Evidence for the Role of Environmental Agents in the Initiation or Progression of Autoimmune Conditions

Jonathan J. Powell, Judy Van de Water, and M. Eric Gershwin

Division of Rheumatology/Allergy and Clinical Immunology, University of California at Davis School of Medicine, Davis, California USA

The concordance of autoimmune disease among identical twins is virtually always less than 50% and often in the 25–40% range. This observation, as well as epidemic clustering of some autoimmune diseases following xenobiotic exposure, reinforces the thesis that autoimmune disease is secondary to both genetic and environmental factors. Because nonliving agents do not have genomes, disease characteristics involving nonliving xenobiotics are primarily secondary to host phenotype and function. In addition, because of individual genetic susceptibilities based not only on major histocompatibility complex differences but also on differences in toxin metabolism, lifestyles, and exposure rates, individuals will react differently to the same chemicals. With these comments in mind it is important to note that there have been associations of a number of xenobiotics with human autoimmune disease, including mercury, iodine, vinyl chloride, canavanine, organic solvents, silica, L-tryptophan, particulates, ultraviolet radiation, and ozone. In addition, there is discussion in the literature that raises the possibility that xenobiotics may also exacerbate an existing autoimmune disease. In this article we discuss these issues and, in particular, the evidence for the role of environmental agents in the initiation or progression of autoimmune conditions. With the worldwide deterioration of the environment, this is a particularly important subject for human health. *Key words:* autoimmunity, environmental agents, MHC, pollution, self-antigen, xenobiotic — *Environ Health Perspect* 107(suppl 5):667–672 (1999).
http://ehpnet1.niehs.nih.gov/docs/1999/suppl-5/667-672powell/abstract.html

Autoimmunity is an inappropriate immune response to self-tissue antigens and, if sustained, can lead to tissue damage that is known as autoimmune disease. Individually, with the exception of diabetes and thyroiditis, autoimmune diseases are relatively uncommon but collectively affect 1 in 31 Americans (*1–3*). It is clear that in both humans and animals the induction and the natural history of autoimmune disease is multifactorial, including both genetic and environmental influences. In some classic autoimmune diseases such as systemic lupus erythematosus (SLE), the concordance among identical twins is generally 25–40% (*4*). The younger the patient at disease presentation, the higher the familial risk. Experiments with different strains of inbred mice clearly reflect the importance of genetics. For example, whereas C57 BL/6 (H-2b) mice are symptom-free following oral challenge with oleic acid anilide, a severe rapid wasting disease is seen in similarly challenged A/J (H-2a) mice (*5*). In addition, however, some data suggest that the incidence of autoimmune disease may be increasing and that differences in various ethnic and geographic populations might be reflective, not only of genetic, but also of environmental influences.

Autoimmune diseases occur predominantly in the Western world, yet immigrants take on the same incidence of disease as the indigenous population and in some cases even exceed it (*4*). Whether the differences in incidence between Western and Third World nations are due to sociologic, nutritional, parasitic, or hygienic reasons is unclear. Although viruses and bacteria are obvious environmental candidates and have been well reviewed elsewhere (*1*), there are a number of physical and chemical environmental factors that clearly impact the immune system and lead to immunopathology. These materials include mercury (Hg), iodine (I), vinyl chloride, canavanine, ultraviolet radiation, ozone, organic solvents, silica, ultrafine particles, analides, and L-tryptophan. In addition, there is the possibility that environmental chemicals may influence autoimmune disease, not only by induction, but also by exacerbation/acceleration of the underlying process.

A major problem in this discussion is the issue of latency time. This may be a particularly important problem for xenobiotics. For example, we know that rheumatic heart disease occurs in genetically susceptible people within a short time following exposure to a specific serotype of β-hemolytic streptococcus. In the latter case, the association between the infection and the clinical disease is obvious based upon this short latency interval. On the other hand, if the latency time were measured not in weeks but in many months to years, the association would be missed and instead of the disease being called rheumatic heart disease secondary to β-hemolytic streptococcus, it would be called an autoimmune carditis with associated arthritis. It is possible that many autoimmune diseases will have long latency times following exposure to xenobiotics and may, therefore, be considered hit-and-run diseases. This makes the identification of causative agents very difficult to prove, particularly for diseases that are relatively uncommon. In this article we focus not on this concept of hit-and-run disease or long latency time but rather on the xenobiotics that induce autoimmunity in humans. Following the discussion of a number of these agents, we will attempt to link them to possible pathogenic mechanisms.

Mercury

Mercury is widespread in the geosphere at low levels but occurs in more concentrated areas as the crude sulfide termed cinnabar. Human intervention of the environment, in the form of mining and coalburning, has released Hg into the biosphere and atmosphere at quantitatively and qualitatively significant levels. Hg compounds are used in fluorescent lights, batteries, and electronic devices, and the metal is still commonly used in dental amalgam for tooth fillings. Indeed, this latter source is the major background source of human exposure to Hg vapor (*6*). Disposal of these products is associated with global redistribution of Hg, chiefly through the atmosphere after incineration, although atmospheric Hg is not considered an important source in direct human exposure (*6*). Awareness of the marked toxicity of Hg compounds now prevents their use in medications and fungicides both of which have been responsible for epidemics of Hg toxicity. Similarly, the discharge of Hg-based products from chemical industries into water bodies has been associated with devastating incidents of toxicity, most notoriously in Minamata and then Niigata, Japan. In these cases, methyl mercury, a particularly bioavailable and toxic form of Hg, was discharged into water bodies and accumulated by fish that were then ingested by the local population (*6*). In fact, the methylation of Hg also

This article is based on a presentation at the Workshop on Linking Environmental Agents and Autoimmune Diseases held 1–3 September 1998 in Research Triangle Park, North Carolina.

Address correspondence to M.E. Gershwin, Division of Rheumatology/Allergy and Clinical Immunology, University of California at Davis School of Medicine, TB 192, One Shields Ave., Davis, CA 95616. Telephone: (530) 752-2884. Fax: (530) 752-4669. E-mail: megershwin@ucdavis.edu

We appreciate the gracious support of Transgenomics, Inc. This work was supported in part by National Institutes of Health grant DK39588.

