Environmentally Induced Autoimmune Diseases: Potential Mechanisms

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Environmental and other xenobiotic agents can cause autoimmunity. Examples include drug-induced lupus, toxic oil syndrome, and contaminated L-tryptophan ingestion. Numerous mechanisms, based on *in vitro* evidence and animal models, have been proposed to explain how xenobiotics induce or accelerate autoimmunity. The majority of these can be divided into three general categories. The first is those inhibiting the processes involved in establishing tolerance by deletion. Inhibiting deletion can result in the release of newly generated autoreactive cells into the periphery. The second mechanism is the modification of gene expression in the cells participating in the immune response, permitting lymphocytes to respond to signals normally insufficient to initiate a response or allowing the antigen-presenting cells to abnormally stimulate a response. Abnormal gene expression can thus disrupt tolerance maintained by suppression or anergy, permitting activation of autoreactive cells. The third is the modification of self-molecules such that they are recognized by the immune system as foreign. Examples illustrating these concepts are presented, and related mechanisms that have the potential to similarly affect the immune system are noted. Some mechanisms appear to be common to a variety of agents, and different mechanisms appear to produce similar diseases. However, evidence that any of these mechanisms are actually responsible for xenobiotic-induced human autoimmune disease is still largely lacking, and the potential for numerous and as yet unidentified mechanisms also exists. *Key words*: anergy, autoimmunity, deletion, mechanisms, suppression, tolerance, xenobiotic.— *Environ Health Perspect* 107(suppl 5):737–742 (1999).
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Irrefutable evidence shows that xenobiotic agents can cause autoimmunity. Examples include drug-induced lupus (*1*), toxic oil syndrome (*2,3*), and contaminated L-tryptophan ingestion (*4,5*). Numerous mechanisms, based on *in vitro* evidence and animal models, have been proposed to explain how xenobiotics may induce or accelerate autoimmunity. This report is a review of potential mechanisms, which are illustrated with specific examples. However, despite the body of literature reviewed herein, evidence that the proposed mechanisms are operant in people with xenobiotic-induced autoimmunity is still largely lacking. For example, the mechanisms involved in the pathologic processes by which drugs induce lupuslike autoimmunity have not yet been elucidated in humans. The multiple potential mechanisms, together with our lack of understanding of mechanisms involved in the human diseases, highlight a need for further study in this area.

Tolerance

Most proposed mechanisms of autoimmunity involve cells of the immune system escaping tolerance. Tolerance refers to the phenomenon in which the immune system responds to and eliminates foreign organisms or molecules but ignores the host. Autoimmunity results when homeostatic mechanisms fail and the immune system responds to the host. The tolerance of host molecules is not inherent to the immune system but is acquired during development and actively maintained throughout life. Multiple mechanisms are used to silence potentially autoreactive lymphocytes. Most of the mechanisms involve either deletion, anergy, or suppression (*6–8*). Deletion refers to the elimination of self-reactive T and B lymphocytes by apoptosis and occurs during maturation as well as in mature lymphocytes. By its nature, deletion is irreversible, but the processes involved must be continuously maintained to prevent the generation of new autoreactive cells. Anergy is the induced unresponsiveness to conventional antigenic stimulation, and the anergic lymphocytes survive as functionally inactive cells. T-cell anergy can be induced by multiple mechanisms such as antigen presentation in the absence of costimulatory signals like those provided by the B7/CD28 molecules, which normally support T-cell activation through the T-cell antigen receptor (TCR) (*9*). Anergy can be reversible (*6–10*) and thus also requires continuous maintainence. Suppression is also an active process in which responses to self-antigens are suppressed by other cells and factors derived from them (*8*). These processes are discussed in greater detail in the sections that follow. The active nature of immune tolerance implies that events or chemicals that inhibit the mechanisms maintaining tolerance have the potential of allowing the generation or activation of autoreactive cells, with the subsequent development of autoimmunity. Because the systems involved are complex, there are a correspondingly large number of ways by which tolerance can be disrupted.

