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Report from the Society for Women's Health Research

Role of Environment and Sex Differences in the Development of Autoimmune Diseases: A Roundtable Meeting Report

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Abstract

Autoimmune diseases (ADs) impose substantial health and financial burdens in the United States and in many parts of the world. Women are disproportionately affected by many of these disorders, which often contribute to lifelong disabilities. While the number of patients with some ADs appears to be rising, the complexities of conducting epidemiological studies prevent a thorough understanding of the prevalence and incidence of these various conditions. Research on environmental influences of these illnesses is limited, although they are generally hypothesized to result from the interaction of environmental agents in genetically susceptible individuals. Further, there is little known regarding the role of sex and gender in the environmentally influenced mechanisms leading to the development of AD. To address these issues, particularly the roles of environment and sex and gender in ADs and the factors that contribute to the rise in ADs, the Society for Women's Health Research convened an interdisciplinary roundtable of experts from academia, medicine, and government agencies to share their expertise, address knowledge gaps in research, and propose future research recommendations.

Introduction

Autoimmune diseases (ADs) are a diverse group of illnesses characterized by inflammatory responses originating from misdirected attacks of the immune system on the body's organ systems. More than 80 ADs have been identified so far and the list continues to grow.[1] ADs affect ~7%–10% of the U.S. population, of which ~78% are thought to be women.[2,3,4] Collectively, ADs are the fifth leading cause for death in women before age 65.[5] These diseases are a major public health problem due to their chronic nature and associated comorbidities, which increase the societal burden in terms of healthcare costs, loss of work productivity, and reduced quality of life; however, a complete understanding of the extent of the burden is lacking.[6]

Our knowledge of AD etiologies is very limited; however, several studies suggest that the environment acts on genetically susceptible individuals in causing most ADs.[1,7] Genes predisposing individuals to systemic lupus erythromatosis (SLE) or lupus, rheumatoid arthritis (RA) and multiple sclerosis (MS) have been identified by genome wide association studies.[8] Low concordance rates in monozygotic twin studies and the appearance of drug-induced lupus symptoms after treatment with specific prescription medications followed by the disappearance of symptoms upon withdrawal of the medications indicate a role of the environment in ADs.[1,9] Additional evidence for the role of the environment in the development of ADs is reviewed in a series of recent publications originating from the 2012 National Institutes of Environmental Health Sciences (NIEHS) Expert Panel Workshop.[1,9,10,11] While there are known genetic and environmental components to ADs, more studies are needed to understand the spectrum of genes and environmental agents and the interactions between them to understand the etiology of these diseases.

Recent epidemiological trends in the incidence rates of some ADs have been attributed to environmental factors, since large changes over a short period of time cannot be explained by genetic contributions alone.[12,13] Specifically, the incidence of type 1 diabetes (T1D) has doubled every 20 years in the United States and other populations.[13] Similarly, the

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incidence of SLE (female to male ratio of 9:1) has tripled in the past four decades in the Rochester Epidemiology Project (REP), Minnesota.[12] RA rates (with 65%–75% female predominance) among women in the REP were on a sharp decline over a period of four decades (1950s to mid-1990s). Surprisingly, these rates have increased by 2.5% from 1995 to 2005.[14] The reasons for this increase are unknown but have been attributed to cumulative effects of environmental factors, including diminished protective effects of oral contraceptives, smoking, and probably vitamin D deficiency.[14]

Correlating a specific AD with a particular environmental agent is complex and challenging. For example, the risk for a specific AD (such as SLE) has been associated with more than one environmental agent (silica, pesticides, cigarette smoking, and Epstein Barr virus).[15] On the other hand, several ADs (including RA, SLE, Graves' disease, and MS) have been associated with one particular environmental exposure (cigarette smoking).[1,7,15,16,17] Studies demonstrating such correlations between the environmental exposure and ADs are numerous; however, specific mechanisms underlying such correlations have yet to be identified.