Received 28 December 1998; accepted 29 March 1999.

occurs in the environment where aquatic microorganisms, termed methangenic bacteria, are able to transfer methyl groups from methylcobalamine to Hg, forming both monomethyl and dimethyl species of Hg. Additional pathways have been proposed, such as the methylation of homocysteine-bound Hg by bacteria during the normal homocysteine→methionine conversion. Nonetheless, it is likely that man-made changes have resulted in inorganic forms of Hg that are more prone to environmental methylation, compared to the naturally occurring forms. Thus, it is the subtle exposure of Hg to man (Table 1), in qualitatively different forms, that concerns us rather than frank toxicity, which should now be a very rare occurrence.

It is the mercury cation (Hg^{2+}), or elemental Hg, following its absorption (most often as the vapor) and intracellular oxidation ($Hg - 2e^- \rightarrow Hg^{2+}$) that is responsible for the toxicity of inorganic Hg compounds (*6*). Thus, mercuric chloride ($HgCl_2$) is frequently used as a convenient source of inorganic Hg ions in toxicologic experiments. As a general mechanism of toxicity, Hg interacts rapidly and avidly with sulfhydryl (SH) groups that are found, for example, on thiol-containing amino acids and proteins. The methyl mercury cation (CH_3Hg^+) exhibits a similar high affinity for these groups but unlike Hg^{2+} does not induce metallothionein, which is protective to the host. This and its ability to easily cross the blood–brain barrier (*7*) makes CH_3Hg^+ particularly toxic. Methyl mercury is dissociated over time by phagocytic cells reforming Hg^{2+} ions.

Protein–Hg interactions have generated substantial interest among immunologists, especially in autoimmune studies, as Hg can modify self-proteins through Hg^{2+}–thiol interactions. Indeed, following Hg intoxication, a membranous nephropathy that may be autoimmune in nature has been described (*8*). In animals, a widely studied model of Hg-induced autoimmunity is the Brown-Norway rat, as reviewed elsewhere (*8*). In these animals, subcutaneous injections of $HgCl_2$ generate antibodies against the glomerular basement membrane, leading to immune-complex deposition in the kidney as well as proteinuria. Autoantibodies show specificity for extracellular matrix molecules such as laminin, collagen, and fibronectin. The mode of action is unclear, but Hg may act initially on $CD4^+$ T cells to stimulate B-cell production of antibodies. This is suggested by Hg's lack of B-cell antigenic activity *in vitro* and characteristic IgE induction *in vivo*, suggesting a T-cell–dependent isotype switch (*8*). Exact mechanisms are lacking, but recent data with isolated cells suggest that direct interaction of Hg and self-proteins may occur, leading to modification of the molecular and antigenic properties of the protein (*9,10*).

Using a genetically susceptible mouse model in which injected Hg^{2+} interacts with the nucleolar protein fibrillarin, Kubicka-Muranyi et al. suggested that Hg induces antigen presentation of a novel Hg–fibrillarin epitope (*10*). The presentation of unaltered fibrillarin epitopes was also upregulated and fibrillarin-specific T-cell clones were stimulated (*10*). Genetic control of this Hg-induced autoimmune response has been mapped to the H-2A region of the major histocompatability complex (MHC) of susceptible mice, but the manner in which Hg targets its protein–antigen epitope was not demonstrated until recently (*9*). Pollard et al. (*9*) have shown that many cysteine-rich proteins (containing thiol groups) are not modified by Hg. Rather, the fibrillarin molecule contains cysteines that are not disulfide bonded, leaving exposed SH groups at amino acids 105 and 274 allowing their modification by Hg. Substitution of only one cysteine with the non–thiol-containing amino acid alanine appeared to abolish interaction with Hg, which suggested that the overall protein structure is also important in dictating interaction with Hg^{2+}. Hg-modified fibrillarin appears to generate T-cell responses, but subsequent antibodies from T-cell–primed B cells recognize unmodified fibrillarin, leading to potentiation of an immune response. Many patients with scleroderma possess antinucleolar autoantibodies, some of which react with fibrillarin. Although there is little evidence of Hg loading in these patients, similar chemical modification at the exposed cysteines of fibrillarin has been proposed and may lead to reactive antibodies (*9*).

Table 1. Mercury association with immunopathology.

In humans, Hg promotes an autoimmunelike syndrome with immune complex deposition and glomerulonephritis.
$HgCl_2$ inhibits the production of IFN-γ in susceptible Brown-Norway rats and induces the production of IL-4, thus skewing the Th response toward Th2.
Hg^{2+} interacts directly with exposed SH groups of endogenous proteins.
Hg stimulates a T-cell-induced B-cell response against Hg-modified proteins.
As with iodine, Hg-triggered immune responses may then recognize native protein.

Abbreviations: Hg^{2+}, mercury ion; Hg, mercury; $HgCl_2$, mercuric chloride; IFN, interferon; IL, interleukin; Th, T helper.

Table 2. Iodine-associated autoimmune disease.

Human studies suggest iodine involvement in the etiology of some autoimmune thyroiditis in genetically predisposed individuals.
1% of the population is affected by autoimmune thyroiditis, but 15% of healthy individuals have circulating autoantibodies.
Iodine increases the incidence of autoimmune thyroiditis in various animal models.
Iodide is commonly ingested and oxidized to the iodinium ion.
Iodine may increase the immunogenicity of Tg, through conjugation of the iodinium ion, and immune components may then recognize noniodinated Tg.
The oxidizing and halogenating potential of iodine should also be considered in tissue damage.

Tg, thyroglobulin.

Iodine

Thyroglobulin is a 660-kDa glycoprotein iodinated at certain tyrosine residues and then cleaved to produce the two major thyroid hormones triiodothyronine (T_3) and tetraiodothyronine, which is also termed thyroxine (T_4). T_3 and T_4, which differ by their degree of iodination, are carried in plasma primarily by thyroxine-binding globulin. Their production and secretion is regulated by the pituitary-derived thyroid-stimulating hormone termed thyrotropin, which in turn is regulated by feedback inhibition as well as by the hypothalamus.

