

Recent advances and opportunities in research on lupus: environmental influences and mechanisms of disease *

Avanços e oportunidades atuais na pesquisa sobre lúpus: influências ambientais e mecanismos da doença

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Abstract *We summarize research on mechanisms through which environmental agents may affect the pathogenesis of lupus, discuss three exposures that have been the focus of research in this area, and propose recommendations for new research initiatives. We examined studies pertaining to key mechanistic events and specific exposures. Apoptosis leading to increased production or decreased clearance of immunogenic intracellular self-antigens and defective apoptosis of autoreactive immune cells both have been implicated in the loss of self-tolerance. The adjuvant or bystander effect is also needed to produce a sustained autoimmune response. Activation of toll-like receptors is one mechanism through which these effects may occur. Abnormal DNA methylation may contribute to the pathogenesis of lupus. Each of the specific exposures has been shown, in humans or in mice, to act upon one or more of these pathogenic steps. Specific recommendations for the continued advancement of our understanding of environmental influences on lupus and other autoimmune diseases include the development and use of mouse models with varying degrees of penetrance and manifestations of disease, identification of molecular or physiologic targets of specific exposures, development and use of improved exposure assessment methodologies, and multisite collaborations designed to examine understudied environmental exposures in humans.*

Key words *Apoptosis, Epstein-Barr virus, Silica, Systemic lupus erythematosus, Trichloroethylene*

Resumo *Resumimos uma pesquisa sobre os mecanismos pelos quais agentes ambientais podem afetar a patogenia do lúpus. Discutimos três exposições, foco de pesquisa na área, e propomos recomendações para novas iniciativas de pesquisa. Examinamos estudos relativos a eventos mecanicistas e exposições relativas. A apoptose que resulta no aumento da produção ou diminuição da menor depuração de auto-antígenos imunogênicos intracelulares e de apoptoses defeituosas de células imunes auto-reativas sofre implicações com a perda da auto-tolerância. Foi necessário um adjuvante ou efeito do observador para gerar uma resposta autoimune sustentável. A ativação de receptores toll-like é um dos mecanismos através do qual esses efeitos podem ocorrer. A metilação anormal do DNA também pode contribuir à patogenia de lúpus. Cada exposição mostrou, em humanos e camundongos, que age conforme um ou mais desses passos patogênicos. Recomendações específicas ao contínuo progresso do conhecimento sobre as influências ambientais no lúpus e outras doenças autoimunes compreendem o desenvolvimento e utilização de modelos de camundongos com graus variados de penetrância e manifestações da doença, identificação de alvos moleculares ou fisiológicos de exposições específicas, desenvolvimento e uso de melhores metodologias de avaliação de exposição e colaborações multisite para examinar exposições ambientais sobre os humanos menos estudados.*

Palavras-chave *Apoptose, Vírus Epstein-Barr, Sílica, Lúpus eritematoso sistêmico, Tricloroetileno*

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Systemic lupus erythematosus (SLE, or lupus) is a chronic autoimmune rheumatic disease that may involve serious renal, cardiovascular, and neurologic complications. The pathogenesis of lupus, as well as other autoimmune diseases such as systemic sclerosis and rheumatoid arthritis, is thought to involve complex interactions of multiple genes and environmental agents. In this review we summarize recent research pertaining to potential pathogenic mechanisms of environmental exposures that may be involved in the development of SLE in humans and recommendations for new research to better understand environmental influences and gene–environment interactions in lupus.

Mechanisms involved in the pathogenesis of lupus

Recent research has advanced our understanding of mechanisms involved in the loss of tolerance and development of a chronic state of inflammation, with work involving the role of apoptosis in the generation and clearance of immunogenic intracellular self-antigens, adjuvant or bystander effects, toll-like receptors (TLRs) and innate immunity, and abnormal DNA methylation. Environmental exposures may act as an initiating event and influence at other points in the pathogenesis of an autoimmune disease.