Sex and gender disparities are profound for ADs, as the majority of ADs exhibit female bias, with women 2.7 times more likely than men to acquire an AD.[3,5,15,18] Strong female predominance is exhibited by several ADs, including SLE, Sjörgren's syndrome (SS), primary biliary cirrhosis, mixed connective tissue diseases, and autoimmune thyroid diseases. Some ADs, such as T1D, ulcerative colitis, and autoimmune myocarditis, show weak or no female predominance,[15] and conditions such as Guillain Barré syndrome and psoriasis show increased male bias.[19,20] Although multiple hypotheses have been proposed, the underlying cause and mechanisms for sex biases are unknown.[18] Some of the candidate factors proposed to cause the sex bias include sex hormones, X chromosome inactivation, X chromosome abnormalities, and fetal microchimerism.[21,18]

ADs have substantial effects on women's health and quality of life. The age of onset for women to acquire an AD is earlier than for men and in the case of SLE, 90% of cases occur in women during 15–45 years of age.[22] Women living with ADs face many challenges, including uncertainty regarding disease progression and prognosis and ability to function at home and in society. Symptoms such as fatigue, depression, sleep disturbance, pain, and sexual and cognitive dysfunction are common in many ADs[23,24,25] Sadly, maintaining a normal lifestyle is very challenging for women with AD, especially due to the lack of adequate social support and impact on ability to work.

On October 5, 2012, the Society for Women's Health Research convened an interdisciplinary roundtable in Washington, DC of expert researchers (Table 1) (including epidemiologists, basic scientists, clinicians, immunologists, and toxicologists) to share their research interests, address their thoughts on the role of environment on sex and gender disparities, and discuss biological mechanisms underlying the development of ADs. The participants were asked to comment on mechanisms and factors contributing to the rise in AD rates and provide consensus statements on future research recommendations.

Environment, Sex/Gender, and Development of ADs

Dr. Frederick Miller provided an overview of how environment and gender (in this instance gender and sex were used interchangeably) relate to autoimmunity and development of ADs. Autoimmunity is defined by the presence of autoreactive T and/or B cells, which cause the pathological inflammation leading to the development of ADs.

Table 1. Society for Women's Health Research Autoimmune Roundtable Participants List and their Affiliations

Name	*Affiliation*
S. Ansar Ahmed, DVM, PhD	Professor and Head of the Department of Biomedical Sciences and Pathobiology Virginia Tech University
Divaker Choubey, PhD	Professor, Department of Environmental Health University of Cincinnati College of Medicine
Glinda Cooper, PhD	Senior Epidemiologist, National Center for Environmental Assessment U.S. Environmental Protection Agency
DeLisa Fairweather, PhD	Assistant Professor, Environmental Health Sciences Johns Hopkins Bloomberg School of Public Health
Kathleen Gilbert, PhD	Professor, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences
Frederick W. Miller, MD, PhD	Acting Director, Clinical Research Program and Principal Investigator, National Institute of Environmental Health Sciences, National Institutes of Health (NIH)
Marc Monestier, MD, PhD	Director, Temple Autoimmunity Center Professor, Microbiology and Immunology Temple University
Prakash Nagarkatti, PhD	Vice President for Research, Carolina Distinguished Professor Department of Pathology, Microbiology, and Immunology University of South Carolina School of Medicine
Christine G. Parks, PhD	Research Scientist, Epidemiology National Institute of Environmental Health Sciences, NIH
Bruce Richardson, MD, PhD	Professor, Department of Internal Medicine Chief of Rheumatology Veteran's Affairs Ann Arbor Healthcare System

According to Dr. Miller, pathogenesis for ADs likely involves chronic immune activation following environmental exposures in genetically susceptible individuals. Although precise mechanisms for the development of ADs are unknown, several studies suggest that over time, the interaction of the environment with genetic risk factors contributes to the pathogenesis.[26]

Dr. Miller presented the following evidence for the role of the environment in the pathogenesis of ADs: (1) strong temporal associations with some exposures and disease onset following exposure; (2) disease improvement after agent removal; (3) disease reoccurrence when patients or animals are re-exposed to the agent; (4) less than 50% disease concordance in monozygotic twins; (5) correlation between seasonality in birth rates and disease onset in some ADs; (6) geographic clustering with onset of disease or disease prevalence; (7) changes in disease incidence and prevalence over time; (8) biological plausibility from *in vitro* and animal studies; and finally, (9) epidemiological associations between particular exposures and certain diseases.[26]