Autoimmune thyroiditis is characterized by lymphocytic infiltration and generation of autoantibodies that recognize thyroid-related molecules including thyrotropin and its receptors, the Na-I symporter (rNIS), thyroglobulin, T_3, and T_4 (*8,11,12*). Alterations in frequency of $CD5^+$ B cells, $CD8^+$ T cells and γδ T cells have been associated with disease, as have the cytokines tumor necrosis factor-α, interleukin (IL)-2, IL-6, IL-12, and interferon (IFN)-γ. Again, genetic susceptibility is clearly important in the initiation of disease although the determining susceptibility loci have not been identified. In addition, however, there is clear evidence from studies both in man and animals that dietary iodide enhances susceptibility to autoimmune thyroiditis (Table 2).

Iodine (I) is the 64th most abundant element in the earth's crust, existing commonly as iodates and iodides in seawater and marine organisms. As discussed above, iodine has high affinity for thyroid hormones, so 80% of total body iodine is maintained in the thyroid by a specific iodide pump. The element is essential for normal thyroid function and although most individuals tolerate a wide range of dietary levels, some are clearly prone to the development of thyroid dysfunction (*13*). The toxicity of iodide has been related to its requirement for oxidation to the iodinum ion ($2I^- - 2e^- \rightarrow [I_2] - 2e^- \rightarrow 2I^+$) prior to its binding to tyrosine residues of thyroglobulin (*8*). Thus, strong intermediate complexes could be formed with electron donors (phospholipids) and oxidative damage promoted (*8*). However, most data point to the role of iodine in the modification (iodination) of thyroid glycoproteins,

leading to direct autoimmune responses. Abnormal processing by antigen-presenting cells, enhancement of MHC-peptide antigen T-cell receptor interactions, and increasing affinity of antibody–antigen binding have all been suggested as consequences of iodination of thyroid glycoproteins (*11*).

A number of reports from several countries have associated the prophylactic use of iodide supplementation with thyroid disease (*8*). More recently, Kahaly et al. (*13*) demonstrated that iodide supplementation in 31 patients with endemic goiter induced hypo- and hyperthyroidism in 6 of 31 patients with associated high titers of thyroglobulin autoantibodies. Abnormal lymphocytic infiltrates of the thyroid gland were shown by biopsy. Following withdrawal of iodide supplementation, thyroid function normalized, autoantibodies fell, and lymphocytic infiltration decreased, clearly demonstrating the role of iodine in disease induction of these individuals. Consistent with these results, Rose et al. (*11*) have shown that peripheral blood lymphocytes from patients with thyroiditis proliferate in response to iodinated thyroglobulin (i.e., recognize as antigenic) but not in response to normal (noniodinated) thyroglobulin. Although animal studies in susceptible obese-strain chickens (*14*) and nonobese mice (*15,16*) similarly support the role of iodine in autoimmune thyroiditis, other data suggest that a causal relationship must be carefully interpreted. First, Rose et al. (*11*) demonstrated equal proliferation of peripheral blood lymphocytes in response to iodinated thyroglobulin in control patients compared to those with thyroiditis. Indeed, as well as T-cell proliferation, autoantibody responses to thyroid components appear frequently in the general population but without associated disease. Second, autoimmune thyroid disorders may occur even in iodine-deficient populations (*17*). Third, Kong et al. (*18*) have shown that immunogenicity of thyroglobulin peptide epitopes is conventionally dependent upon the amino acid sequence, whereas the degree of iodination may only enhance antigenicity. Together, these data suggest that although iodide is not prerequisite for thyroid-related autoimmune disease, excess iodination of thyroid glycoproteins may trigger autoimmune responses against the iodinated and noniodinated epitope that manifests as disease only in susceptible individuals.

Vinyl Chloride

Most reports on the synthetic resin vinyl chloride (VC) are concerned with its mutagenic rather than autoimmune properties (*19*). However, Lernmark proposes that although autoreactivity is important in preventing tumor formation, this may be at the cost of developing autoimmune disease (*20*). Indeed, occupational exposure to VC has been associated with mixed connective tissue disease (*21*) and most notably scleroderma (see "Organic Solvents"). Individuals susceptible to the effects of vinyl chloride carry human leukocyte antigen (HLA) DR5, whereas the haplotype A1B8 DR3 defines disease severity (*8*). Markers of disease include autoantibodies to ribonuclear proteins, Raynaud syndrome, and arthritis (*8*). Although VC may bind to nucleotides, its oxidized metabolites, chloroethylene oxide and chloroacetaldehyde, are also highly reactive and bind to SH groups (*19*). As with Hg, binding to SH groups of protein–thiols may significantly modify the protein, thus altering its antigenicity. In addition, preferential inactivation of cytotoxic ($CD8^+$) T cells has been proposed following oxidation of cellular thiols (*8*). The inactivation of $CD8^+$ cells and loss therefore of suppressor activity, might be a contributing factor to the autoimmune response.

Canavanine

Canavanine is an analogue of L-arginine produced by plants to reduce their palatibility. Dietary canavanine, obtained through alfalfa, has been implicated in SLE-like syndromes (*22*) and SLE has even been induced experimentally in macaques by feeding them alfalfa seeds (*8*). The monkeys developed autoimmune hemolytic anemia, antibodies to double-stranded DNA, immune complex-mediated glomerulonephritis, and arteritis. Autoantibodies continued for up to 2 years after discontinuation of alfalfa from the diet. *In vitro*, high doses of canavanine inhibit DNA synthesis in murine leukocytes; lower doses selectively inhibit B-cell function (*22*). In contrast to L-arginine, which has a pI of 10.8, the pI for canavanine is 8.2. Following its ingestion and uptake, it may substitute for arginine in endogenous proteins, modifying their structure and function. Surface changes in the B cells of autoimmune mice were altered by dietary canavanine (*23*) and other studies have shown that proliferative responses of human peripheral T cells, notably cytotoxic ($CD8^+$) T cells, are inhibited by this nonessential amino acid (*24*). The combination of surface modification of B cells and inhibition of $CD8^+$ cells could potentially lead to loss of tolerance. More directly, however, the substitution of arginine by canavanine, perhaps at the level of transfer RNA^{arg} in mammals, would render self-molecules immunogenic.