Apoptosis as a source of self-antigens

Immune responses against self-antigens are fundamental to lupus pathogenesis, and dead and dying cells are a major source of the selfantigens targeted in lupus¹. Consistent with this idea, immunization of test animals with preparations of dead cells, or preparations expressing epitopes found on dead cells, can induce lupuslike immunity and clinical manifestations². Moreover, mutations that lead to impaired nonimmune clearance of dead cell debris have been shown to be strong risk factors for the development of lupus in animal models³. Experimental evidence shows that the clearance of dead cells can be overwhelmed when encountering extremely high rates of cell death, leading to the induction of lupuslike disease⁴. Some environmental agents have been shown to induce periods of increased cell death (e.g., ultraviolet light, viral infections)⁵. Notably, ultraviolet-B exposure and viral infections can also lead to the production of novel forms of autoantigens [such as ultraviolet radiation–induced, covalently linked protein–RNA conjugates⁶] that may be particularly favorable for

the induction of autoimmunity. It is also plausible that environmental exposures may lead to periods of defective or depleted dead cell clearance mechanisms (caused by, for example, complement consumption or reduced local macrophage levels).

Impaired elimination of autoreactive cells via apoptosis

Although dead cell debris promotes autoreactivity, the process of programmed cell death is also critical to preventing and limiting an autoimmune response. Specifically, apoptosis of immune cells participating in autoimmune responses (e.g., T cells, B cells, dendritic cells) is a major mechanism for the induction and maintenance of self-tolerance^{7,8}. Just as gene mutations leading to defects in immune cell apoptosis have been linked to increased risk for the development of lupus⁹, environmental factors influencing the set point at which autoreactive cells undergo apoptosis also appear to influence lupus susceptibility. An example in mice is the ability of estrogen to protect autoreactive B cells from programmed cell death¹⁰. Conversely, impairments in the ability of cytotoxic cells to eliminate autoreactive immune cells appear to enhance the risk of lupus¹¹. Environmental exposures that selectively impair apoptosis of immune system cells may predispose to lupus in a manner that synergizes with exposures that increase target tissue apoptosis. Once established, the development of autoreactive immune memory cells (with impaired apoptosis compared with naïve cells) and the induction of lupus target organ injury (with increased local cell death due to the effects of lupus) can lead to a self-sustaining and selfamplifying cycle of lupuslike autoimmunity.

The adjuvant or bystander effect

What mechanisms prevent the unlimited proliferation of T cells that are capable of recognizing self-antigens? T-cell receptor engagement alone is ineffective in activating the pathways needed to produce the proinflammatory cytokines and other growth factors required for the induction of the pathogenic autoimmune response. A second, non-antigen-specific signal determines whether an encounter with a potential selfantigen is aborted at an early stage, proceeds only to a limited, harmless autoimmune response, or progresses to a pathogenic outcome. The secondary signals involved in modulating the immune response have collectively been termed the “adjuvant effect”; another term for these signals is the “bystander effect”¹².

Information has accumulated rapidly in recent years that allows us to better understand the adjuvant effect at a molecular level. A vivid illustration of the importance of the adjuvant effect in the induction of autoimmune disease was revealed by studies of autoimmune myocarditis. This disease can be induced in susceptible strains of mice by infection by Coxsackievirus B3 or, alternatively, by immunization with purified cardiac myosin¹³. The cardiac myosin immunization must, however, be accompanied by a powerful adjuvant, complete Freund adjuvant, which includes the mycobacterium component. Incomplete Freund adjuvant results in the production of myosin-specific antibodies without the occurrence of inflammatory lesions in the heart muscle. In resistant strains of mice that do not develop disease after Coxsackievirus B3 infection or cardiac myosin immunization, cotreatment with bacterial lipopolysaccharide results in a florid disease¹⁴. This disease is dependent upon the prompt production of the key early proinflammatory cytokines, interleukin-1 and tumor necrosis factor- α ¹⁵. Mast cells are critical players in the initiation of the adjuvant effect that occurs early after viral infection¹⁶.

Another series of experiments used a surrogate marker of the adjuvant effect: a sudden drop in the thyroid hormone thyroxine that occurs after immunization using complete Freund adjuvant¹⁷. These studies indicate that TLRs or similar receptors of the innate immune response are critical for mounting the adjuvant effect. Thus, active infection or products of the infection can provide the adjuvant effect necessary for the induction of many autoimmune disorders. The adjuvant effect depends upon early nonantigen-specific signals that initiate an innate immune response. It shapes the later adaptive response that is directly responsible for pathogenic autoimmunity. The potential role of other environmental agents in the induction of an adjuvant effect of this type and the role of an adjuvant effect specifically in lupus are important areas of research.

