Outer Causes of Inner Conflicts: Environment and Autoimmunity

Autoimmune disease is one of the most common yet least understood groups of maladies that confront medicine today. Some estimates place the number of sufferers of such diseases as high as 20% of the U.S. population, most of them women. Autoimmune disorders range from the familiar, such as rheumatoid arthritis, to the relatively uncommon, such as systemic lupus erythematosus (SLE, or lupus) or Behçet disease, as well as a host of obscure disorders that affect only a few people in a million. What these disorders have in common is that they cause the immune system, which is designed to protect the body, to attack the body's own tissues.

Because the origins and mechanisms of autoimmune diseases are largely a mystery, scientists have posed the question of what role, if any, environmental factors play in these diseases. Pose such a question to a group of immunologists, clinicians, epidemiologists, molecular biologists, and toxicologists—as was done in September 1998 at a workshop convened at the NIEHS—and the consensus answer is that they don’t know. The primary leads that researchers have to follow are widely scattered occupational, accidental, and pharmaceutical exposures that have led to autoimmune syndromes, along with some animal models that exhibit autoimmune syndromes mimicking aspects of human autoimmune disease. Researchers stress that the field of autoimmune study as it relates to environmental exposures is in its infancy. “I don’t think we’re at a point that we could say that there are known environmental causes of autoimmune disease,” says NIEHS epidemiologist Glinda Cooper. “There are potential environmental links, but we don’t have a combination of a theoretical framework and experimental and human data needed to fully support them.”

Genetic studies show genes to be only partly responsible for autoimmune diseases. Identical twin studies have consistently found that if one twin has an autoimmune disease, there is a 20–30% chance the genetically identical sibling will also have the same disease. Although this is considerably higher than the 3–5% risk for nonidentical twins and other siblings, says Noel Rose, an immunologist at The Johns Hopkins University in Baltimore, Maryland, “It suggests that, at most, only half of the risk of developing an autoimmune disease is an inherited trait.
or, more likely, an inherited series of traits with a number of genes involved.” The other half, then, may be an environmental component of autoimmune disease. Researchers believe that agents from outside the body, whether microbes, chemicals, or radiation, may also be found to play a major role in the development of autoimmune disease.

**Where to Start?**

Uncertainty in this new field reaches even to the fundamental questions of what an autoimmune disease is and how many people are affected. Researchers debate the number of autoimmune diseases and how many people they affect due partly to different definitions and purposes for the estimates. When a group of epidemiologists at Johns Hopkins, including Rose, prepared to review the epidemiology of autoimmune disease in the United States, they came up with 28 diseases with “direct or indirect evidence of autoimmune pathogenesis” (of these, 4 had no epidemiologic data to review). By contrast, the American Autoimmune Related Diseases Association, an advocacy group, suggests that autoimmunity is the underlying cause of more than 80 chronic diseases that afflict some 50 million Americans. Some researchers believe there may be evidence to support the higher estimates and suggest that as more is learned about autoimmune disorders, individual diseases may turn out to be part of large categories of rare diseases with similar manifestations.

One of the reasons for these widely differing numbers is a paucity of basic epidemiologic data that is largely due to the fact that most individual autoimmune diseases are quite rare. By the admittance of at least one John Hopkins researchers, published in the September 1997 issue of *Clinical Immunology and Immunopathology*, only three autoimmune diseases—rheumatoid arthritis, Graves disease (hyperthyroidism), and Hashimoto thyroiditis (hypothyroidism)—afflict as many as 1 or 2 Americans in 100. Another handful of autoimmune diseases, such as multiple sclerosis, type 1 diabetes mellitus, and vitiligo, are estimated to affect between 1 and 5 Americans in 1,000, but most of the remaining diseases afflict less than a few Americans per 100,000. The true numbers are probably higher.

Some of the relatively minor symptoms of autoimmune diseases such as fatigue, joint and back pain, and muscular ache are simply withstood by sufferers without visits to the doctor’s office or hospital. Even when patients enter the medical system, the often complex manifestations of autoimmune diseases resemble more common nonimmune disorders and are likely to lead to misdiagnoses.