Ultraviolet Radiation and Ozone

Ozone is one of the most powerful oxidizing agents known and one of the most difficult air pollutants to control (*25*). The major source of ozone in the atmosphere is from the use of petroleum products, although it occurs naturally in the stratosphere and, ironically, forms a protective layer to our exposure to UV radiation (*26*). Both ozone and UV radiation have significant immunosuppressive activity. Exposure of experimental animals to ozone leads to decreased numbers of resident alveolar macrophages, probably through direct cell lysis (*27*). Their mobility and phagocytic activity are also decreased, as are intracellular antimicrobial enzymes such as lysozyme and acid phosphatase (*27*). Splenocytes lose responsiveness to antigens and T-cell mitogens, and both thymic and splenic atrophy are seen (*27*). In children, ozone decreases CD4–CD8 T-cell ratios (*28*). Ozone damage appears to be mediated through reactive intermediates such as hydroxyl radicals, singlet oxygen, and peroxides that may oxidize lipid membranes and thiols and alter methylation and protein-binding sites on DNA (*25*). On the basis of these observations, it is intriguing to consider a putative role of ozone in either the establishment or the progression of an autoimmune disease. However, at present, there are no substantial data that address this issue.

UV light is also associated with the induction of systemic immunosuppression, and exposure of mice to UVB radiation (280 to 320 nm) results in the development of epidermal carcinoma (*29*). Indeed, UVB radiation is well established as a high risk factor in skin cancers in man. Reduction in delayed-type hypersensitivity response and bacterial resistance has also been shown for UVB exposure, although adaptation may occur with prolonged exposure (*30*). *In vitro*, UVB radiation inhibits mixed leukocyte reactions. Exposed allogeneic stimulators display decreased MHC class II expression and IL-2 release, whereas UVB-irradiated responders do not proliferate in response to allogeneic cell stimulators (*29*). Hence, immunosuppression appears to be caused by direct cellular damage and not induction of suppressor mechanism. There is no evidence as yet for the role of ozone or UVB radiation in the induction of autoimmune disease. Nonetheless, these are included as they may have potential effects, and ozone has already been shown to increase antigenicity of proteins, leading to increased IgE (*31*). Further work on UV irradiation is clearly required. At present, the association with autoimmune disease is primarily speculative based on *in vitro* data and extrapolation of immune responses in mice.

Organic Solvents

Systemic sclerosis incorporates a broad spectrum of immune-mediated disease. Its major diagnostic criterion, abnormal thickening of the skin (scleroderma) proximal to metacarpophalangeal joints, belies both its severity and the frequent involvement of

internal organs (*32*). Diffuse cutaneous and limited cutaneous forms of systemic sclerosis are widely accepted subdivisions of disease, and in both, antinuclear autoantibodies are common (~ 90%) (*32*).

An increased risk of systemic sclerosis appears to be associated with occupational exposure to organic solvents. A recent epidemiologic study confirmed solvent–disease associations with systemic sclerosis, particularly among patients positive for antibodies against the enzyme Scl-70 (a DNA topoisomerase I) (*33*). One-quarter of all systemic sclerosis patients in the United States have antibodies to Scl-70; these are marginally more prevalent in patients with the diffuse cutaneous form of disease (~35%) compared to those with limited cutaneous disease (*32*). The former showed a stronger association with organic solvent exposure than the latter (*33*). Although solvents may be categorized into different chemical groups such as aromatic hydrocarbons, aliphatic hydrocarbons, and chlorinated solvents, distinct association between disease and a single group is difficult. Solvents are often mixed, and their very nature as solvents means that the role of dissolved solutes cannot be excluded in disease. The mechanisms and genetics of susceptibility in VC disease may provide clues to the pathogenesis of organic solvent disease.

Silica

The most common misconceptions that have confused biologic data on silicon-containing molecules are either that silicon, silica, silicate, and silicone are roughly interchangeable terms, or that silica, silicate, and silicone describe single chemical entities. Through covalent bonds with oxygen, silicon shows extensive diversity in its degree of polymerization, which is mirrored in its subsequently varied chemistry. It is as incorrect, therefore, to ascribe a single biologic property to silicon-containing molecules as it would be for carbon-containing molecules.

Oxygen and silicon are the two most abundant elements of the earth's crust. Silicon rarely occurs in elemental form but binds with oxygen to form silica of general formula $[O–Si–O_3]^{4-}$. In its simplest form, silica exists as monomeric silicic acid ($SiOH_4$) which accounts for the vast majority (> 90%) of environmental soluble silica. However, this occurs in equilibrium with much lower levels of dimeric or trimeric soluble silica (*34*). Silicic acid is probably the major absorbable form of silica in man and has no known toxicity. In fact, experiments in rats and chickens suggest that it is essential for normal growth and development because of its interaction with collagen (*35,36*).

Silicic acid is stable in solutions up to 2 mM, but it rapidly polymerizes at higher concentrations (pH < 10) where the thermodynamically stable species is insoluble (solid-phase) silica (*34*). Degree of hydration (e.g., glass vs sand) and age determine the crystal structure (e.g., quartz vs amorphous silica), surface characteristics, and pathogenicity of particulate silica. Often cations such as sodium, potassium, magnesium, and calcium become incorporated within the silica matrix, forming silicates; invariably this also involves aluminum, which may partly substitute for silicon, forming aluminosilicates (*37*). Aluminosilicates are themselves a diverse group of silicates that make up rock and soil minerals of the earth, and have varying crystal structures, elemental composition, and pathogenicities. The weathering and cycling of silica and silicates provide a diverse range of silica species with physical forms and properties between soluble monomeric and solid-phase silicas (*34,38*). However, pathologic responses have only been associated with particulate forms of silica and silicates.

Silicone describes a family of man-made polymers containing the silicon–oxygen backbone and organic side groups such as methyl groups (*38,39*). Silicones are not of environmental origin and will not be further discussed here. It should be mentioned, however, that Naim et al. (*40*) have shown that different formulations of silicone, some of which are used in breast implants, have different adjuvant effects in delayed-type hypersensitivity reactions in rats. Clearly, as with silica, the varying chemical makeup and physical forms of silicones should be considered when assessing their toxicity.