In addition to providing antigenic stimuli to B cells and T cells, nucleic acid components associated with dead cell debris have been shown to activate the adjuvant effect through TLR activation¹⁸. RNA activation of TLR7 on B cells and plasmacytoid dendritic cells has been particularly strongly associated with lupus pathogenesis, based on its role as the genetic risk gene for a murine model of inherited lupus¹⁹ and its ability to induce lupus-associated type I interferon production²⁰. The RNA-sensing TLR3 has also been implicated as an inducer of inflammation in a variant form of lupuslike dis-

ease²¹, and the DNA-sensing TLR9 has been associated with lupus nephritis in some models²². However, these innate immune receptors have also been associated with downregulation of immune responses under some circumstances²³. Adjuvant effect signals may influence the tissues targeted by autoantigen-specific innate immune responses by selectively increasing inflammatory responses in some tissues and decreasing the inflammatory responses in others²⁴.

Environmental factors could influence TLR responses and hence the adjuvant effect in lupus in multiple ways. Radiation, chemical toxins, or microbial products may selectively activate or inhibit general innate immune response pathways or selectively influence a single TLR pathway to influence the induction of lupus. Moreover, recent identification of lupus risk factor genes with functions in interferon responses²⁵ and as more general mediators of innate immune signaling, as in the case of STAT4²⁶, suggests that innate immune pathways subject to environmental influences in addition to TLRs may prove to be relevant to lupus pathogenesis. Deficiencies in the complement system may result in the aberrant clearance of apoptotic cells, and specific deficiencies have been implicated in the pathogenesis of SLE²⁷.

Epigenetics: DNA demethylation and pathogenic T and B cells

Environmental factors may also induce autoimmunity through epigenetic mechanisms. Epigenetics is defined as heritable changes in gene expression that occur without a change in DNA sequence, and the best-characterized mechanism is DNA methylation. DNA methylation, the postsynthetic methylation of cytosines in CG pairs, silences genes by altering chromatin structure into a transcriptionally repressive configuration. The methylation of previously unmethylated sequences is mediated by the *de novo* DNA methyltransferases Dnmt3a and Dnmt3b. A more detailed discussion of methylation and epigenetics is provided in a recent review²⁸.

DNA methylation patterns are established during development and suppress genes that are inappropriate or detrimental to the function of any given cell type. Inhibiting lymphocyte DNA methylation during mitosis alters gene expression, resulting in immunogenic changes that can alter the response to self-antigens, including overexpression of the adhesion molecule LFA-1 (CD11a/CD18), the cytotoxic molecule perforin, and B-cell costimulatory molecules CD70 and CD40L²⁹. LFA-1

overexpressing T cells are autoreactive, resembling the T cells that cause lupuslike chronic graft-versus-host disease. Perforin overexpression contributes to autologous macrophage killing by the autoreactive cells, with subsequent release and impaired clearance of antigenic nucleosomes, whereas CD70 and CD40L overexpression overstimulate B-cell immunoglobulin production²⁸⁻³⁰. The CD40L overexpression occurs only in women, as CD40L is encoded on the X chromosome³⁰. Injecting demethylated T cells into genetically identical mice causes a lupuslike disease with anti-DNA antibodies and an immune complex glomerulonephritis^{28,31}. These functional changes suggest a model in which hypomethylated T cells kill macrophages and perhaps other antigenpresenting cells (APCs), causing an increase in apoptotic material that promotes an anti-DNA response, which is further augmented by increased antibody production.

Studies in lupus patients also suggest that T-cell DNA demethylation may be fundamental to the disease process. CD4⁺ T cells from patients with active lupus overexpress LFA-1, perforin, CD70, and CD40L because of demethylation of the same sequences demethylated by a Dnmt inhibitor³². Further, CD4⁺ lupus T cells demonstrate functional changes identical to T cells demethylated *in vitro*, with perforin-dependent autoreactive macrophage killing and CD70/CD40L-dependent B-cell overstimulation²⁸⁻³⁰.