Identifying specific environmental risk factors for autoimmune diseases is highly speculative. What data exist from epidemiologic studies are often contradictory or show weak effects of individual risk factors. In fact, most candidates for environmental links to autoimmune disease come primarily from a few well-known large-scale chemical or drug exposures that produced autoimmune syndromes. For example, in 1981, 35,000 people in Spain developed acute “toxic oil syndrome” after ingesting contaminated rapeseed oil. Their initial symptoms, including fever, fatigue, and joint and muscle pain, were similar to symptoms seen in autoimmune diseases. In most cases, patients were eventually categorized as having a “lupus-like” or “scleroderma-like” disorder. (Systemic sclerosis is an autoimmune disease that manifests itself through a thickening of the skin.) A similar syndrome was caused in the United States in 1989 by contaminants in the dietary supplement L-tryptophan. A large number of medicinal drugs, approximately 40 of which are still in use today, have also produced lupus-like syndromes in some patients. Most prominent among the more than 70 drugs that have caused lupus-like syndromes are procainamide, a cardiac antiarrhythmic medicine, and hydralazine, an antihypertensive drug.

Occupational exposure to vinyl chloride also has been linked to a lupus-like syndrome, and silica dust is known to induce scleroderma. But because these syndromes differ in some respects from the spontaneous diseases and because they generally resolve when the chemical or drug exposure is ended, researchers wonder whether these syndromes provide real insight into the etiology of the spontaneous or idiopathic diseases.

**The Prototypical Autoimmune Disease**

SLE is considered the prototypical systemic autoimmune disease. It is a highly variable disease, with symptoms that change over time as the immune system attacks different internal organs, the skin, joints, and muscles. SLE patients may manifest only a few lupus symptoms, rather than the four or more that may generally be required for inclusion in a study. This is one reason that epidemiologic researchers, who have to select subjects using well-defined and standardized criteria, fail to include many patients whom rheumatologists are treating for SLE.

Symptoms of SLE range from mild to life-threatening. The disease can involve joints, muscles, and the skin and, in more serious cases, major organs (such as the heart, lungs, and kidneys) and the nervous system. Major symptoms such as kidney or nervous system inflammation can be treated and resolved with corticosteroids or immunosuppressive drugs. However, these drugs have significant side effects and so are not used for other symptoms, forcing patients to live with joint pain, overwhelming fatigue, and general achiness throughout the body that can be only partly relieved by nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen. “It’s very much like coming down with the flu,” says Virginia Ladd, executive director of the American Autoimmune Related Diseases Association and a lupus patient herself. “That’s how a person with lupus feels a lot of the time: not sick enough to go to bed, but with that flu-like feeling.”

The joint, skin, and muscle symptoms can be traced to chronic inflammation, which may be mediated by antibodies generated against the body’s own cells. In fact, the presence of large numbers of such antibodies, particularly antinuclear antibody and anti-double-stranded DNA, is one of the strongest diagnostic criteria for SLE. The most common organ involvement is a kidney disorder called glomerulonephritis, wherein antibodies direct white blood cells to destroy kidney tissue and to form complexes with other molecules that clog up the filtration system of the kidney. If the kidneys are involved to a significant degree in SLE, patients may have to undergo dialysis and may even die from the disease.

SLE, despite its relatively low prevalence, has been pushed to the forefront of autoimmune research with respect to environmental links because lupus-like syndromes are among the most common autoimmune disorders related to side effects of medicinal drugs or to accidental or occupational exposures to toxicants. Although none of these agents has yet been shown to be a risk factor in idiopathic SLE, they do provide candidates for study.