Freshly fractured particulate silica, released typically during mining, is recognized as a cause of lung disease (silicosis) in certain exposed individuals (*41*). Its possible role in the induction of autoimmune disease is less well understood. In a survey of over 3,000 gold miners, Steenland and Brown (*42*) showed that although silicosis and tuberculosis were associated, as expected, with silica exposure, risk was also increased for autoimmune diseases, including arthritis, SLE, and scleroderma. In the opposite manner, Koeger et al. (*43*) reviewed 764 patients with autoimmune connective tissue disease and showed that in 3% of cases (n = 24), patients were exposed to silica chiefly through mining and sandblasting but also through less obvious routes such as sculpture work. Systemic sclerosis (see "Organic Solvents") was most prevalent, but rheumatoid arthritis, SLE, and dermatomyositis also occurred. Frequency of lung fibrosis and the crystalline silica content of bronchoalveolar lavage specimens confirmed exposure to silica in these patients, and in some, cessation of exposure induced disease remission, strongly suggesting a cause-and-effect relationship (*43*). In a Scandinavian study, Brown et al. (*44*) confirmed an increase in autoimmune disease among hospitalized silicotic patients, and others have specifically implicated the development of SLE in this patient group (*45,46*). Evidence is clearly increasing for a role of particulate, crystalline silica in the development of autoimmune disease in susceptible individuals. Epidemiologic studies aimed at further investigating these links need careful planning, as associations may previously have been missed because of inappropriate study designs (*47*).

Mechanisms of autoimmune induction following exposure to particulate silica are unclear, although an adjuvant effect is likely (see "Particulates"). Acquired humoral immunity (*48*), upregulation of soluble Fas (*49*), increased macrophage sensitivity to lipopolysaccharides (*50*), and reduced antigen presentation (*50*) have all been demonstrated. Observations on soluble Fas are particularly interesting given the recent findings that Fas and Fas ligand may be critically involved in the pathogenesis of Hashimoto's thyroiditis (*51*). Membrane Fas is expressed by many cells and induces apoptosis in the host once bound to the Fas ligand of an effector cell. The Fas ligand is expressed not only by cytotoxic T cells but also by cells in immunologically privileged sites such as the eye chamber and testes. Such cells protect themselves by inducing apoptosis in attacking T cells that bear membrane Fas. Indeed, the apoptotic elimination of immunocompetent effector cells is considered an important mechanism in the prevention of autoimmunity (*52*). In the thyroid and under select cytokine conditions, cells may express both Fas ligand and membrane Fas, leading to locally induced (fratricidal) apoptosis, so dysregulation of this mechanism would similarly have implications in autoimmunity (*51*). Soluble Fas, which is an alternatively spliced product of the *Fas* gene, antagonizes the binding between membrane Fas and the Fas ligand; elevated levels could inhibit apoptosis of self-recognizing clones, precipitating autoimmune responses at a later stage (*49*).

Particulates

There is increasing evidence from epidemiologic studies that microparticles (ultrafine or submicron particles) are associated with disease. Interest has focused on the lung because of the association between airborne microparticles and asthma (*53*). However, microparticles also make up an increasing proportion of the Western diet and have been implicated in the etiology of Crohn's disease (*54*). On average, 5 mg of titanium dioxide, for example, are now ingested per person per day in the United Kingdom (*55*), which alone makes an individual's daily intake about 10^{12} particles (*56*). Submicron-sized particles can be surprisingly

well absorbed in the gastrointestinal tract and their translocation is distinct from that of soluble species (*57*). Three distinct particle types have been consistently demonstrated in the intestinal lymphoid aggregates of man: aluminosilicates, mixed silicates, and titanium dioxide (*54*). These are food additives, naturally occurring contaminants from soil, or particles formed de novo in the environment or intestinal lumen (*54*). Similarly, in the atmosphere, car exhaust fumes, industrial pollution, and cigarette smoke have markedly increased our exposure to neoparticulates (*53*). A number of suggestions have been made to explain the pathogenicity of ultrafine particles, but we propose that their relationship with morbidity is due mainly to their influence on mucosal antigen processing.

In contrast to soil-derived particles, those that are newly formed in the environment or are man-made additives have active, charged surfaces and may adsorb luminal or environmental antigens (*58*). The differential processing and presentation of soluble and particulate antigens have been well described and may greatly affect the immune outcome. The processing of particle-bound antigen is qualitatively and quantitatively distinct to that of soluble antigen (*59–62*). Particulate antigen, processed and presented by macrophages or B cells, stimulates class II restricted T-cell hybrids up to 10^5-fold more efficiently than soluble antigen (*59*). Macrophages process a greater size range of particulate antigens compared to B cells, but both antigen-presenting cells (APCs) generate epitopes distinct from those from soluble antigen. Furthermore, Sedlik et al. (*60*) have shown that both T helper (Th)1 and Th2 cytokines are produced in response to immunization with particle-bound antigen, but in the presence of appropriate cofactors (e.g., IL-12), Th1 cells are selectively activated. In addition, particulate antigen, unlike soluble antigen, may gain entry to an alternative class I MHC pathway, most notably in macrophages (*61*). Following phagocytosis, solubilized antigen may escape the phagosome and enter conventional cytosolic class I processing. Alternatively, post-Golgi combination of phagocytosed, degraded antigen with class I MHC may occur either intravesicularly or at the cell surface following regurgitation. Recent data support an intravesicular mechanism (*61*). Although the trafficking of nascent class I MHC is rare, conventionally coupled class I MHC peptide may undergo pH-induced decoupling during passage of the acidic trans-Golgi network, allowing peptide exchange (*63*). Fewer studies have specifically investigated the processing of particulate antigen *in vivo*, although Maloy et al. (*64*) have shown that oral ovalbumin delivered in poly (lactide coglycolide) microparticles results in ovalbumin (OVA)-specific cytotoxic lymphocyte responses and generates intestinal IgA. Clearly, the influence of the physical form of antigen in dictating cellular uptake and processing in the lung and gastrointestinal tract should be carefully considered.