The evidence summarized above suggests that aberrant T-cell DNA methylation contributes to lupus pathogenesis. DNA methylation silences one X chromosome in women, and suppresses parasitic viral DNA. Thus DNA methylation may represent one pathway that may influence the marked sex difference in incidence of lupus in humans, as seen with the example of sex-specific CD40L overexpression and B-cell stimulation. Further support for the association between T-cell DNA methylation and lupus can be found in reports that hydralazine and procainamide, which cause antinuclear antibodies in most people and a lupuslike disease in a subset, are DNA methylation inhibitors. Procainamide acts through inhibition of the DNA methyl transferase enzyme Dnmt1³³, and hydralazine acts through the extracellular signal-regulated kinase (ERK) signaling pathway³⁴. The signaling defect in lupus and in hydralazine-treated T cells maps to protein kinase C (PKC)- δ ³⁵, supporting commonality of mechanisms. Ultraviolet light also triggers lupus flares, inhibits ERK pathway signaling, and is a DNA methylation inhibitor²⁸. These examples suggest that other xenobiotics could contribute to the develop-

ment or exacerbation of lupus in some patients by similar mechanisms. Agents (e.g., dietary deficiencies) that deplete the pool or biosynthesis of the methyl donor or that inhibit key enzymes or the signaling pathways (e.g., increased homocysteine levels) could lead to increased inhibition of the reaction. Finally, reports that methyl donor restriction during fetal development can cause lifelong effects³⁶ raises the possibility that nutritional deficiencies or exposure to toxicants during pregnancy may affect lupus susceptibility later in life.

Viral, solvent, and particulate exposures and the pathogenesis of autoimmune disease

In the previous section we described mechanisms through which different environmental exposures could affect the development of lupus and other systemic autoimmune diseases. Here we discuss three examples of exposures. Although consensus does not exist regarding all of the issues with respect to each exposure and the development of lupus, these three diverse exposures have generated interest from lupus researchers, based on potential connections to elements identified in the lupus disease pathway. Of these, the environmental exposure with the most developed literature suggesting a link with SLE is Epstein-Barr virus (EBV). EBV is a nearly ubiquitous pathogen that has been associated with lupus in studies using serologic and DNA measures. Its high worldwide prevalence raises the key question of how a common environmental exposure leads to disease in only a very small subset of infected individuals³⁷ and suggests that differences in EBV infection or differences in the host immune response to the infection may be key considerations. Although the first study of occupational respirable silica exposure and systemic autoimmune disease was published almost 100 years ago [reviewed by Brown *et al.*³⁸]; Parks *et al.*³⁹], it is only in the past decade that this relationship has become the focus of mechanistic studies in animal models, taking its historical label as an adjuvant to the molecular level. These examples further illustrate how exposures can lead to a series of events from the initial initiating impact to multiple levels of the immune response. Trichloroethylene is a commonly used solvent and potential air and water contaminant; solvents have been most consistently associated in epidemiologic studies with systemic sclerosis⁴⁰. In experimental studies of trichloroethylene using the MRL^{+/+} mouse model, however, autoimmune hepatitis, skin inflammation, and alopecia were seen. These

observations highlight the critical gaps in our understanding of the expression of autoimmune disease in animals and humans.

EBV: a summary of three decades of research

EBV was offered as a potential environmental factor in SLE as early as 1972. Pediatric lupus patients, used as controls in a study of EBV and childhood lymphoma, were found to have an enriched frequency of EBV serology compared with other children⁴¹. During the following few decades, debate raged [for a historical review, see McClain *et al.*⁴²]. Evidence of induced models, molecular mimicry, adjuvant or bystander effects, viral DNA association, increased viral loads, differential EBV gene expression, and abnormal EBV T-cell and B-cell responses in lupus have recently all provided additional support for potential roles for EBV in lupus [reviewed by Poole *et al.*⁴³].