In 2012, Dr. Miller led a NIEHS expert panel workshop to review 30 years of epidemiological literature to examine the role of environment in the development of ADs. Using predetermined criteria, the panel reached a consensus about several specific environmental agents with respect to their role in the development of specific ADs, including (1) crystalline silica exposure contributes to the development of several ADs (RA, SSc, SLE etc.); (2) solvent exposure contributes to development of SSc; (3) smoking contributes to the development of seropositive RA; and (4) an inverse relationship exists between increased ultraviolet radiation exposure and decreased risk for MS. Knowledge gaps identified by the panel were summarized by Dr. Miller and include a need for (1) cost-effective, validated methods for assessing human exposures; (2) more research on genotypes, phenotypes, and multiple exposures; (3) a critical understanding of the effects of the timing of exposures and dose responses; and (4) increased resources to define associations of environmental agents with disease. Unfortunately, carefully controlled and adequately powered epidemiological studies are limited and warrant additional study.[1]

According to Dr. Miller, genes that respond to environmental exposures tend to be major risk factors for the development of ADs. Dr. Miller discussed some supporting genetic evidence, such as (1) increased prevalence in certain families and ethnic groups, (2) gradients of disease concordance in pedigrees, and (3) AD associations with many genes (such as the *HLA-DR* gene haplotypes), some of which are shared among multiple diseases.[27] Dr. Miller pointed out that these shared genetic risk factors for multiple ADs suggest a common pathogenic mechanism. For instance, epidemiological studies have shown that the HLA-*DR3* haplotypes are important for disease predisposition for SLE, MS, and T1D, while *DR9* haplotypes are shared in T1D and RA.[27] Further, Dr. Miller elaborated that sometimes certain genes and environmental exposures induce protective effects. For example, ingestion of L-tryptophan in some individuals led to the development of eosinophilia–myalgia syndrome, whereas some individuals remained unaffected following the ingestion. Multivariate analyses showed the HLA *DRB1*03* gene as a risk factor in affected individuals and HLA *DRB1*07* gene appeared to be protective as it was found frequently in unaffected individuals. Understanding protective genes, and possibly protective environmental exposures, may one day provide mechanistic clues for minimizing the development of AD.[28,26]

In addition to environmental influences, studies have revealed that possible mechanisms influencing female predominance in ADs may include both hormonal and genetic effects. Sex hormones are primarily responsible for the hormonal effects, with estrogen and prolactin tending to be anti-inflammatory, and androgens tending towards pro-inflammatory effects. Interestingly, prolactin leads to the production of interferon (IFN)-γ and high levels of IFN-γ have been associated with increased AD activity.[29,30,31] The use of hormone replacement therapies is thought to be associated with an increased risk of some ADs.[32] Interestingly, children exhibit female predominance in ADs as well, suggesting involvement of other factors besides estrogen and progesterone. Suggested genetic effects for female predominance include epigenetics, incomplete or skewed X chromosome inactivation in females, and the role of as-yet unknown genes. Other possible effects attributed to female predominance include microchimerism, preferential exposures to environmental agents, and certain infections or drugs.[29] Evidence for female predominance in ADs comes from the National Health and Nutrition Examination Survey (1994–2004), a population-based study, which estimated the prevalence of antinuclear antibodies (ANA; a clinical indicator of autoimmunity) across age by sex in the United States. As Dr. Miller explained, there was a female predominance of ANA at every age group in this study, with female:male prevalence peaking for the 40–49 years age group and decreasing at later ages. This result was true across all ethnic and socioeconomic groups.[33]

In summary, Dr. Miller emphasized that ADs are on the rise and possibly result from the interaction of environmental and genetic factors over time. Both hormonal and nonhormonal factors contribute to female predominance. Advances in other complex diseases, such as novel technologies, statistical approaches, and development of collaborating consortia and focused resources, need to be applied to environmental studies with the goal of interrupting AD associated pathogenesis before the onset of illness.