Mechanisms Disrupting Tolerance

Most of the mechanisms by which xenobiotics disrupt tolerance can be grouped into three general categories. The first is those inhibiting the processes involved in establishing tolerance by deletion, permitting release of autoreactive cells in the periphery. The second is modification of gene expression in the cells participating in the immune response, permitting lymphocytes to respond to signals normally insufficient to initiate a response or allowing the antigen-presenting cells to abnormally stimulate a response. Abnormal gene expression can thus disrupt tolerance maintained by anergy or suppression and permit activation of autoreactive cells. The third is the modification of self-molecules such that they are recognized by the immune system as foreign. These categories are not necessarily mutually exclusive, and some xenobiotics may affect tolerance by multiple mechanisms. Examples illustrating these concepts are presented in the sections that follow and are summarized in Table 1.

Abnormalities of Tolerance Induction by Deletion

The *lpr* and *gld* mouse strains offer two of the clearest demonstrations that abnormalities of deletion can result in systemic autoimmunity (*11–13*). These strains develop a lupuslike disease that includes the production of antinuclear antibodies as well as massive lymphoid proliferation. The *lpr* and *gld* mutations result in the functional loss of genes encoding Fas and Fas ligand (FasL), respectively, which are involved in triggering apoptotic cell death (*11,12*). During normal thymic development, potentially autoreactive thymocytes are eliminated by apoptosis, triggered by Fas–FasL interactions (central tolerance). A similar process can occur in the periphery (peripheral

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Table 1. Potential mechanisms of xenobiotic-induced autoimmunity.

Xenobiotics may break tolerance by
Inhibiting deletion of autoreactive cells
Modifying gene expression to reverse anergy or prevent suppression by
Modifying chromatin structure
Stimulating or inhibiting signaling molecules
Stimulating or inhibiting cytokine receptors
Acting as endocrine disruptors
Altering antigenicity of self-molecules by
Acting as haptens
Cleaving self-molecules to generate cryptic epitopes
Acting as superantigens

tolerance) (*12*). Mice with mutations in Fas or FasL fail to eliminate the autoreactive T cells, resulting in the subsequent development of autoimmunity (*11–13*). Humans with defects in these genes develop autoimmune hemolytic anemia, thrombocytopenia, and neutropenia, supporting the association with autoimmunity (*14*). These examples provide persuasive evidence that inhibiting deletion can produce an autoimmune disease and raise the possibility that xenobiotics might have a similar effect. Because the mechanisms identifying the potentially autoreactive cells are not well understood, the possibility also exists that xenobiotics could disrupt the selection process, resulting in release of autoreactive cells. However, this will remain speculative until the mechanisms identifying the autoreactive cells are better characterized.

Other animal models demonstrate that exogenous agents can affect central tolerance to produce autoimmunity. Cyclosporine A (CsA), an immunosupressant believed to act by blocking T-cell signal transduction (*15*), can interfere with central tolerance induction. Lethally irradiated, bone marrow-reconstituted mice will develop autoimmunity if given high-dose CsA. These mice develop T-lymphocyte and macrophage-rich inflammatory lesions in the colon, stomach, liver, and pancreas. The autoimmune disease can be tranferred by injecting T lymphocytes from affected mice into naive genetically identical (syngeneic) recipients, indicating that autoreactive lymphocytes are responsible. In this model, CsA appears to prevent thymic deletion by blocking signaling during the negative thymic selection step. This prevents activation of autoreactive thymocytes by self-molecules and thus prevents their subsequent apoptotic elimination (*16*).

Radiation can also interfere with tolerance. High-dose fractionated total lymphoid irradiation causes a variety of organ-specific autoimmune diseases in BALB/c mice. These include autoimmune gastritis with antiparietal cell antibodies, thyroiditis with antithyroglobulin antibodies, sialoadenitis, and orchitis, depending on the radiation dose. $CD4^+$ T cells from these mice adoptively transfer the autoimmunity to naive syngeneic mice, suggesting that irradiation is interfering with mechanisms inducing T-cell tolerance or anergy in these mice (*17*). Neonatal thymectomy produces a similar $CD4^+$ T-cell–mediated autoimmune disease (*18*), which further supports this concept. An autoimmune disorder characterized by skin rash and occasionally autoimmune thrombocytopenia or hemolytic anemia has been reported in breast cancer patients receiving autologous stem cell transplantations (*19*), suggesting that a similar disorder may occur in humans.

