and B-cell populations) and routine tests of delayed-type skin hypersensitivity and selected autoantibody titers to nuclear antigens. If tier-1 test results are abnormal, tier-2 procedures may be applied. If a large population of exposed persons is involved, only a small fraction of the population might receive tier-2 testing. Tier-2 tests would include tests for induction of a primary antibody response to protein and polysaccharide antigens and induction of a primary delayed skin test type reaction and assessment of the proliferation response to both mitogen and a special antigen. In addition, quantitation of other lymphoid cell subtypes can be performed, including monocytes and NK cells, plus T and B cells bearing the marker CD5, CD11, CD16, CD19, CD23, CD64, and class 2 major histocompatibility complex on T cells. Serum levels of cytokines, secreted activation markers, and receptors are also included among the tier-2 tests.

Tier-3 tests may be applied to persons with abnormalities appearing in the tier-2 battery of tests. An example of these tier-3 tests is performance of nonspecific killing of a tumor cell line to test for function of NK cells. In addition to performing these tests on a single occasion, any groups of environmentally exposed persons would have to be followed sequentially to both confirm the initial abnormalities and show evidence of a persistent immune system dysfunction.

The American Academy of Allergy and Immunology Committee emphasizes that application of these tier tests or any portions of them to persons who allege environmental injury has historically led to their misuse when departures from the normal values were considered evidence of injury. The Committee also emphasized that the wide range of normal values, the variations in normal ranges in different laboratories, and the influence of multiple confounding variables such as cigarette smoking, viral infection, stress, drugs, associated diseases, age, and sex must be considered in the interpretation of test results. They stress that the lack of standardization of flow cytometry, which is only now under consideration, should also be appreciated.

Autoantibodies

A variety of antinuclear antibodies (ANA) *may* serve as potential indicators of activity of certain diseases. A few of the many well-known diseases associated with organ-specific and nonorgan-specific antibodies are as follows: systemic lupus erythematosus, autoimmune hemolytic anemia, thyroiditis, myasthenia gravis, insulin-dependent type 1 diabetes, mixed connective tissue disease, multiple sclerosis, rheumatoid arthritis, Sjögren's syndrome, and scleroderma. Monitoring the titer of certain autoantibodies in serum may allow clinicians to follow the process or treatment of specific diseases. One example is the assessment of antineutrophilic cytoplasmic antibodies in vasculitis and Wegener's granulomatosis. ¹⁴

Certain elements and their salts, such as mercuric chloride, cadmium, and gold thiomalate, can induce nonorgan-specific autoantibody formation, autoimmune disease, and hypergammaglobulinemia in experimental animals. Mercuric chloride induces autoimmune disease in rats and mice with production of autoantibody to the nuclear antigen fibrillen and hypergammaglobulinemia, particularly IgE. Cadmium has induced ANA formation in approximately 90% of mice exposed to this agent orally in low concentrations. Many of these animals have developed amyloidosis and antiglomerular basement membrane antibody. Cadmium also has known immunosuppressive effects in animal models. Gold thiomalate, likewise, has induced ANA formation in experimental animals.^{15 17}

Initial studies on autoantibodies in systemic lupus erythematosus were concerned with autoantibodies reactive with DNA and the histone components of deoxyribonucleoprotein. Other so-called nonhistone acid nuclear protein antigens were later described. These include Sm and ribonucleoprotein, which result in a speckled pattern of autoantibody staining on indirect immunofluorescence assays.^{18 19} These are collectively referred to as small nuclear ribonucleoproteins. Evidence suggests that small nuclear ribonucleoproteins regulate such important intracellular events as premessenger RNA splicing. Other nucleoprotein autoantigens and their respective autoantibodies have been important in studying mechanisms of DNA synthesis and replication. There is speculation that perturbation of these nuclear constituents by environmental agents, such as toxins or infectious agents, might render them immunogenic. Thus it has been hypothesized that the immune response to these autoantigens may be primarily driven or directed by external environmental agents.^{20 21} Some clinical clues to this puzzle may be provided from mercuric chloride-, cadmium-, and gold thiomalate-induced autoantibody formation in experimental animals and from the antihistone antibodies of drug-induced lupus in humans. In some other cases, drugs and chemicals may cause immunoregulatory aberrations. Methyldopa, which has effects on T suppressor cells, silicon dioxide, which can induce autoimmune phenomena and autoantibody formation in man, and asbestos fibers, which are known to simultaneously act as polyclonal B-cell stimulants and suppressors of certain T-cell functions, are illustrative examples of such agents.²²

Some practitioners have extended observations based on experimental model data to develop cause and effect relationships that incriminate chemicals from air pollution in the home or workplace as indices of autoimmune disease in humans. At present, however, no sound evidence exists that autoantibodies are operative in the pathogenesis of diseases of humans associated with exposure to environmental chemicals or pollutants or that autoantibodies are in fact induced by such exposure in humans.

The demonstration of autoantibodies per se does not necessarily indicate autoimmune disease. The titer and pattern of certain ANAs may be correlated with objective signs and symptoms of disease activity and other factors (i.e., age), but the major reason for performing these tests is to aid in the diagnosis and assist with monitoring after diagnosis of various autoimmune connective tissue diseases.

Approximately 35% of healthy persons will have a positive test result for ANAs if their serum is examined at a dilution of less than 1:10. At dilutions equal to or greater than 1:10, 4% of healthy persons will have a positive test result. Positive results in healthy persons tend to occur in those

older than 60 years (38%).²³ In addition, a specific causal relationship cannot be drawn between environmental exposure and the presence of antibodies without completing a significance assay, including specificity and sensitivity considerations.

There are growing research needs in the broad area of immunotoxicology as it relates to possible induction of immunoregulatory abnormalities. As noted in the recent National Institute of Allergy and Infectious Diseases Task Force on Immunology and Allergy, "Although scientists recognize the significant threat of environmental toxins, they have not yet determined the links between certain agents and specific diseases. Researchers need to elucidate the mechanisms whereby certain classes of toxicants produce deviations in the immune system, and the consequences thereof, and to quantitate aspects of their toxicity in terms of dose and duration of exposure. Even if scientists undertake extensive animal testing to detect noxious substances, humans probably will continue to be exposed to new immunotoxins that have not been defined by animal research. Therefore, to define emerging problems and to guide surveillance and prevention measures, researchers [also] need to develop ways of ensuring early recognition and evaluation of immunotoxins."²³

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Ritchie C. Shoemaker, Harriett Ammann, Richard Lipsey, Edward Montz

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ABSTRACT

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