Early targets of key lupus autoantigens are often restricted and then diversify over time in a concept termed epitope spreading⁴⁴⁻⁴⁶. These initial lupus autoantigen humoral targets have proven quite interesting in that immunization of select animal strains with these sequences constructed on a polylysine backbone develop not only antibodies to the peptide of immunization, but also antibodies that bind to the parent protein and eventually develop other autoantibody specificities and clinical features of systemic autoimmunity⁴⁶. Interestingly, initial targets of Sm B' and 60kD Ro are cross-reactive with sequential regions of EBV nuclear antigen-1 (EBNA-1), the key latent protein of EBV. This work suggests potential pathways for molecular mimicry to lead to subsets of lupus.

Using new sensitive ELISA assays, studies of pediatric and adult lupus patients and healthy controls drawn from relatives or a large pedigree study have shown an association of EBV seroconversion and SLE [reviewed by James *et al.*⁴⁷; McClain *et al.*⁴⁸; Poole *et al.*⁴³]. In addition, several studies also evaluated the presence of EBV DNA in peripheral blood mononuclear cells and showed an association between EBV DNA presence and lupus⁴⁹⁻⁵¹. Lupus patients had a 15-fold⁵¹ to 30-fold⁵³ increase in EBV DNA in the peripheral blood compared with controls; however, no difference in EBV DNA levels was found in mouthwash samples⁵¹. The controls in these studies were described as healthy and were matched by demographic factors, but the recruitment process was not described in detail.

More recent studies using isolated B cells from lupus patients and controls showed a 10-fold in-

crease in infected cells as well as differences in EBV gene expression⁵⁴. These increased levels of EBV infection and gene expression in lupus patients could lead to *a*) a stronger or altered immune response to EBV proteins with resultant crossreactive self immune responses, *b*) an increased activation state of the host immune response, *c*) a proinflammatory cytokine environment, which could result in easier breaks in tolerance, or *d*) potential EBV-infected autoreactive cells, which could lead to autoantibody formation and/or pathogenic responses.

The host immune response to EBV is also different in lupus patients compared with lupus-affected controls. Lupus patients have higher numbers of CD4+ T cells but lower numbers of CD8+ T cells, which produce interferon- α in response to EBV⁵³. These abnormal responses could contribute to the changes in EBV load seen in lupus patients or provide additional help for abnormal B-cell responses. Antibody responses to EBV are also different. Lupus patients have antibodies against a broad spectrum of early diffuse EBV proteins⁵⁵ as well as a higher frequency of antibodies to a larger number of latent nuclear antigens such as EBNA-2 and EBNA-3^{56,57}. At least two groups to date have shown higher IgA responses in lupus patient sera^{58,59}. One of these studies involved a comparison group recruited through a population-based sampling procedure⁵⁹. SLE reactivities are most similar to patients with chronic viral reactivation. Interestingly, pediatric lupus patients have a broader humoral immune response against EBNA-1 with a larger number of specific humoral epitopes. Areas of reactivity outside of the commonly targeted glycine-alanine repeat are cross-reactive with common early epitopes of self-antigens and are potential targets of molecular mimicry⁶⁰. Other groups have also found SLE unique humoral immune responses to EBNA-1⁶¹.

A variety of bystander (or adjuvant) effects as outlined above for different mechanisms could also be quite important, serving as key links between EBV, and potentially other pathogens, and lupus^{43,62}. EBV is known to act through different TLRs, which could lead to interferon production, abnormal self-antigen presentation, T-cell activation, cytokine production, and loss of tolerance. EBV has a viral interleukin-10 homolog, which could induce inappropriate APC activation, as well as a bcl-2-like homolog, which can inhibit apoptosis of infected cells. Additional evaluation of unique SLE-specific bystander responses to pathogens are warranted.

Silica: modeling overlapping pathologies for mechanistic clues

Inhalation of silica is associated with overlapping pathologies of inflammation, fibrosis, and autoimmunity. Critical genetic risk factors that confer either susceptibility or, perhaps more intriguingly, protection from silicate-induced autoimmune changes, have not been identified, but clues to early events in autoimmunity may come from studies of the pulmonary effects of silica and the immune dysfunction related to fibrosis^{63,64}. Mouse strains with different susceptibilities to fibrosis and inflammation make them valuable for teasing apart early processes related to macrophage activation, cytokines, and gene regulation related to silica⁶⁵⁻⁶⁷.