Complexities and Challenges Associated with Epidemiological Studies in ADs

Epidemiological data are typically presented as prevalence (proportion of cases for disease in a population at a given time) and incidence (the number of new cases occurring over a given time). Prevalence of ADs has risen in the last few decades. Initially, Jacobson used literature reviews of published studies (1965–1995) to estimate the total AD prevalence as approximately 3.2%, across 24 selected ADs in the United States.[3] Using hospital registry data from 1977 to 2001, investigators in Denmark estimated the total prevalence across 31 diseases to be 5.2%.[34] Dr. Glinda Cooper discussed a more recent analysis using published results from 1989 to 2008, which resulted in an estimated prevalence of 7.6%–9.4% for 29 ADs.[4] Dr. Cooper noted that changes in diagnosis and test ordering could contribute to the changes in incidence of some ADs over time, but these types of diagnostic changes are unlikely explanations for the rising

incidence seen in T1D. Improvements in the surveillance methods and quality of epidemiologic research may improve our understanding of disease burden and temporal trends in the future.[4]

In order for the environmental exposures to cause a change in the incidence of ADs, Dr. Cooper explained that the exposure must be common throughout the population and should have a relatively strong impact on risk of disease. For example, prevalence of smoking men and women in the United Kingdom decreased dramatically from 1950 to 1998.[35] This decrease could result in a decrease in diseases for which smoking was a relatively strong risk factor, such as RA. Further, Dr. Cooper mentioned that trends in environmental exposures are affected by variations within populations. For example, while occupational silica exposure is on the decline in the United States, women are entering new work roles that put them at risk for silica exposure.[36] Thus, the population-wide decrease in exposure does not reflect the gender-specific increase in exposure. Research on multiple environmental exposures is critical to our understanding of the incidence trends. For example, use of multiple agents such as pesticides, solvents, and phthalates increased dramatically post–World War II and may have had combined effects on incidence and prevalence rates of ADs. Dr. Cooper suggests that disease associations can be observed even with small levels of exposure. In conclusion, Dr. Cooper stressed the importance of both collecting better incidence data for ADs and the need for new methodologies to predict the effects of exposures on the development of ADs.

Dr. Christine Parks addressed the role of sex, gender, and the environment in systemic ADs and focused her discussion on sex and gender-specific mechanisms related to farming/pesticides. A modest association has been previously observed, mostly in men, between farming and the risk for RA and SLE.[37,38,39] However, Dr. Parks explained a potential role for pesticides as plausible triggers for these ADs. In addition to pesticides, other immune-modifying exposures are present in a farming environment, such as animals/infections, ultraviolet rays, dusts, metals, diesel, and noise.[40] In discussing the role of gender in relation to farming and pesticides, Dr. Parks expressed that women may not self-identify as farmers, though they are often involved in farm work and are thus exposed to potential triggers. Farmers and their families often live where they work, so the occupational exposure of the farm environment is actually shared among those who do and do not self-identify as farmers. This makes it difficult to identify associations between farming and gender since many of the environmental exposures associated with the farming are not adequately captured on surveys simply using the occupational title of "farmer." Farmers and their spouses often grow up on farms and may have had early exposures that lead to the development of ADs, making causal relationships between current exposures and AD development difficult to detect and once again making it challenging to establish associations between farming and gender.

Because many agricultural pesticides are also used in residential settings (albeit at lower concentrations), Dr. Parks looked at the effects of residential pesticides and RA/SLE risk in Women's Health Initiative (WHI) observational study, where ~76,000 post-menopausal women were considered at risk of RA/SLE.[41] Dr. Parks and colleagues examined self-reported residential or workplace insecticide use in relation to risk of RA/SLE (based on self-report and concurrent use of antirheumatic drugs). The results indicated that for women without prior farm experience, personal exposures to insecticides were associated with increased risks of SLE and RA. The smaller subgroup of women who lived on farms had an even higher risk for SLE and RA. Since it was not possible to study early life exposures in the WHI, Dr. Parks evaluated the Sister Study cohort, which included more than 50,000 women aged 35–74. Dr. Parks found that RA risk was associated with more frequent residential pesticide application and personal use of insecticides in childhood, in addition to markers of low socioeconomic factors such as household education, young maternal age, and paternal smoking, emphasizing the importance of studying early life exposures.[42]

Dr. Parks addressed the difficulties in assessing early life exposures, difficulty with extrapolating findings from animal studies to humans, and determining the *in utero* and childhood exposures in epidemiological studies. For these reasons, Dr. Parks proposes designing better questionnaires to assess early exposures, along with studies to determine biologic effects associated with the questionnaires.