Anilides and L-Tryptophan

A number of mechanisms have been proposed by which environmental agents can modulate immune function and induce disease. These include oxidative damage, augmentation of antigen-specific immune responses that may cross react with self-tissues, and direct modulation of either the afferent or efferent immune system. It is also becoming clear that as with most other illnesses, environmental impacts will likely turn out to be under genetic control. Many of these principles are best illustrated by the epidemics of eosinophilic myalgia with shared characteristics that occurred nearly 20 years ago (*8*). For example, the toxic oil syndrome of Spain in 1981 involving cooking oil led to both acute and chronic disease as well as formation of autoantibodies to collagen, DNA, and skeletal muscle. Patients who developed the disease were exposed to adulterated rapeseed oil (*8*). The primary candidate for disease induction appeared to be fatty acid esters of oleylanilide and 3-(*N*-phenylamino)-1,2-propanediol. Patients had high circulating levels of IgE and autoantibodies to collagen, DNA, and skeletal muscle. Disease association was related to the HLA DR3 and DR4 haplotypes. The pathogenesis of toxic oil syndrome remains unclear. Recent studies of cytokine mRNA expression in lung tissue of affected patients have suggested that, as in animal models, both Th1 and Th2 cytokines are activated, although the balance may be in favor of Th2 expression (*65*). Indeed, it has been hypothesized that the associated increase in IFN-γ production may have activated indothiamine-2,3-dioxygenase, causing abnormally rapid metabolism of tryptophan (*8*). A more plausible mechanism of disease was provided by a mouse model of toxic oil syndrome (*66*), in which increased levels of mRNA for IFN-γ, IL-1β, and IL-6 occurred in splenocytes and circulating IgE was induced. It was suggested that anilide treatment led to a breakdown in tolerance, allowing polyclonal activation of B cells (*66*).

In 1989, a second epidemic of eosinophilic myalgia occurred from the intake of L-tryptophan supplements. Eosinophilia, myalgia, urticaria, pruritus, and fasciitis, as well as elevated C-reactive protein and antinuclear antibody were hallmarks of disease; again, genetic associations were noted (*8*). Supplements contaminated with 1-1′-ethylidenebis[tryptophan] were suspected, but purer L-tryptophan has also induced similar symptoms in animals and humans, suggesting that L-tryptophan per se contributes to disease induction. Following L-tryptophan administration, the Lewis rat exhibits many symptoms of eosinophilic myalgia, including enhanced tryptophan metabolism and fasciitis but not peripheral eosinophilia (*8*).

A number of mechanisms have been proposed, including increased secretion of indoleamines with decreasing IFN-γ secretion, leading to increased collagen synthesis and eosinophil activation. However, as above, other reports suggest that IFN-γ is important in the pathogenesis of eosinophilic myalgia. The metabolic products of L-tryptophan, kynurenine and serotonin, are considered fibrinogenic agents and the latter has been associated with scleroderma and toxic oil syndrome. Another L-tryptophan metabolite, picolinic acid, may activate macrophages for cytokine production. However, mechanisms of autoimmune disease caused by L-tryptophan and contaminated oil remain unclear (*8*).

Conclusion

There may be multiple mechanisms involved in the initiation and progression of autoimmune diseases and it is therefore unlikely that the study of autoimmune diseases will consistently generate "one inducer, one disease" etiologies (Table 3). Because nonliving agents do not have genomes, disease characteristics involving nonliving xenobiotics are primarily host functions. And because host genetic susceptibilities (e.g., MHC and differences in

Table 3. Proposed mechanisms for the induction of autoimmune disease by xenobiotics.

Alteration of self-molecules by xenobiotics or their metabolites to increase immunogenicity
Induction of immune responses that cross-react with self-molecules
Direct activation of Th cells that lead to autoimmune cytokine network
Oxidative cell damage
Incorporation into protein resulting in loss of function or increased immunogenicity
Alteration of apoptosis
Change in idiotype network

Th, T helper.

Table 4. Variables in onset and/or exacerbation of environmental autoimmune disease.

Environmental agents
Chemical characteristics
Route of exposure
Dose and duration of exposure
Host factors
Age and sex
General immune competence
Concomitant infection/environmental exposure
Cellular cytokine environment
Genetic factors
Major histocompatibility complex
Gene polymorphisms
Level of gene expression

toxin metabolism), lifestyles, and exposures to toxic agents differ, individuals may react differently to the same toxic compounds (Table 4). Alternatively, people may produce similar overt responses to different inducers. This could occur if different chemical species break tolerance mechanisms and expose common or similar autoreactive components. Such chemical inducers may not necessarily be immunogenic themselves. Autoantigens tend to be evolutionarily highly conserved and this might partly explain the immunologic similarities found among a wide array of autoimmune diseases. One might add that if the immune system evolved in response to infectious microbes, then it was not specifically designed to cope with the physical and chemical stresses augmented by recent human activity. Presently, the ability to categorize unique immunologic diseases with particular inducers may be more difficult with toxic agents than with microbial pathogens. However, the diagnosis and prevention of these chemically induced problems should improve after sufficient information has accumulated on the nature and fate of these inducers.