Disruption of central tolerance may play a role in drug-induced lupus. Procainamide can be metabolized to procainamide hydroxylamine (PAHA) by reactive oxygen species. Injecting PAHA into the thymus of nonautoimmune mice induces IgG antichromatin antibodies that are sustained over several months (*20*). *In vitro* studies have shown that PAHA transiently prevents the induction of anergy in mature T lymphocytes (*21*), suggesting that PAHA may similarly prevent tolerance established during thymic development. The PAHA model illustrates the principle that small organic molecules can disrupt central tolerance to result in the production of antinuclear antibodies. However, evidence that this process contributes to human drug-induced lupus is still lacking.

Modification of Cellular Gene Expression

Evidence indicates that xenobiotics can break tolerance by modifying gene expression in cells participating in immune responses. In several of these systems a valid link to autoimmunity has been established using animal models. Xenobiotics can modify gene expression in lymphocytes as well as antigen-presenting cells by acting on receptors, their signaling pathways, or at the DNA level. Examples include endocrine disrupters and agents that mimic or inhibit the effects of cytokines on their receptors, agents affecting kinases and phosphatases to activate or inhibit signaling, and agents that modify chromatin structure to affect gene expression. Changes in gene expression caused by any of these mechanisms may either break tolerance by modifying mechanisms involved in suppression or anergy or augment a tendency to develop autoimmunity.

Xenobiotic Modification of Chromatin Structure and Gene Expression

Changes in T-cell DNA methylation have been implicated in the development of autoimmunity. DNA methylation refers to the postsynthetic methylation of deoxycytosine (dC) residues at the 5 position. Methylation of dC residues in promoter sequences can suppress gene expression through mechanisms involving specific methylcytosine-binding proteins and changes in chromatin structure (*22,23*). These changes prevent transcription factors from interacting with promoter sequences, thereby suppressing gene expression. DNA methylation patterns are established during ontogeny, then maintained through subsequent mitoses by DNA methyltransferase (MTase), which replicates methylation patterns in newly synthesized DNA (*24*). Pharmacologic inhibition of DNA MTase with the nucleoside analog 5-azacytidine causes overexpression of the adhesion molecule lymphocyte function-associated antigen 1 (LFA-1) as well as autoreactivity in cloned, antigen-specific $CD4^+$ T cells (*25*). The autoreactivity is due to the LFA-1 overexpression because causing LFA-1 overexpression by transfection causes a similar autoreactivity (*26,27*). Therapeutic concentrations of procainamide and hydralazine also inhibit DNA methylation, increase LFA-1 expression, and cause autoreactivity in T-cell lines (*26–29*). Adoptive transfer of murine T cells rendered autoreactive by treatment with 5-azacytidine, procainamide, or hydralazine into nonautoimmune syngeneic mice leads to widespread autoimmune disease with anti-DNA antibodies, proliferative glomerulonephritis, pulmonary alveolitis, liver lesions resembling primary biliary cirrhosis, and histologic changes in the brain resembling central nervous system lupus (*30,31*). Ultraviolet light, implicated in triggering lupus flares, has a similar effect on T cells (*26*). These findings suggest a model for xenobiotic induction of lupuslike autoimmunity in which inhibiting DNA methylation in T lymphocytes modifies gene expression and induces autoreactivity, and the autoreactive cells can then cause a lupuslike disease. In support of this, T cells from patients with active lupus have a reduction of total genomic deoxymethylcytosine content and overexpress LFA-1 on an autoreactive T-cell subset, the size of which strongly correlates with disease activity (*25,32*). However, these findings have yet to be identified in patients with drug-induced lupus.

Xenobiotics can also affect histone acetylation with effects on gene expression. The acetylation of histones is associated with gene expression, whereas deacetylation correlates with transcriptional suppression (*33*). An association between DNA methylation and histone deacetylation has been proposed, as methylcytosine-binding proteins physically associate with histone deacetylase, directing the deacetylase activity to regions of chromatin destined for inactivation (*23*). Similar to DNA MTase, histone deacetylase is susceptible to inhibition by xenobiotics with effects on gene expression, which are

frequently synergistic with DNA MTase inhibitors (*34*). Inhibitors of histone deacetylase include sodium butyrate (*35*) and trichostatin A (*34,35*). Treating cells with histone deacetylase inhibitors can alter cell morphology, induce hemoglobin F production, and modify T-cell cytokine expression (*34,36*). Altered cytokine production can affect tolerance (vide infra), suggesting a mechanism by which histone acetylase inhibitors might contribute to autoimmunity. Histone deacetylase inhibitors also reactivate retroviral expression (*36*). Reactivation of latent retroviruses has been proposed as a mechanism contributing to the development of autoimmunity (*37*), although this link to autoimmunity also remains theoretical.