In summary, Dr. Parks remarked that women might have unique exposures to environmental factors requiring further research into gender-specific exposure assessment methods and determination of female susceptibility. If women are more susceptible, then an understanding of the factors that set the stage for sex-specific susceptibility is needed.

Biological Mechanisms in the Development of ADs

Sex differences, sex hormones, and immune regulation in ADs

In Dr. DeLisa Fairweather's opinion, unlike the cancer field, the AD field is splintered and would benefit from the grouping of ADs based on common themes such as pathology, age, and sex to help understand AD etiology. For example, pathology needs to be evaluated in a context dependent manner, as it is important to consider the organ involved in a specific AD and also the type of immune response that is being generated. Dr. Fairweather discussed a review from 2008, in which she grouped ADs based on age and sex and clearly showed sex differences in the pathological mechanisms of ADs for acute and chronic pathologies.[2] Regardless of sex, acute immune pathology involves inflammatory immune responses, while chronic pathology is characterized by fibrosis, the formation of scar tissue as a result of inflammation or repair from injury. Interestingly, grouping ADs based on male or female predominance reveals that the types of inflammatory immune responses driving acute versus chronic pathology differ. Male-predominant ADs that manifest before age 50 are characterized by a mixed Th1/Th17 immune response resulting in acute pathology through cell-mediated acute inflammation and presence of autoantibodies (autoAbs). In contrast, acute pathologies of female predominant ADs are driven by Th2, antibody-mediated responses. Both male and female predominant AD pathologies progress to a chronic condition in people around age 50. Regardless of the T helper (Th)1/Th17 or Th2 acute pathology, the chronic pathology is characterized by chronic fibrosis.[2] As this analysis shows, evaluating epidemiological data based on the grouping of ADs and subsequent analysis

by sex, pathology, and age can provide information regarding disease mechanisms that would be otherwise impossible, due to low incidence rates for specific diseases.

In addition to the Th1/Th2 bias for men and women respectively, Dr. Fairweather discussed an important component of the innate immune system, which may play a role in AD pathology: mast cells. Mast cells are located throughout the body, interface directly with sex hormones and other immune factors, and are known to drive both acute and chronic immune responses through Th1-, Th2-, and Th17-mediated mechanisms. Further, mast cells activate the inflammasomes in response to "danger signals" from damaged "self". These cytoplasmic multiprotein complexes are found in innate immune cells and have been implicated in AD pathology. However, our knowledge of mast cells and the inflammasome responses to environmental factors is poorly understood and more research is required to understand their possible role in AD pathogenesis. In conclusion, Dr. Fairweather emphasized the need for future research studies to analyze data by sex, examine exposures for "groups" of ADs, examine sex differences in antibody/autoAb responses in patients and animal models, and study inflammasome-driven responses to pertinent environmental agents.

Dr. Divaker Choubey further discussed the role of the immune system in AD pathogenesis, focusing on the role of IFNs and inflammasomes in environmentally induced inflammation. IFNs are a family of cytokines that play a role in inflammation and bind to cell surface receptors ultimately leading to activation of IFN-inducible genes and translation of IFN-inducible proteins such as the p200 protein family. Upon sensing cytoplasmic DNA, these proteins assemble the inflammasomes. Interestingly, female predominant ADs are associated with elevated levels of IFN messenger RNAs (mRNAs) ("IFN signature"), which encode the p200 proteins.[31] Simultaneously, autoAb–DNA complexes (clinical hallmarks of ADs) are taken up by the macrophages and other innate immune cells, leading to DNA localization in the cytoplasm. Detection of this cytoplasmic DNA by certain p200 proteins results in the activation of inflammasomes, leading to increased secretion of proinflammatory cytokines such as interleukin (IL)-1β and IL-18.[31, 43] The inflammasomes may provide a point of intersection for sex factors—such as the IFN signatures in women—and environmental exposures.