Cooper and colleagues at the University of North Carolina at Chapel Hill, Duke University Medical Center in Durham, the East Carolina University School of Medicine in Greenville, and the Medical University of South Carolina in Charleston are conducting an epidemiologic study of recently diagnosed SLE patients in North Carolina and South Carolina. They have cast a wide net, looking for both hormonal and environmental risk factors. Based on animal studies on the effects of estrogen on disease progression and evidence that estrogen replacement therapy may be associated with an increased risk of SLE in postmenopausal women, the researchers are looking at a number of factors involving both hormone medication and a woman’s own hormonal profile, including
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Suspected Risk Factors For Developing Systemic Lupus Erythematosus

Genetic Susceptibility
- immune function (MHC)
- metabolism (PM450, NAT, GST)

Nutritional Susceptibility
- antioxidants
- fatty acids
- dietary fat

Systemic Lupus Erythematosus

Hormonal Susceptibility
- age at menarche
- menstrual cycle patterns
- medications (hormone replacement, oral contraceptives, fertility drugs)
- smoking
- endogenous estrogen, androgen, prolactin levels

Environmental Exposures
- silica dust
- solvents
- smoking
- environmental endocrine modulators

Infectious Exposures
- herpetic viral (Epstein-Barr, cytomegalovirus, herpes zoster)
- retroviral (endogenous or infectious)

Abbreviations: MHC, major histocompatibility complex; NAT, N-acetyltransferase; GST, glutathione S-transferase. Source: Cooper GS, Dooley MA, Treadwell EL, St. Clair EW, Parks CG, Gilkeson GS. Hormonal, environmental, and infectious risk factors for developing systemic lupus erythematosus. Arthritis Rheum 41(10):1714-1724 (1998). Virus photo credit: CDC.

Age at menarche, menstrual cycle patterns, and age at menopause. Other candidate risk factors such as exposure to silica, UV light, and some solvents and metals were chosen based on evidence from occupational and experimental studies. Data collection from the 250 SLE patients and 350 controls, involving interviews and blood sampling, was recently completed and the researchers are now analyzing the data of what will be the largest population-based epidemiologic study of SLE to date.

A More Selective Disease

In contrast to SLE, type 1 diabetes (also known as insulin-dependent diabetes mellitus (IDDM)) is an organ-specific disease; only cells in the pancreas appear to be attacked by the immune system. What’s known about how type 1 diabetes acts is that antibodies to the insulin-producing beta cells of the pancreas direct white blood cells to infiltrate the region and destroy the beta cells. When the beta cells have been destroyed, patients must then control glucose metabolism by regular injections of insulin. This is an imperfect solution, and unexpected fluctuations in glucose concentrations can put great stress on other organs.

Patients are thus forced to live within rigid schedules: missing a scheduled snack or meal or undergoing unexpected exertion or excitement can quickly trigger drastic hypoglycemia (low blood sugar), which can result in coma and possibly permanent brain injury. Even when patients follow their regiments strictly, they are at the mercy of their injected insulin and other medications, which are hard pressed to supply the body’s tissues with the precise glucose levels they require. This means that many patients are subject to long-term complications of diabetes such as increased risk of heart attack and stroke, deteriorating vision, kidney failure, and infection.

Type 1 diabetes is the second most common chronic childhood disease and, unlike most autoimmune disorders, afflicts a slightly higher number of males than females. It affects approximately 150,000 children in the United States and typically is diagnosed between the ages of 10 and 13. But Roland Tisch, an immunologist at the University of North Carolina at Chapel Hill, says, “When a person is diagnosed with overt diabetes, the disease process has most likely been going on for a number of years.”

A number of environmental factors, primarily viruses and dietary factors, have been linked to type 1 diabetes. Among the virus candidates are those that cause mumps, mononucleosis, and rubella (German measles). There is even a good candidate mechanism, called molecular mimicry, for
how a virus might induce autoimmune type 1 diabetes. The theory is based on the observation that some viruses have elements in common with beta cells. For example, a protein found on the rubella virus is structurally similar to one found in pancreatic cells; it is thought that this mimicry might confuse the immune system into attacking beta cells. But no virus has been definitively shown to cause type 1 diabetes in humans.

The only nonmicrobial agents that have been implicated in autoimmune type 1 diabetes are foods, and the evidence has not been particularly strong. Cow's milk, N-nitroso compounds, gluten, fat, and proteins have all raised red flags in small epidemiologic studies, but subsequent study has not conclusively shown any of these agents to be a clear risk factor for type 1 diabetes. Echoing researchers of other autoimmune diseases, Tisch says, "No one is going to dispute that there is an environmental link, but there isn't any definitive proof out there linking IDDM and viruses or any other environmental agents."

