

Meeting report

Epidemiologic associations between occupational and environmental exposures and autoimmune disease: Report of a meeting to explore current evidence and identify research needs

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Abstract

To advance understanding of autoimmunity associated with exposure to environmental factors, an "Exploratory Meeting Epidemiology on Occupational and Environmental Factors Associated with Autoimmunity" was organized in Bilthoven, the Netherlands, from May 10–12, 2000.

Even if no firm conclusions can be drawn on a role of certain chemicals in the environment and in the work place in causing or exacerbating autoimmune responses and illnesses, many indications of this to occur exist. The aim of the meeting was to determine the optimal methodology for assessment of autoimmunity associated with occupational or environmental exposures in the human population, and to set up interdisciplinary and collaborative epidemiological studies to investigate the association of exposure to silica, hexachlorobenzene, ultraviolet radiation, and other agents with autoimmunity and autoimmune diseases in the human population. These agents were selected as carrying particular suspicion at present.

It was concluded that there is a need for experimental studies in laboratory animals and for clinical investigations to improve scientific knowledge about the causes and mechanisms of environmentally-induced autoimmune disorders and their treatment; in addition there is a need for an interdisciplinary approach to epidemiological studies of the environmental and other causes of these disorders in human populations. Specific designs for epidemiological studies in this context, as well as laboratory assays for health outcomes, were reviewed.

Several recommendations for the epidemiological approach to evaluating effects of environmental or occupational agents on autoimmunity were made. The prime recommendations are the following: 1) systematic descriptive epidemiological data on autoimmunity and autoimmune disorders

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are required; 2) the establishment of disease-reporting registries should be encouraged; 3) the development of internationally accepted standard diagnostic criteria for all autoimmune diseases should be encouraged; 4) the social impact of these disorders should be evaluated and estimations of direct and indirect economic costs should also be made; 5) the methods of exposure assessment used in epidemiological studies should be standardized; 6) laboratory methods for measurement of biological responses should be standardized; and 7) the inclusion of indicators of autoimmunity and autoimmune diseases and of relevant environmental exposures in ongoing epidemiological studies should be encouraged.

The importance of studying environmental causes of autoimmune diseases and autoimmunity lies in the identification and prevention of risks to the public health, and in improving our knowledge of basic mechanisms of health and disease.

Key words: Environmental exposure – autoimmunity – epidemiology – silica – hexachlorobenzene – ultraviolet radiation

Introduction

Autoimmune disorders are characterized by a common feature – inappropriate immune reactivity against self antigens. In aggregate autoimmune diseases are estimated to affect approximately 3–5 % of contemporary western populations. These diseases often occur among young and middle aged adults and cause significant and chronic morbidity and disability. Additionally, autoimmunity may play a role in the development and expression of other diseases, including atherosclerosis, infectious diseases and cancer. The autoimmune conditions arise as a result of a breakdown in self-tolerance which probably results from combinations of predisposing and/or contributing factors. It is believed now that certain environmental factors are involved in the development of immune dysregulation and autoimmunity. Thus, understanding the role of environmental factors in provoking and modulating autoimmune conditions will result in health benefits and cost savings that extend far beyond those relating solely to the autoimmune diseases themselves.

In this report, “autoimmunity” and “autoimmune disease” are distinguished as follows:

1. Autoimmunity: The state in which various laboratory tests show immunologic reactions against self antigens but are not necessarily accompanied by clinical disease.
2. Autoimmune disease: A clinical disorder, meeting standard diagnostic criteria, in which there is frank illness of varying severity, and laboratory evidence of autoimmunity.

Relatively little research attention has been paid to studying the patterns and causes of autoimmune conditions. This reflects several circumstances in the recent past: (i) the lack of agreed definitions of some of the clinical conditions, (ii) the seemingly complex and ill-defined nature and genesis of these conditions, (iii) the

fact that autoimmune diseases are often not recorded as the underlying cause of an affected individual's death, and (iv) the lack of population- and disease-based registries that would show incidences of autoimmune diseases.

In Table 1 several autoimmune disorders are listed, as well as inclusion definitions used in epidemiology of autoimmune diseases as they are given in References.

Many lines of evidence suggest that genetic factors likely play an important role in the development of autoimmune diseases. Although no genes or alleles have been described that are unique to any autoimmune disease, a growing number of specific genes have been identified as risk factors on the basis of their increased frequency in patients with certain autoimmune disorders compared to that seen in racially-matched control populations. Most genes that have been identified to influence the risk for the development of autoimmune disorders are those that regulate immune responses (immunogenetic markers) or the metabolism of drugs and toxins (pharmacogenetic markers). The polymorphic human leukocyte antigen (HLA) genes within the major histocompatibility complex (MHC) are the best documented genetic risk factors for the development of autoimmunity. The roles of many other genes in the induction of autoimmune diseases – including those encoding immunoglobulins, T-cell receptors, cytokines and their receptors, and target autoantigens – are less well understood. A number of environmentally-associated autoimmune disorders have been linked to HLA alleles differing from those seen in the idiopathic variety of the same autoimmune disease, strongly suggesting that gene-environment interactions are important in the pathogenesis of many forms of autoimmunity.