Dr. Choubey discussed a new congenic mouse model (B6.*Nba2*) that develops lupus-like disease and exhibits both sex bias and the IFN signature associated with lupus. These mice have increased levels of autoAbs against nuclear antigens and double stranded DNA; yet, they do not develop kidney disease.[44] Dr. Choubey discussed a region of DNA, the Nba2 interval that contains three candidate lupus susceptibility genes, including the *ifi200*, encoding the p200 family proteins. Interestingly, interactions among the three lupus susceptibility genes are required to develop autoAbs.[45] Recent discoveries from this murine model of autoimmunity include (1) sex hormones regulate the expression of p200 family proteins,[46,47,48] (2) IFN signaling regulates the expression of estrogen receptor α, and (3) polymorphisms in interferon-regulated factor 5 (IRF5) predisposes individuals to increased production of IFNs with estrogen further up-regulating the IRF5 expression[49,50] In conclusion, Dr. Choubey stressed the need for further studies to assess the role of p200 family proteins in autoimmunity induced by both estrogen and environmental exposures.

Epigenetic alterations and microRNA regulation in ADs

Epigenetics involve alterations in gene expression through mechanisms other than DNA sequence modification. Common epigenetic mechanisms are DNA methylation, histone modifications, and microRNA (miRNA) regulation. The role of epigenetics in the development of ADs has been a subject of recent interest and investigation. SLE-inducing drugs, such as procainamide and hydralazine, cause lupus-like autoimmunity in mouse models through T-cell DNA demethylation.[51] Similar epigenetic changes have been identified in some SLE patients, sparking interest in a possible role for epigenetics in the development of SLE.[52] Dr. Bruce Richardson addressed how epigenetic alterations in immune cells may lead to AD etiology. He explained that environmental agents such as drugs and ultraviolet light induce epigenetic changes in the CD4+ T-cell genome causing inhibition of DNA methylation of T-cell DNA.[51] This leads to the overexpression of number of genes causing the T cells to become autoreactive, cytotoxic, and pro-inflammatory. Dr. Richardson showed striking data that in women with SLE, the promoter of the immune associated gene, CD40L, is demethylated, resulting in overexpression of CD40L.[53] Interestingly, this phenomenon is not seen in men with lupus. Since CD40L is found on the X chromosome, this finding creates an interesting parallel with the idea that partial demethylation of the X chromosome, and consequent overexpression of some X chromosome genes, may contribute to the sex bias observed for several ADs.[54] Dr. Richardson proposed that since having two X chromosomes is the strongest predisposing factor for the development of lupus, with 90% of affected individuals being women, men suffering from lupus must require a greater degree of T-cell DNA demethylation, a greater total genetic risk, or both, to develop a lupus flare of equal severity as those experienced by women.

Dr. Ansar Ahmed discussed the role of another epigenetic mechanism, miRNAs, in AD development. MicroRNAs or miRNAs are small, evolutionarily conserved, noncoding RNAs that interact with mRNAs leading to mRNA cleavage and degradation or direct inhibition of translation. According to Dr. Ahmed, dysregulation of a subset of miRNAs involved in both innate and adaptive immune functions contributes to autoimmunity in RA, lupus, MS, systemic scleroderma (SSc), and Sjörgen syndrome, each with a unique pattern of miRNA dysregulation.[55–59] For example, in SLE, genetic, hormonal, and environmental factors dysregulate miRNAs, resulting in changes in gene expression that culminate in the breakdown of self-tolerance, induction of inflammatory cytokines, production of autoAbs, aberrant DNA hypomethylation, and dysregulated T regulatory cells.[57] Dr. Ahmed discovered a common set of dysregulated miRNAs (miRNA182-96-183 cluster, miR31, and miR155) in three different murine lupus models on differing genetic backgrounds (MRL-lpr, B6-lpr, and NZB/WF1).[60] Interestingly, sex differences were evident in the NZB/WF1 mice, where the expression of the miRNA 182-96 cluster correlated with the onset of lupus in female mice. Dr. Ahmed has also shown that a particular miRNA (miRNA146a) is selectively regulated by estrogen in immune cells, suggesting a role for miRNAs in estrogen-mediated

immune regulation.[61] According to Dr. Ahmed, miRNAs may help to understand AD pathogenesis and also have the potential to serve as biomarkers for diagnosis.