Not Just Your Grandmother's Disease

Rheumatoid arthritis, thought by many to be a disease of elderly women, actually affects people of all ages, including a considerable number of men (about 25% of cases). Rheumatoid arthritis is considered a systemic disease because it can affect joints throughout the body and has other systemic effects including fatigue, weight loss, and anemia. But it can also be thought of as organ-specific because it selectively destroys a membrane called the synovium that lines the joints. An antibody called rheumatoid factor appears to initiate destruction of the synovium. The ensuing inflammation involves the abnormal growth and division of synovial cells as well as the arrival of white blood cells, which release enzymes that are thought to destroy the cartilage and cause the joint to fuse.

The first symptoms of this process—joint inflammation (arthritis) and pain in the extremities—typically appear in patients between 25 and 50 years of age. The disease is usually symmetrical, affecting both hands or both feet equally. Patients find that their joints are stiff for hours each morning. The typical treatment for mild arthritis is rest and NSAIDs. Corticosteroids and immunosuppressive drugs are reserved for severe discomfort or disability and must be reduced and discontinued as soon as possible. These measures provide relief for 75% of patients, but the drugs do not slow the progression of the disease, and 10% of patients will ultimately become disabled as their joints are destroyed.

As with other immune diseases, the only risk factors for rheumatoid arthritis that have been extensively studied are genetic. There is strong evidence that variations in certain genes, especially in combination, predispose the bearers to develop rheumatoid arthritis. Researchers are now turning to the question of which environmental factors might interact with a susceptible genetic profile. Among the candidates are infectious agents, immunizations, estrogen replacement therapy, smoking, and alcohol consumption. Various studies have failed to turn up strong or even consistent effects for any of these risk factors. However, researchers believe that combinations of different low-risk environmental factors interacting with a susceptible genetic profile are needed to trigger the disease. "It may be that you have to have the right genes that put you at high risk, and it could then be 25 different things in the environment that give that susceptible person the disease," says rheumatologist Beth Karlson of Brigham and Women's Hospital in Boston, Massachusetts.

The Difference between the Sexes

Rheumatoid arthritis predominantly strikes women, usually later in life. This is the common factor that links most autoimmune diseases. The ratio of women to men afflicted is 3:1 for rheumatoid arthritis, 9:1 for lupus, and 25:1 for autoimmune thyroiditis. Researchers point out that women's immune systems appear to be more aggressive. "Females live longer and their immune systems are better," says David Lawrence, an immunologist at the Wadsworth Center, a part of the New York State Department of Health in Albany. "But being able to make antibodies better and to make a stronger cell-mediated immune response [means] they also have an increased possibility of getting autoimmune disease."

Suspicion for the difference in incidence between the sexes understandably falls on hormones, especially estrogen. However, attempts to elucidate the role of estrogen in human autoimmune disease have typically turned up weak effects or conflicting results. For example, of five epidemiologic studies of perimenopausal hormone replacement therapy and the risk of rheumatoid arthritis, one showed an increased risk, one showed a protective effect, and three found no effect. On the other hand, studies of SLE, scleroderma, and Raynaud phenomenon (periodic spasms of the smallest arteries in the hands and feet that lead to sensations of cold or pain) have consistently shown slight increases in risk after hormone replacement therapy. Researchers are also continuing to look for links between women's hormonal profiles and autoimmune disease because animal studies have demonstrated that estrogen can theoretically alter regulation of the major immune system cells, the T and B lymphocytes (see EHP 107(Suppl 5):681–686 (1999)). Determining the exact role of estrogen will depend on finding the mechanisms by which the hormone helps turn the immune system against the body.

Where Things Go Awry

The immune system consists of a vast array of defense mechanisms: large white blood cells such as macrophages, neutrophils, and leukocytes, which can ingest antigens such as invading microbes; smaller white blood cells that include T lymphocytes (or T cells), which scan for and attack specific antigens, and B lymphocytes (or B cells), which generate individualized antibodies to ensure continued and rapid responses to antigens; and a host of small molecules called cytokines, which complement and assist the leukocytes in these processes.