New Zealand mixed (NZM) mice showing a mild lupus-prone phenotype⁶⁸ exhibit exacerbation of lupus pathology after intratracheal silica exposure⁶⁹. Survival in silicaexposed NZM mice was decreased, with increased proteinuria levels and immunoglobulin (Ig) G deposition suggesting exacerbated kidney damage. The lungs in these mice also had increased inflammatory infiltrates and fibrotic lesions that were well established after 14 weeks, concurrent with significant elevation of autoantibody levels⁶⁹. Although there are limited data in mice regarding their chronological relationship, there is some evidence that autoantibodies precede and contribute to fibrotic changes in humans [reviewed by Jindal and Agarwal⁷⁰].

It has been hypothesized that these overlapping outcomes may be related at the level of innate immune responses (adjuvant effect) to silica, possibly via scavenger receptors such as SR-A and MARCO on macrophages and mast cells^{71,72}. Mice lacking either SR-A or MARCO show different responses to silica versus titanium dioxide, which causes inflammation but not fibrosis or autoimmunity. In addition, there are striking differences in expression of these receptors and silica uptake by cells from Balb/c and C57Bl/6 mice⁷², offering strain and receptor explanations for differential fibrotic susceptibility. The role of scavenger receptors as proinflammatory pathways is complicated by their additional roles in apoptosis induction and the clearance of apoptotic debris. A possible unifying model implicates silica-induced apoptosis of the very cells needed for apoptotic clearance, in an environment of silicaenhanced antigen presentation by dendritic cells or alternately activated macrophages^{66,73}, similar to the autologous macrophage killing caused by demethylated T lymphocytes²⁸.

The silica-induced exacerbation of

lupus pathology in NZM mice was ameliorated in mice coinstilled with rottlerin, a putative PKC- δ inhibitor⁷⁴. Although rottlerin can have PKC- δ -independent effects⁷⁵, as a PKC- δ inhibitor, it is anti-apoptotic⁷⁶. Further supporting the role of apoptosis in the exacerbation of the NZM lupus model, autoantibodies in silica-exposed mice were shown to bind to macrophages undergoing apoptosis⁷⁷. In addition, the Fas/Fas ligand system is up-regulated with silica exposure in both humans and rodents, which could affect its autoimmune effects by increasing apoptosis^{78,79}. Because silica can cause oxidative stress and apoptosis in macrophages^{80,81}, it is possible that these events lead to clustering or proteolytic cleavage of autoantigens. Studies have shown that silica can lead to altered proteosomal processing of specific scleroderma autoantigens^{82,83} and activation of apoptotic pathways involving various caspases⁸⁴.

Much of the recent data regarding the effects of silica on lymphocyte populations in mouse models of lupus were recently reviewed³⁸. In silica-exposed NZM mice, lymph nodes had local reduction in regulatory T cells despite a dramatic increase in CD4+ T cells⁸⁴. Wu *et al*⁸⁵ have shown that the function of regulatory T cells is reduced in silicosis patients. Recently, Carlsten *et al*⁸⁶ evaluated several serologic measurements for their potential as early markers of immunologic effects of occupational silica exposure in 11 men and found a reduction of CD25+ T cells and some increases in T-helper cell (Th)1 and Th2 serum cytokines. However, the study design did not distinguish the CD25+ cells as activated versus regulatory T cells. In NZM mice, a relative reduction in serum IgG1, along with elevated tumor necrosis factor- α in lung lavage, suggested a possible Th1 skewing by silica⁸⁴, consistent with studies in rats and non-lupus-prone mice^{87,88}. Although the roles of tumor necrosis factor- α and Th1/Th2 cytokines in lupus remain unclear^{89,90}, reported increases of interferon- γ with silica are consistent with development of lupus^{86,88}.

Clearly, silica affects the immune system at several levels that could play roles in lupus pathogenesis.