Dr. Prakash Nagarkatti further explained that miRNAs expression in immune cells is altered following exposure to environmental contaminants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Dr. Nagarkatti's laboratory performed miRNA arrays in fetal thymocytes folloowing prenatal expsure to TCDD. They screened 608 mouse miRNAs and found that 106 miRNAs were altered significantly in fetal thymocytes post-TCDD exposure when compared with vehicle controls.[62] Furthermore, several of the miRNAs that were downregulated contained highly complementary sequence to the 3'-untranslated region of several genes including AhR, CYP1A1, Fas, and FasL. Because Fas and Fas ligand are involved in the regulation of apoptosis and AD, these data suggested that prenatal exposure to TCDD may alter the immune response and increase the susceptibility to AD. These data were consistent with their earlier studies using an HY-T-cell receptor transgenic mouse model to study the development of T cells, in which it was noted that TCDD altered the negative and positive selection of T cells in the thymus.[63] Inasmuch as these processes play a critical role in the regulation of AD, the data suggested that prenatal exposure to TCDD may increase susceptibility to AD. This was also corroborated by demonstrating that TCDD exposure may enhance immune response to self-antigens.[63]

Epigenetic modification by CpG methylation at specific sites in the promoters of various genes, expressed in T cells, is known to regulate T cell differentiation into various subsets. Both Foxp3 and IL-17 genes possess CpG islands in their promoter regions. Dr. Nagarkatti's laboratory examined the methylation status of CpG islands present in Foxp3 and IL-17 promoters following aryl hydrocarbon receptor (AhR) activation. Activation of T cells from $AhR^{+/+}$ but not $AhR^{-/-}$ mice, in the presence of TCDD, promoted increased differentiation of regualtory T cells (Tregs) while inhibiting Th17 cells. This correlated with the findings that TCDD caused decreased methylation of CpG islands of Foxp3 and enhanced methylation of IL-17 promoters.[64] In contrast, another AhR ligand, a specific photoproduct of tryptophan, 6-formylindolo[3,2-*b*]carbazole (FICZ) was shown to have the exact opposite effects. Thus, FICZ increased the differentiation of Th17 cells while inhibiting Tregs. While both TCDD and

Table 2. Proposed Research Recommendations in Autoimmune Disease Research from the SWHR Autoimmune Roundtable

Analyze epidemiological data to:
1. Estimate disease burden from a woman's health perspective;
2. Group autoimmune diseases by pathology, sex, and race in order to evaluate relationship among them;
3. Determine role of environmental agents in influencing autoimmune disease incidence trends in adults;
4. Detect and measure early life/emerging exposures in males and females;
5. Identify disease phenotypes and risk factors by sex;
6. Determine role of emerging technologies in altering immune responses.

Examine biological mechanisms to:
1. Understand role of different periods in lifespan (from *in utero* to menopause and beyond) and autoimmune disease;
2. Determine relationship between immune dysregulation and autoimmunity, including sex differences if any;
3. Determine role of biomarkers to predict disease onset and progression from autoimmunity to autoimmune diseases;
4. Study epigenetics and epigenetic modulators in autoimmunity;
5. Understand role of lung and immune responses within the lung in different autoimmune diseases;
6. Understand role of microbiome in autoimmune disease;
7. Determine role of obesity in response to environmental factors;
8. Link endogenous estrogen variability to disease manifestation;
9. Elucidate sex differences in
   a. Phenotypic expression and disease severity of particular autoimmune diseases;
   b. Adaptive and innate immune sentinel cells (that interact with environmental factors);
   c. Autoantibody titers and more general immune responses;
   d. Inflammasome biology;
   e. Regulatory mechanistic pathways that prevent autoimmune diseases;
   f. Obesity and fat deposition (and how that affects autoimmune disease development and progression);
   g. Animal models of toxicity;
   h. Multiple exposures and their interactions;
   i. Both genetic and environmental protective factors.