In order for this aggressive system to work, the immune system must learn to distinguish "self" antigens (specific molecules on the body's own cells) from "nonself" antigens (microbes or molecules from outside the body) in a process called tolerance. For T cells, which have primary surveillance responsibility, this education takes place in the thymus. In order to leave the thymus, T cells must recognize and therefore not react to the major histocompatibility complex (MHC)—unique to each person—that is found on the surface of thymus cells. By a process that science has still not completely described, T cells that react strongly to self-MHC molecules are functionally suppressed or eliminated in the thymus. The current assumption is that they are stimulated to undergo apoptosis, or programmed cell death. A parallel process in the bone marrow is believed to remove autoreactive B cells.

But the occasional autoreactive lymphocyte makes it out into the blood or lymphatic circulation, even in healthy people. The body then appears to call on a complex set of interactions between the lymphocyte and other molecules and cells to neutralize the lymphocyte. Two main mechanisms have been uncovered: anergy, whereby the T cell is made less responsive to antigens, and suppression, whereby other lymphocytes and natural killer cells use cytokines to inhibit the capacity of autoreactive lymphocytes to respond to self-antigens.

While much mystery remains regarding how the immune system operates, there has been enough progress that it is possible to suggest where environmental factors might disrupt the immune system's tolerance for its own antigens. Bruce Richardson, a rheumatologist and immunologist at the University of Michigan and the Ann Arbor Veterans Affairs Medical Center, describes three broad opportunities for environmental substances.
to interfere with this tolerance. The first is to prevent the establishment of tolerance in the thymus, the second is to alter gene expression in the cells that participate in the immune response, and the third is to alter the MHC proteins that signal "self" on the surface of the body’s cells, either by directly modifying the proteins or by altering the genes that encode them [see EHP 107(Suppl 5):737–742 (1999)].

Although no one study is considered a front-runner at this point, there are some animal data to support each of these mechanisms. For example, the MRL-lpr mouse has a defective form of Fas, a protein necessary for apoptotic cell death in the thymus. Without Fas, autoreactive T cells are not weeded out by apoptosis, and these mice develop a lupus-like disease and die at an early age. Humans with defects in the gene that encodes Fas develop autoimmune disorders in which red blood cells and other blood components are destroyed. However, there is, as of yet, no evidence to link these disorders with Fas-mediated apoptosis of T cells in the thymus, much less to suggest that an environmental agent contributed to the process.

An idea that particularly intrigues researchers of the first mechanism has been advanced by Robert Rubin, an immunologist at The Scripps Research Institute in La Jolla, California. Rubin studies drugs that have been shown to cause lupus-like syndromes in patients. Research into the mechanism of these syndromes has been stymied by the fact that the various drugs have no obvious similarities of structure or pharmacologic activity. Rubin hypothesized that metabolites of these drugs could interfere with the mechanisms that teach T-cell tolerance in the thymus. (This idea arose from recent revelations that T cells are generated in the thymus throughout life; it had been believed that T-cell production ceased soon after birth.) In an article in the 15 April 1997 issue of the Journal of Clinical Investigation, Rubin demonstrated that injecting procainamide hydroxylamine (a metabolite of procainamide) directly into the thymus of mice will produce a syndrome like that found in human drug-induced lupus [see EHP 107(Suppl 5):803–806 (1999)]. Whether this mechanism is at work in the human drug-induced disease or whether it could be a mechanism that other environmental agents use to contribute to idiopathic lupus is conjecture. "We’re quite optimistic but we don’t have much evidence for it," says Rubin. "It’s preliminary and quite controversial."

Richardson has demonstrated that environmental agents could alter gene expression in T cells outside the thymus—the second possible mechanism for interference with tolerance—to help cause autoimmunity. Based on suggestions that the methylation of DNA—which is essential for proper expression of proteins—is altered in autoimmune disease, Richardson and his colleagues exposed T cells to a DNA methylation-inhibiting drug in vitro. The resulting autoreactive cells were transplanted to healthy mice with identical MHC profiles, where they induced a disease that resembled lupus. This research was reported in the June 1996 issue of the Journal of Clinical Investigation.