Trichloroethylene effects in a lupus mouse model

The female MRL+/+ mouse develops a lupuslike disease late in life (50% mortality at 17 months) and has been used in a series of studies of trichloroethylene. Short-term intraperitoneal trichloroethylene exposure (10 mmol/kg every 4 days for 6 weeks) resulted in increased spleen weight, as well as some serum markers of systemic

autoimmunity including total IgG and antinuclear antibodies⁹¹. A chronic oral exposure study (21, 100, or 400 mg/kg/day trichloroethylene in drinking water for 32 weeks) also resulted in an accelerated autoimmune response, with increased antinuclear antibodies after 4 weeks of exposure to concentrations as low as 21 mg/kg/day⁹². Chronic treatment with trichloroethylene did not accelerate the development of the lupus nephritis, but after 32 weeks of exposure, tissue pathology commensurate with autoimmune hepatitis was seen. In both mice and humans the majority of trichloroethylene absorbed into the circulation is metabolized by an oxidative pathway in the liver⁹³, converting trichloroethylene to trichloroacetaldehyde, which in solution is in equilibrium with trichloroacetaldehyde hydrate. Female MRL+/+ mice treated for 40 weeks with drinking water containing concentrations of trichloroacetaldehyde hydrate that encompassed the molar equivalents of previous lowlevel trichloroethylene exposure did not develop autoimmune hepatitis or lupus nephritis but did develop a dose-dependent alopecia and skin inflammation⁹⁴.

Trichloroethylene and trichloroacetaldehyde hydrate were also shown in these experiments to increase percentages of activated interferon- α producing CD4+ T cells^{92,95}. A recent study that reported higher levels of interleukin-2 and interferon- α levels in 35 trichloroethylene-exposed workers (mean exposure levels 35 mg/m³) compared with 70 nonexposed workers⁹⁶ provides evidence of similar early immune responses in humans and the MRL+/+ mouse model.

During its metabolism, some trichloroethylene is converted to a trichloroethylene oxide reactive intermediate, which may ultimately lead to the formation of N6-formyl lysine or N6-dichloroacetyllysine adducts. These adducts have been detected as stable neoantigens in the liver of trichloroethylene-treated MRL+/+ mice⁹⁷, and adduct-specific antibodies have been detected in trichloroethylene-treated MRL+/+ mice⁹⁸. Trichloroethylene treatment also promoted the development of antibodies specific for unmodified liver microsomal proteins⁹⁹. Thus, it appeared that trichloroethylene exposure could trigger an immune response against both unmodified and trichloroethylene modified liver proteins. Trichloroethylene may also perturb the immune system through the induction of oxidative stress, as seen by the increased serum levels of inducible nitric oxide synthase (iNOS) and nitrotyrosine in a chronic duration (48 weeks) drinking-water exposure study in female MRL+/+ mice¹⁰⁰. Antibodies against lipid peroxidation-de-

rived aldehydes malondialdehyde and 4-hydroxynonenal were also seen. At least some of the oxidative stress generated by trichloroethylene occurred in the liver⁹⁹. Although trichloroethylene-induced adducts and oxidative stress can be immunogenic, the role of these altered selfantigens and the resulting antibodies in disease pathology remains to be determined.

T-cell resistance to activation-induced apoptosis has been seen in patients with lupus, alopecia, and scleroderma¹⁰¹⁻¹⁰³ and was also seen in these studies of trichloroethylene-exposed mice. Almost 88% of the activated CD4+ T cells isolated from control MRL+/+ mice at the 4-week time period were induced to undergo activation-induced apoptosis *in vitro*. In contrast, only 57% of CD4+ T cells from mice exposed for 4 weeks to trichloroacetaldehyde hydrate underwent apoptosis. This effect was subsequently linked to a decrease in FasL expression on the CD4+ T cells¹⁰⁴. A trichloroacetaldehyde hydrate-induced downregulation of FasL could enable activated selfreactive CD4+ T cells to escape Fas-mediated deletion but retain effector function.

Gilbert *et al* developed a model to synthesize the results from these experiments. Metabolism of ingested trichloroethylene leads to the generation of adducts on liver proteins such as CYP2E1. Trichloroethylene also induces oxidative/nitrosative stress in the liver. The damaged liver cells expressing chemically modified antigens may be taken up by phagocytic cells such as Kupffer cells or hepatic stellate cells. Chemokines secreted by the phagocytic cells help recruit CD4+ T cells, which are then presented with unmodified and/or modified liver antigens. Normally, liver-specific CD4+ T cells would be deleted by activation-induced apoptosis before they mediated pathology. However, trichloroethylene works via metabolite trichloroacetaldehyde hydrate to downregulate expression of FasL on the CD4+ T cells, thereby decreasing their susceptibility to Fasmediated apoptosis. This effect increases longevity of liver-specific CD4+ T cells and thus promotes liver damage commensurate with autoimmune hepatitis. Mice treated with trichloroacetaldehyde hydrate directly do not undergo hepatic adduct formation and/or oxidative stress. Consequently, trichloroacetaldehyde hydrate-mediated inhibition of activation-induced T-cell apoptosis does not manifest itself as hepatitis. The reason why the inflammatory disease is instead directed to the skin and the applicability of this model to specific autoimmune diseases in humans (including lupus, scleroderma, and autoimmune liver disease) remain to be determined.