Adsenosine disphosphate (ADP)-ribosylation is yet another mechanism involved in chromatin formation with effects on gene expression. Poly(ADP-ribosyl) (PADPR) transferase is a chromatin-bound enzyme that catalyzes the transfer of ADP-ribose moieties from nicotinamide adenine dinucleotide (NAD) to chromatin proteins, principally histones H1 and H2B. ADP-ribosylation prevents methylation of genes by modifying histone H1 (*38,39*), thus participating with DNA methylation and histone acetylation in modifying chromatin structure. Several authors have implicated changes in polyADP-ribosylation with autoimmunity. Histones coupled to nucleic acids exhibit augmented immunogenicity, and antibodies against poly(ADP-ribose) occur in idiopathic systemic lupus erythematosus (SLE) and drug-induced lupus (*40*). In addition, both procainamide and hydralazine upregulate PADPR polymerase activity in lymphocyte cell lines (*41*), which could inhibit DNA methylation indirectly (*38,39*). Besides affecting chromatin, poly(ADP-ribosylation) of T-cell–surface proteins including LFA-1, CD8, CD27, CD43, CD44, and CD45 is known to occur. NAD treatment increases poly(ADP-ribosylation) of cell-surface proteins and inhibits antigen-stimulated responses (*42*). Further, NAD-treated cells fail to efficiently home to lymphoid organs, suggesting direct effects on adhesion molecule expression or function (*42*). Finally, a gene contributing to familial lupus has recently been identified as PADPR polymerase (*43*), and defects in PADPR polymerase have been associated with idiopathic SLE (*44,45*). These observations provide tantalizing clues as to how ADP-ribosylation might contribute to autoimmunity, but again, mechanisms directly linking changes in histone ADP-ribosylation to autoimmunity have not been established.

In summary, DNA methylation, histone acetylation, and histone polyADP-ribosylation all contribute to chromatin structure and thereby to gene regulation. All are susceptible to modification by xenobiotics, usually in a synergistic fashion, and at least two lupus-inducing drugs, procainamide and hydralazine, have effects on this regulatory mechanism. This area appears to represent a potentially fruitful approach to modification of cells by xenobiotics, with direct relevance to autoimmunity.

Endocrine Disruptors

Considerable experimental evidence supports an immunomodulatory role for female sex hormones (*46*). Females generally have more robust humoral and cellular immune responses than males. This has been proposed as an explanation for the increased prevalence of autoimmune disease in women (*46*). Female sex hormones have been directly implicated in contributing to increased disease severity in murine models of lupus (*46–48*), and oral contraceptives have been associated with human lupus (*49*). Thymocytes, T cells, B cells, macrophages, and endothelial cells express estrogen receptors (*50,51*), and treating immunocytes with estrogens causes enhanced protooncogene expression, modified cytokine production, changes in immune cell apoptosis, and altered adhesion molecule expression, which may augment an immune response (*50*). Recently, lymphocytes have been shown to traffic differently in male and female mice, and the differential trafficking to be directly related to increased severity of autoimmunity in the female mice. Oophorectomy diminished disease severity and produced a trafficking pattern resembling that of males, providing yet another mechanism by which female sex steroids may affect autoimmunity (*52*). This sensitivity of the immune system to hormonal modulation suggests a mechanism by which xenobiotic endocrine disrupters may contribute to autoimmunity. Numerous examples of chemicals with estrogenlike activity have been described and include resveratrol, a phytoestrogen present in grapes and a variety of medicinal plants (*53*), and phenol red, a widely used pH indicator (*54*). Others are discussed elsewhere (*53*). Although the mechanism by which xenobiotic endocrine disrupters contribute to autoimmunity remains obscure, the potential contribution of these agents to autoimmunity remains a valid concern.