The question of whether this would happen in humans obviously couldn’t be tested, so in experiments published in the August 1997 issue of Arthritis and Rheumatism, the researchers asked a related question: do the drugs that cause lupus in people have these same effects, namely, inhibiting DNA methylation and making mouse T cells autoreactive? Says Richardson, "We looked at procainamide and hydralazine, both of which cause lupus in a significant number of people, and the answer was yes." Although such animal results are a long way from proving that the drugs induce lupus in humans by inhibiting DNA methylation or that this mechanism is at work in idiopathic lupus, they have provided Richardson’s group with the motivation to search for the genes and gene products that may be affected by these drugs.

As for the third autoimmune mechanism, whereby the MHC proteins that signal "self" on the surface of the body’s cells are altered, thereby making them targets for lymphocytes, there are a number of examples that implicate a process called haptenization. Certain small, foreign molecules called hapten, although not antigens by themselves, are able to interact with MHC proteins and change their structure. T and B cells identify cells with altered MHC proteins as nonself antigens and mount immune attacks on them. The mechanism by which a hapten binds to a larger protein to produce a recognizable antigen has been demonstrated for a number of molecules, including vinyl chloride, metabolites of phenytoin, and ions of mercury, gold, and nickel. There is no evidence yet that haptenization contributes to autoimmune disease in vivo, but hapteneses have been shown to alter self-proteins and to induce auto-antibodies in some experimental in vitro systems.

Autoimmune Animal Models

To illuminate whether any of these mechanisms are actually used by environmental agents to contribute to autoimmune diseases, immunologists and other scientists hope to use models of animals that have genetic predispositions to develop such diseases. It is generally agreed that lupus research may benefit the most from such models as there are several mouse strains that develop lupus-like syndromes. The best known model is the New Zealand black/New Zealand white mouse—50% of the female mice of this species die of glomerulonephritis by 34 weeks of age. The MRL-lpr mouse, with its mutation in the gene for Fas, also develops a very severe autoimmune syndrome early in life, though females and males are equally affected.

Lawrence suggests that newer animal variations may be the best models for studying the subtle effects of environmental agents. For example, many strains of New Zealand mixed mice have been created by selective

Color is the key. Two chromosomal loci for susceptibility to SLE have been linked to coat color in New Zealand black/New Zealand white mice. Different strains of these mice have varying susceptibilities to developing lupus and are being bred to study the possible effects of environmental exposures on the development of SLE in humans.
breeding of the New Zealand black/New Zealand white mice and their descendants. Among these, there are some strains in which only a small percentage of the mice develop autoimmune disease and in which the disease progresses slowly, more like the human disease profile. Similarly, the MRL-lpr mouse without the Fas gene mutation develops a less severe disease later in life.

The Long Road Ahead
At the close of the September 1998 NIEHS workshop on environmental links to autoimmune diseases, the participants came up with research needs in five main categories that included development of innovative and appropriate research tools, establishment of an autoimmune disease registry or surveillance system, development of strategies for screening chemicals for autoimmune activity, development of an emergency response strategy to gain information on accidental exposures that may lead to autoimmune responses, and autoimmune hypothesis-driven research in occupationally exposed groups and in experimental animals [see EHP 107(Suppl 5):811–814 (1999)]. The participants clearly felt that the first four needs would have to be met before the last could begin to be addressed.

Researchers appear to be setting out into this new field of study—autoimmunity and environmental health—with only a few bits of intriguing information and a multitude of basic questions. They know some of the genes involved and they have hints about a few of the mechanisms and chemicals that might be involved. But as immunologist Frederick Miller of the U.S. Food and Drug Administration points out, “You may have multiple environmental exposures interacting with multiple genetic risk factors to result in the complete expression of an autoimmune disease.” This suggests that answers will be slow in coming, but Miller is hopeful: “The beauty of immune diseases is that they are theoretically preventable because they are all gene-environment interactions.” The key, he says, will be to find the genes and environments that combine to cause diseases, after which it will be possible to tell susceptible people which drugs, chemicals, dietary supplements, or foods they need to avoid.

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