Research gaps and recommendations

Federal agencies and private foundations have organized several meetings in recent years concerning many aspects of lupus, including the sex and ethnic disparities and prospects for development of new treatments. The goal of one workshop, “Lupus & the Environment: Disease Development, Progression and Flares” (held 8–9 September 2005 in Washington, DC), was to appraise the state of the science and produce recommendations for new research to better understand environmental influences and gene–environment interactions in lupus. The workshop produced a prioritized list of recommendations for research support. Similar issues are described in *Future Directions of Lupus Research*, a recent publication from the National Institute of Arthritis and Musculoskeletal Diseases¹⁰⁵. Progress has been made and some interesting research has been published since this workshop, but the recommendations that came out of the workshop continue to apply:

. Continued development and increased use of improved lupus-prone and non-prone animal models that are appropriate for research on mechanisms linking environmental exposures to lupus. Models with varying degrees of penetrance and varying manifestations of disease are needed.

. Identification of molecular or physiologic targets of exposures leading to either incidence or progression of lupus, building on studies of emerging concepts such as epigenetics, posttranslational steps, metabolic mechanisms, understanding bystander/adjuvant effects of exposure, investigation into “inappropriate” autoimmune responses to common exposures, and multiple exposure studies that test synergy of various factors or agents.

. Development and dissemination of improved technologies and instrumentation to assess environmental exposures integrated over the relevant etiologic time period, including geocoding methods for use with geographic information systems¹⁰⁶ and biosensors¹⁰⁷, techniques involving specific biomarkers of exposure, and questionnaire- or interviewbased derivation of specific exposure histories¹⁰⁸.

. Multisite collaborations with standardized protocols for collection of environmental exposure data focusing on the period before development of clinically expressed disease. These large-scale studies are needed to address the considerable heterogeneity among lupus patients in genotypic profile, in serologic and phenotypic expression, and potentially in etiologic pathways.

. Further study of gene–environment interactions in both human and animal settings. Of particular interest is the identification of lupus-specific versus more general autoimmune disease genes and the exposures that trigger flares for particular genomic profiles.

Our understanding of the mechanisms involved in the pathogenesis of lupus continues to expand. This understanding provides the opportunity to begin to assess whether and how environmental exposures contribute to this process. Clearly the specific environmental exposures discussed in this review are unlikely explanations for the extreme disparity in disease rates seen among women and among ethnic minorities. It will take much more work from a variety of disciplines to address these issues. We believe that acting on these recommendations will enhance our ability to design research studies that address both how and why the pathology of lupus arises, so the devastating impact of this disease can be ameliorated.

Acknowledgments

This article was originally published by *Environ Health Perspect* 116:695–702 (2008). doi:10.1289/ehp.11092 available via <http://dx.doi.org> [Online 5 March 2008] and is part of the scientific collaboration between *Cien Saude Colet* and *EHP*. This manuscript is based on presentations and discussions from the workshop “Lupus and the Environment: Disease Development, Progression and Flare” (Washington, DC, 8–9 September 2005) produced by the Federal Interagency Working Group on Women’s Health and the Environment and supported by the U.S. Department of Health and Human Services Office of Women’s Health, the National Institute of Environmental Health Sciences, and the Lupus Foundation of America. The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. This article may be the work product of an employee or group of employees of the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH); however, the statements, opinions, or conclusions contained therein do not necessarily represent the statements, opinions, or conclusions of NIEHS, NIH or the U.S. government. The authors declare they have no competing financial interests.

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Received 20 November 2007

Accepted 5 March 2008