Establish resources for:
1. A national-based registry and repository for all autoimmune diseases;
2. Interdisciplinary research group with various stakeholders for cost-effective collaboration;
3. Curriculum for allied health professionals;
4. Appropriate NIH study section and special emphasis panel on sex differences research;
5. Autoimmune disease parameters within the National Toxicity Program and examine autoimmune disease outcomes by sex.

Roundtable participants were asked to provide key recommendations based on existing knowledge gaps in the autoimmune disease field to facilitate progress in the autoimmune disease research. Consensus statements provided by the roundtable participants have been summarized into three categories.

NIH, National Institutes of Health.

FICZ are potent ligands of AhR, why these ligands behave differently remains an enigma. Preliminary studies indicated that this may result from differential induction of miRNAs. If environmental or dietary AhR ligands were to increase Th17 induction through epigenetic regulation, they could trigger or enhance certain inflammatory and ADs. Clearly, additional studies are necessary to investigate the effect of AhR ligands on epigenetic regulation of genes involved Treg and Th17 cell differentiation as well as miRNAs because such regulation may play a key role in the pathogenesis of a large number of ADs.

Chemical exposures

Trichloroethylene (TCE) is an industrial organic solvent used in the production of hydrofluorocarbons and is a major environmental pollutant. Epidemiological studies have linked TCE to increased incidences of ADs such as SLE, SSc, and autoimmune hepatitis (AH). Dr. Kathleen Gilbert observed a direct effect of TCE in lupus-prone mice following chronic exposure to TCE, though surprisingly, accelerated AH rather than SLE was observed in these mice. AH is characterized by an infiltration of T cells in both mice and humans. The disease, although rare, is found more often in women (70% cases) than in men.[65] In the mouse model, a 4-week TCE exposure increased CD4+ T cell IFN-γ expression and synthesis.[65] Dr. Gilbert hypothesized that TCE exposure leads to global gene methylation, resulting in the observed increase in IFN-γ. Her current working hypothesis for sex bias is that TCE metabolism by the liver leads to gender-associated alterations in the methylation pathways in CD4+ T cells, eventually causing AH.

Dr. Marc Monestier discussed a connection between mercury, autoimmunity, and gender. Currently, there is insufficient evidence to show that exposure to metals, including mercury, leads to the development of any particular AD.[1] High exposure to mercury induces changes in the central nervous system and the cardiovascular system, and toxic effects of mercury have been described following exposure from seafood, skin lightening creams, and gold mining.[66–69] Mercury-exposed gold miners in Brazil were found to have higher levels of ANA antibodies compared with non-mercury miners, suggesting that mercury exposure can lead to antibody responses that are characteristic of autoimmunity.[70] Further, Dr. Monestier described divergent effects between female and male mice offspring following postnatal exposure of the mother to mercury, with female offspring exhibiting a decrease in regulatory T cells. He further stated that the biodistribution, toxiocokinetics, and mercury accumulation differ between the sexes, though this research area remains largely unexplored.

Research Recommendations and Conclusion

The roundtable participants identified future research recommendations, based on existing knowledge gaps in the field of ADs (Table 2). While progress has been made in the understanding of AD etiology, it has been slow and primarily limited to only few ADs. Further, identification of environmental agents and studies on their interaction with genetic components in the development of ADs may help prevent the rise in AD rates. Several challenges still exist in AD research, including (1) sub-optimal coordination among researchers, agencies, and nations; (2) inadequate validated exposure biomarkers/assessment tools and training in environmental studies; and (3) limited population-based prevalence, incidence, and demographic information, including lack of standardized AD phenotype databases and registries. A clear knowledge of the basic mechanisms underlying these diseases is much needed to understand the role of environment and sex bias in ADs.

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