Cytokines

Cytokines can also modify gene expression to break tolerance. For example, interferon (IFN)-γ upregulates class II major histocompatability complex (MHC) molecule expression on nonlymphoid tissues such as murine intestinal epithelial cells, allowing antigen presentation to $CD4^+$ T lymphocytes (*55*). Overexpressing IFN-γ on islet cells leads to the development of insulitis and diabetes in mice, presumably by this mechanism, demonstrating the pathologic consequences of inappropriate cytokine secretion or other activity that induces expression of these molecules (*56*). Xenobiotics have the potential of having similar effects on cells. For example, pharmacologic concentrations of alimemazine, a phenothiazene, induces de novo MHC class II molecule expression on cultured thyroid epithelial cells, offering a novel explanation for the induction of thyroid gland-specific autoreactivity (*57*), although *in vivo* evidence supporting this hypothesis remains to be established. Other xenobiotics could have similar effects.

In a related example, exposure to silica has been associated with the development of fibrotic and other connective tissue diseases, including rheumatoid arthritis, scleroderma, and lupus (*58*). Pulmonary interstitial fibrosis is also encountered. *In vitro* studies have shown that silica can activate macrophages to secrete cytokines including interleukin (IL)-1 and tumor necrosis factor (TNF), and increase surface Fc receptor and MHC class II molecule expression (*58,59*). It has been proposed that secretion of these cytokines and possibly other functions of silica-activated macrophages contribute to the fibrosis and possibly the induction of silica-induced autoimmunity.

Xenobiotics could also interfere with tolerance mediated by suppression, through effects on cytokines and chemokines. The concept of immune suppression has been resurrected by the recent observation that certain cytokines and chemokines have suppressive effects in selected systems. For example, transforming growth factor β (TGF-β) can suppress immune responses, and knockout mice lacking TGF-β develop inflammatory lesions in various organs including the heart and stomach (*60,61*). Local TGF-β production can also establish localized tolerance (*62*). These observations raise the theoretical possibility that xenobiotics interfering with TGF-β actions could induce autoimmunity. Another example involves T-cell subsets. $CD4^+$ T cells can be divided into subsets defined by their cytokine secretion repertoire. T-helper (Th)0 precursor cells produce a relatively wide variety of cytokines but mature into Th1 cells participating in delayed-type hypersensitivity responses and secreting IFN-γ, IL-2, and lymphotoxin, or mature into Th2 cells promoting antibody synthesis and secreting IL-4, IL-5, IL-6, and IL-10 (*63*). The maturation of Th0 into Th1 or Th2 cells can be influenced by cytokines, with IFN-γ suppressing IL-4 production and promoting Th1 differentiation, and IL-4 and IL-10 suppressing IFN-γ and promoting Th2 differentiation (*63*). This paradigm has been successfully applied to experimental systems such as collagen-induced arthritis, where a Th1 response is responsible for disease manifestations and cytokines

promoting Th2 differentiation suppress the disease (*64*). Xenobiotics have the potential to similarly modify immune responses. A recent example is the demonstration that DNA methylation inhibitors together with histone deacetylase inhibiors will modify IFN-γ and IL-4 expression in Th1 and Th2 cells (*65*).

Modification of chemokines can have a similar regulatory effect. For example, corneal endothelial and lens epithelial cells express little or no MHC class I molecules and are thus susceptible to natural killer (NK) cell-mediated killing. This is prevented by local inhibitory factors in the aqueous humor such as macrophage inhibition factor (MIF), which prevents perforin granule release from NK cells; authors have proposed that this suppressive mechanism prevents immune-mediated destruction of these cells (*66*). Agents interfering with MIF action would thus permit an autoimmune reaction.

In another example of suppression, TNF inhibits lupus nephritis in animal models, suggesting a suppressive role for this cytokine in lupuslike diseases (*67*). Administering anti-TNF agents to patients with rheumatoid arthritis has induced lupuslike serologies (*68*), raising the possiblity that xenobiotic agents that inhibit TNF production or action could remove this suppressive effect and contribute to the development of lupuslike diseases, further supporting the concept that interference with suppression may contribute to human autoimmunity.