References and Notes

1. Luppi P, Rossiello MR, Faas S, Trucco M. Genetic background and environment contribute synergistically to the onset of autoimmune diseases. J Mol Med 73:381–393 (1995).
2. D'Haens GR, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 114:262–267 (1998).
3. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology 115:182–205 (1998).
4. 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 41:1714–1724 (1998).
5. Berking C, Hobbs MV, Chatelain R, Meurer M, Bell SA. Strain-dependent cytokine profile and susceptibility to oleic acid anilide in a murine model of the toxic oil syndrome. Toxicol Appl Pharmacol 148:222–228 (1998).
6. Clarkson TW. The toxicology of mercury. Crit Rev Clin Lab Sci 34:369–403 (1997).
7. Aschner M, Aschner JL. Mercury neurotoxicity: mechanisms of blood-brain barrier transport. Neurosci Biobehav Rev 14:169–176 (1990).
8. Yoshida S, Gershwin ME. Autoimmunity and selected environmental factors of disease induction. Semin Arthritis Rheum 22:399–419 (1993).
9. Pollard KM, Lee DK, Casiano CA, Bluthner M, Johnston MM, Tan EM. The autoimmunity-inducing xenobiotic mercury interacts with the autoantigen fibrillarin and modifies its molecular and antigenic properties. J Immunol 158:3521–3528 (1997).
10. Kubicka-Muranyi M, Griem P, Lubben B, Rottmann N, Luhrmann R, Gleichmann E. Mercuric-chloride-induced autoimmunity in mice involves up-regulated presentation by spleen cells of altered and unaltered nucleolar self antigen. Int Arch Allergy Immunol 108:1–10 (1995).
11. Rose NR, Saboori AM, Rasooly L, Burek CL. The role of iodine in autoimmune thyroiditis. Crit Rev Immunol 17:511–517 (1997).
12. Morris JC, Bergert ER, Bryant WP. Binding of immunoglobulin G from patients with autoimmune thyroid disease to rat sodium-iodide symporter peptides: evidence for the iodide transporter as an autoantigen. Thyroid 7:527–534 (1997).
13. Kahaly GJ, Dienes HP, Beyer J, Hommel G. Iodide induces thyroid autoimmunity in patients with endemic goitre: a randomised, double-blind, placebo-controlled trial. Eur J Endocrinol 139:290–297 (1998).
14. Bagchi N, Brown TR, Sundick RS. Thyroid cell injury is an initial event in the induction of autoimmune thyroiditis by iodine in obese strain chickens. Endocrinology 136:5054–5060 (1995).
15. Rasooly L, Burek CL, Rose NR. Iodine-induced autoimmune thyroiditis in NOD-H-2h4 mice. Clin Immunol Immunopathol 81:287–292 (1996).
16. Many MC, Maniratunga S, Varis I, Dardenne M, Drexhage HA, Denef JF. Two-step development of Hashimoto-like thyroiditis in genetically autoimmune prone non-obese diabetic mice: effects of iodine-induced cell necrosis. J Endocrinol 147:311–320 (1995).
17. Knobel M, Barca MF, Pedrinola F, Medeiros-Neto G. Prevalence of anti-thyroid peroxidase antibodies in autoimmune and nonautoimmune thyroid disorders in a relatively low-iodine environment. J Endocrinol Invest 17:837–842 (1994).
18. Kong YC, McCormick DJ, Wan Q, Motte RW, Fuller BE, Giraldo AA, David CS. Primary hormonogenic sites as conserved autoepitopes on thyroglobulin in murine autoimmune thyroiditis. Secondary role of iodination. J Immunol 155:5847–5854 (1995).
19. Chiang SY, Swenberg JA, Weisman WH, Skopek TR. Mutagenicity of vinyl chloride and its reactive metabolites, chloroethylene oxide and chloroacetaldehyde, in a metabolically competent human B-lymphoblastoid line. Carcinogenesis 18:31–36 (1997).
20. Lernmark A. Immune surveillance: paraneoplastic or environmental triggers of autoimmunity. Crit Rev Immunol 17:437–447 (1997).
21. Veltman C, Lange CE, Juhe S, Stein G, Bachner V. Clinical manifestations and course of vinyl chloride disease. Ann NY Acad Sci 246:6–17 (1975).
22. Montanaro A, Bardana EJ Jr. Dietary amino acid-induced systemic lupus erythematosus. Rheum Dis Clin North Am 17:323–332 (1991).
23. Prete PE. Membrane surface properties of lymphocytes of normal (DBA/2) and autoimmune (NZB/NZW) F_1 mice: effects of L-canavanine and a proposed mechanisms for diet-induced autoimmune disease. Can J Physiol Pharmacol 64:1189–1196 (1986).
24. Alcocer-Varela J, Iglesias A, Llorente L. Effects of L-canavanine on T cells may explain the induction of systemic lupus erythematosus by alfalfa. Arthritis Rheum 28:52–57 (1985).
25. Pryor WA, Church DF. Aldehydes, hydrogen peroxide, and organic radicals as mediators of ozone toxicity. Free Radic Biol Med 11:41–46 (1991).
26. Piver WT. Global atmospheric changes. Environ Health Perspect 96:131–137 (1991).
27. Wright ES, Dziedzic D, Wheeler CS. Minireview. Cellular, biochemical and functional effects of ozone: new research and perspectives on ozone health effects. Toxicol Lett 51:125–145 (1989).
28. Zwick H, Popp W, Wagner C. Effects of ozone on the respiratory health, allergic sensitization, and cellular immune system in children. Am Rev Respir Dis 144:1075–1079 (1991).
29. Deeg HJ. Overview. Ultraviolet irradiation in transplantation biology. Manipulation of immunity and immunogenicity. Transplantation 45:845–851 (1988).
30. Jeevan A, Kripke ML. Alteration of the immune response to Mycobacterium bovis BCG in mice exposed chronically to low doses of UV radiation. Cell Immunol 130:32–41 (1990).
31. Behrendt H, Becker WM, Fritzsche C, Sliwa-Tomczok W, Tomczok J, Friedrichs KH, Ring J. Air pollution and allergy: experimental studies on modulation of allergen release from pollen by air pollutants. Int Arch Allergy Immunol 113:69–74 (1997).
32. Clements PJ, Furst DE, eds. Systemic Sclerosis. Baltimore:Williams & Wilkins, 1996.
33. Nietert PJ. Is occupational organic solvent exposure a risk factor for scleroderma? Arth Rheum 41:1111 (1998).