Signaling Modifiers

There are examples of xenobiotics modifying signaling with the potential of contributing to autoimmunity. Small organic molecules capable of specifically inhibiting signaling pathways have recently been described, raising the possibility that xenobiotics could have similar effects. For example, a selective Mek1 inhibitor is available that inhibits signaling through the Ras-MAPK pathway (*69*). Treating T lymphocytes with this compound decreases DNA MTase levels, which theoretically contributes to the development of lupuslike diseases (*70*).

Programmed cell death is also regulated by signaling pathways that could be susceptible to interference by xenobiotics similar to those affecting other signaling pathways. The observation that benzo[*a*]pyrenes alter signaling (*71*) and induce apoptosis (*72*) *in vitro* supports this concept. Abnormal cell death with release of normally sequestered antigens could theoretically contribute to autoimmunity. Release of sequestered antigens as the antigenic stimulus in lupuslike diseases has support from the DNA hypomethylation model of drug-induced lupus. In this model, the autoreactive T cells home to lymphoid tissue where they induce macrophage apoptosis. Release of DNA from the apoptotic cells has been proposed as the source of antigenic DNA for anti-DNA antibody synthesis in this model (*30,31,52*). Others have proposed that apoptotic cells provide autoantigens as well (*73,74*).

Finally, small organic molecules can also modify gene expression directly. Pyrrole and imidazone polyamides can permeate living cells and inhibit transcription of specific genes (*75*), which could potentially play a role in autoimmune as well as other diseases. The immunologic effects of these agents have not yet been explored.

The DNA hypomethylation, endocrine disruption, and cytokine models all demonstrate that modification of cells participating in immune responses can contribute to autoimmunity. The number of potential mechanisms by which xenobiotics can modify cells is large. It is likely that as yet unanticipated mechanisms will be identified as our understanding of signaling pathways and regulation of gene expression improves. This topic represents an underdeveloped but potentially interesting area for study.

Antigen Modification

Another category of potential mechanisms for xenobiotic-induced autoimmunity is the modification of self-proteins to break tolerance and induce an immune response. This concept has its roots in early studies on haptens and has been extended by the newer concepts of cryptic epitopes and epitope spreading. A cryptic epitope is a new antigenic determinant, or epitope, created by the cleaving of a molecule. Epitope spreading refers to the generation of immune responses to antigenic determinants, or epitopes, physically adjacent to the immunizing epitope. Extensive experimental evidence exists to support the concepts of haptenization and cryptic epitopes in autoimmunity, although as in the other proposed mechanisms, direct proof of causation needs confirmation in patients or animals with xenobiotic-induced diseases.

Haptenization

The general concept of haptenization is that proteins can be chemically modified by combination with small reactive compounds called haptens, improving antigenicity. Early studies demonstrated that covalent coupling of haptens such as trinitrophenol to proteins produced potent antigens (*76*). Urushiol, the irritant in poison ivy, is a clinically relevant example of this (*76*). Once a response is initiated, the phenomenon of epitope spreading may occur, in which antigenic epitopes associated with but physically distinct from the hapten are progressively recognized by responding T and B cells (*77*). The phenomenon of epitope spreading is clearly demonstrated in the development of the immune response to small ribonucleoproteins in lupus (*78*). The concept of haptenization of self-proteins, together with epitope spreading, has prompted the hypothesis that some xenobiotics induce autoimmunity to self-proteins through a similar mechanism.

Numerous examples illustrating this concept have been described. Heavy metals have been implicated in triggering some forms of autoimmunity (*76*). Heavy metal ions of mercury, gold, and nickel will bind proteins, altering molecular and antigenic properties. Mercuric chloride can induce antifibrillarin antibodies in susceptible mouse strains (*79*), and mercury ions will bind fibrillarin, potentially altering its antigenicity (*80*). Hydralazine, procainamide, and to a lesser degree, isoniazid and D-penicillamine change the usual right-handed helical configuration of DNA (B DNA) to a more immunogenic left-handed helical configuration (Z DNA) *in vitro* (*81*). This conversion has been proposed as a mechanism contributing to the development of anti-DNA antibodies in drug-induced lupus (*81*). Others have shown that bioreactive metabolites of phenytoin can covalently bind and modify MHC molecules (*82*). These authors have proposed that the modified MHC molecules trigger a T-cell response analogous to chronic graft versus host disease, which has features of a variety of systemic autoimmune diseases including lupus, rheumatoid arthritis, and scleroderma (*83*). Similarly, oxidized metabolites of vinyl chloride, implicated in inducing a sclerodermalike disease, can bind sulfhydryl and amino groups, which also potentially modifies antigenicity or function (*84*). However, persuasive evidence demonstrating that these mechanisms contribute to autoimmunity *in vivo* is still lacking.