34. Iler RK. The Chemistry of Silica Solubility, Polymerisation, Colloid and Surface Properties, and Biochemistry. New York:John Wiley & Sons, 1979.
35. Carlisle EM. Silicon as an essential element for the chick. Science 178:619–621 (1972).
36. Schwarz K, Milne DB. Growth promoting effects of silicon in rats. Nature 239:333–334 (1972).
37. Hem JD. Geochemistry and aqueous chemistry of aluminum. In: Kidney International, Vol 29 (Coburn JW, Alfrey AC, eds). New York:Springer-Verlag, 1986:S3-S7.
38. Taylor PD, Jugdaohsingh R, Powell JJ. Soluble silica with high affinity for aluminum under physiological and natural conditions. J Am Chem Soc 119:8852–8856 (1997).
39. Yoshida SH, Teuber SS, German JB, Gershwin ME. Immunotoxicity of silicone: implications of oxidant balance towards adjuvant activity. Food Chem Toxicol 32:1089–1100 (1994).
40. Naim JO, Ippolito KM, van Oss CJ. Adjuvancy effect of different types of silicone gel. J Biomed Mater Res 37:534–538 (1997).
41. NashT, Allison AC, Harington JS. Physico-chemical properties of silica in relation to its toxicity. Nature 210:259–261 (1966).
42. Steenland K, Brown D. Mortality study of gold miners exposed to silica and nonasbestiform amphibole minerals: an update with 14 more years of follow-up. Am J Ind Med 27:217–229 (1995).
43. Koeger AC, Lang T, Alcaix D, Milleron B, Rozenberg S, Chaibi P, Arnaud J, Mayaud C, Camus JP, Bourgeois P. Silica-associated connective tissue disease. A study of 24 cases. Medicine 74:221–237 (1995).
44. Brown LM, Gridley G, Olsen JH, Mellemkjaer L, Linet MS, Fraumeni JF Jr. Cancer risk and mortality patterns among silicotic men in Sweden and Denmark. J Occup Environ Med 39:633–638 (1997).
45. Wilke RA, Salisbury S, Abdel-Rahman E, Brazy PC. Lupus-like autoimmune disease associated with silicosis. Nephrol Dial Transplant 11:1835–1838 (1996).
46. Conrad K, Mehlhorn J, Leuthke K, Deorner T, Frank KH. Systemic lupus erythematosus after heavy exposure to quartz dust in uranium mines: clinical and serological characteristics. Lupus 5:62–69 (1996).
47. Steenland K, Goldsmith DF. Silica exposure and autoimmune diseases. Am J Ind Med 28:603–608 (1995).
48. Cojocaru M, Niculescu T, Spaetaru E. Study of lung antibodies in patients with silicosis. Romanian J Int Med 33:243–247 (1995).
49. Otsuki T, Sakaguchi H, Tomokuni A, Aikoh T, Matsuki T, Kawakami Y, Kusaka M, Ueki H, Kita S, Ueki A. Soluble Fas mRNA is dominantly expressed in cases with silicosis. Immunology 94:258–262 (1998).
50. Rao TD, Frey AB. Administration of silica sensitizes lipopolysaccharide responsiveness of murine macrophages but inhibits T and B cell priming by inhibition of antigen presenting function. Immunol Invest 27:181–199 (1998).
51. Giordano C, Stassi G, De Maria R, Todaro M, Richiusa P, Papoff G, Ruberti G, Bagnasco M, Testi R, Galluzzo A. Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. Science 275:960–963 (1997).
52. Strater J, Moller P. Pathogenesis of primary biliary cirrhosis: CD95-induced apoptosis at last? Eur J Gastroenterol Hepatol 10:539–541 (1998).
53. Fubini B. Surface reactivity in the pathogenic response to particulates. Environ Health Perspect 105:1013–1020 (1997).
54. Powell JJ, Ainley CC, Harvey RSJ. Characterisation of inorganic microparticles in pigment cells of human gut associated lymphoid tissue. Gut 38:390–395 (1996).
55. Ministry of Agriculture Fisheries and Food. Dietary Intake of Food Additives in the UK: Initial Surveillance. Food Surveillance Paper no 37. London:HMSO, 1993.
56. Churg A. The uptake of mineral particles by pulmonary epithelial cells. Am J Respir Crit Care Med 154:1124–1140 (1996).
57. Desai MP, Labhasetwar V, Amidon GL, Levy RJ. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharmacol Res 13:1838–1845 (1996).
58. Li XY, Gilmour PS, Donaldson K, MacNee W. *In vivo* and *in vitro* proinflammatory effects of particulate air pollution (PM_{10}). Environ Health Perspect 105:1279–1283 (1997).
59. Vidard L, Kovacsovics-Bankowski M, Kraeft SK, Chen LB, Benacerraf B, Rock KL. Analysis of MHC class II presentation of particulate antigens by B lymphocytes. J Immunol 156:2809–2818 (1996).
60. Sedlik C, Deriaud E, Leclerc C. Lack of Th1 or Th2 polarization of $CD4^+$ T cell response induced by particulate antigen targeted to phagocytic cells. Int Immunol 9:91–103 (1997).
61. Song R, Hardin CV. Roles of proteasomes, transporter for antigen presentation (TAP) and β_2-microgobulin in the processing of bacterial or particulate antigens via an alternative class I MHC processing pathway. J Immunol 156:4182–4190 (1996).
62. Kovacsovics-Bankowski M, Clark K, Benacerraf B, Rock KL. Efficient major histocompatibility complex class I presentation of exogenous antigen upon phagocytosis by macrophages. Proc Natl Acad Sci USA 90:4942–4946 (1993).
63. Stryhn A, Pedersen L, Romme T. pH dependence of MHC class I-restricted peptide presentation. J Immunol 156:4191–4197 (1996).
64. Maloy KJ, Donachie AM, O'Hagan DT, Mowat AM. Induction of mucosal and systemic immune responses by immunization with ovalbumin entrapped in poly(lactide-co-glycolide) microparticles. Immunology 81:661–667 (1994).
65. Del Pozo V, de Andraes B, Gallardo S, Caardaba B, de Arruda-Chaves E, Cortegano MI, Jurado A, Palomino P, Oliva H, Aguilera B, et al. Cytokine mRNA expression in lung tissue from toxic oil syndrome patients: a TH2 immunological mechanism. Toxicology 118:61–70 (1997).
66. Bell SA, Hobbs MV, Rubin RL. Isotype-restricted hyperimmunity in a murine model of the toxic oil syndrome. J Immunol 148:3369–3376 (1992).