Cryptic Epitopes

The cryptic epitope hypothesis is based on experiments demonstrating that T cells will respond to peptide fragments of self-molecules not normally presented by antigen-presenting cells. In general, proteins are degraded enzymatically and the resultant peptide fragments presented in MHC molecules at the cell surface. The immune system is usually tolerant to these self-peptides. However, if the peptides are cleaved differently then presented, novel epitopes may be revealed and a response generated that may then also undergo epitope spreading (*85*).

Gold ions are capable of cleaving proteins to generate cryptic epitopes. Gold salts are implicated in autoimmune thrombocytopenia and immune complex glomerulonephritis through unknown mechanisms (*76*). Au(III), a reactive metabolite of gold, can cleave bovine ribonuclease A *in vitro* and generate cryptic epitopes capable of T-cell sensitization (*76*). Heavy metals can also catalyze oxidation

reactions mediated by reactive oxygen species. Several scleroderma autoantigens, including topoisomerase I, the large subunit of RNA polymerase II, and the 70-kD subunit of the U1 small nuclear ribonuclear protein are cleaved by metal-catalyzed (Fe, Cu) oxidation reactions, which may produce cryptic epitopes. Because episodic ischemia–reperfusion such as that seen in the vasospasm associated with scleroderma can generate reactive oxygen species, this mechanism may theoretically contribute to some of the autoantibodies seen in this disease (*86*), although *in vivo* evidence is still lacking.

Other Mechanisms

Mechanisms proposed for infectious or other etiologies of autoimmunity may also be applied to xenobiotics, although experimental evidence for these is largely absent. Antigenic mimicry has been proposed as a mechanism potentially causing autoimmunity (*87*). This concept is usually applied to infectious agents having molecules bearing one or more epitopes resembling human antigens, such that responses to the foreign antigen cross react with self-antigens. It is conceivable that a xenobiotic could behave in a similar fashion through haptenic effects. Superantigens, which noncovalently cross link MHC molecules with the TCR, have also been nominated as potentially triggering autoimmunity (*88*). It is possible that haptenic modification of a protein binding MHC molecules or the TCR, such as CD4 or CD8, could similarly stabilize TCR–MHC interactions. Xenobiotic-induced mutations in self-proteins could also occur and serve as a novel antigen and induce a response through epitope spreading. Finally, immune responses to antigenic determinants on antibodies or the TCR, known as idiotypes, have been proposed as a mechanism contributing to the development of anti-DNA antibodies (*89*), and it is possible that the haptenic modification of an immunoglobulin idiotype could induce an autoimmune response. Given the large number of ways xenobiotics can potentially modify proteins, additional mechanisms probably exist as well.

Conclusions

Several conclusions may be derived from this review. First, the majority of the mechanisms proposed for xenobiotic-induced autoimmunity can be arbitrarily classified into three broad categories: defects in lymphocyte deletion, modification of gene expression by the cells participating in the immune response to break tolerance, and direct interactions with self-proteins leading to either expression of new epitopes or unveiling of cryptic epitopes. These general concepts suggest additional mechanisms by which xenobiotics might trigger autoimmunity. Second, some mechanisms may be common to a variety of agents. The ability of different metal ions to cleave proteins and of chemically distinct molecules to inhibit DNA methylation and induce autoimmunity support this contention. Third, it would appear that different mechanisms can produce similar diseases. The intrathymic injection of PAHA and the treatment of activated T cells with DNA methylation inhibitors both induce anti-DNA antibodies, most likely through distinct mechanisms. Fourth, despite the large body of literature addressing potential mechanisms by which xenobiotics may induce autoimmunity, persuasive evidence demonstrating that these mechanisms are actually operant in humans with xenobiotic-induced autoimmunity is still lacking. Finally, the potential for numerous and as yet unidentified mechanisms exists, highlighting a need for further work in this area. It is hoped that the material presented at the workshop Linking Environmental Agents and Autoimmune Disease will stimulate further studies on this topic.

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