The rapid growth in our understanding of the biology and genetics of the human immune system, along with new mechanistic insights into the nature of autoimmune disorders, now creates the possibility for better formulated studies on the causation of those disorders.

Table 1. Autoimmune disease definitions used for study inclusion in the References^a.

Autoimmune disease	Definition employed
Addison's disease	Addison's disease (non-tuberculosis related, non-iatrogenic)
Autoimmune hemolytic anemia	Blood test for warm and cold antibodies
Chronic active hepatitis	Liver disease, serum LKMI antibody, exclusion of other types of liver disease
Glomerulonephritis	Immunofluorescence and microscopy of renal biopsy
Goodpasture's syndrome	No articles
Graves' disease/hyperthyroidism	Graves' type thyrotoxicosis with appropriate thyroid function tests or hyperthyroidism
Idiopathic thrombocytopenia purpura	No articles
Insulin dependent diabetes	Diabetes diagnosis based on insulin dependence
Multiple sclerosis	Definite or probable multiple sclerosis by clinical criteria
Myasthenia gravis	Osserman's criteria, or muscle weakness and response to anticholinesterase drug
Myocarditis	Dallas criteria or autopsy diagnosis
Pemphigus vulgaris	Direct or indirect immunofluorescence assays or by histology
Pernicious anemia	Schilling test positive
Relapsing polychondritis	No articles
Polymyositis/dermatomyositis	Bohan and Peter criteria or equivalent
Primary biliary cirrhosis	Anti-mitochondrial antibody or liver biopsy
Rheumatic fever and Rheumatic heart disease	Jones or modified Jones criteria, initial and recurrent attacks included heart disease
Rheumatoid arthritis	Chest radiographs for rheumatic heart disease
Scleroderma	Definite or classical disease determined by ARA ^b , Rome, New York, Bennett and Wood, CIDMS
Sjögren's	ARA ^b or equivalent criteria for definite disease
Systemic lupus erythematosus	Copenhagen or equivalent criteria
Thyroiditis	Thyroiditis with appropriate thyroid function tests or hypothyroidism
Uveitis	Ophthalmological examination for anterior, posterior, or generalized uveitis
Vitiligo	Clinical diagnosis

^a Adapted from Jacobson et al, 1997.^b ARA, American Rheumatological Association.

ders. Further leverage comes from the advent of various new serological, molecular biological, and genetic assays. Meanwhile, animal experimental studies of autoimmune phenomena have enhanced our insight into the underlying biology and the plausibility of various environmental factors as causes of autoimmune diseases in humans.

Many exogenous factors are known to influence the immune system, both in laboratory animals and in humans. They include compounds such as drugs, as well as environmental agents such as ultraviolet radiation (UVR) and chemicals. While inadvertent modulation of the immune response may lead to altered resistance to infectious agents, by the same token such influences may lead to (exacerbated) allergic or autoimmune conditions. In particular systemic lupus erythematosus (SLE) is often found associated with exposure to certain drugs, while SLE may be exacerbated by UV radi-

ation. It should be noted here that drug-induced and non-drug induced lupus may not be the same diseases, as the former is reversible, while the latter is not. Scleroderma has also been associated with exposure to a variety of chemical agents. The toxic oil syndrome, as occurred in Spain, which includes severe myalgia, arthralgia, Raynaud's syndrome, and scleroderma-like changes was probably caused by rapeseed oil denaturated with aniline. An epidemic of what has become known as the eosinophilia-myalgia syndrome which has occurred in Mexico and the USA has been linked to consumption of contaminated L-tryptophan. Occupational acro-osteolysis is linked to vinyl-chloride monomer exposure. Drugs account for 10–20% of cases of dermal vasculitis, and this figure is increasing.

It should be noted that environmental factors such as of microbiological origin may also influence the immune system, and in fact have an impact on autoim-

munity. These latter factors were beyond the scope of the meeting, and hence of the report.

Environmental factors may have an impact on the prevalence of autoimmunity and autoimmune diseases in the population. In light of these research developments, and given the increasingly recognized burden of population disease and disability due to autoimmune disorders, it is now possible to pursue a targeted programme of epidemiological research. This research would give particular emphasis, first, to describing the pattern and extent of autoimmune diseases in human populations, and second, by drawing on a range of scientific disciplines, to elucidating the causal factors.

In order to further advance the field of autoimmunity associated with exposure to environmental factors, an "Exploratory Meeting Epidemiology on Occupational and Environmental Factors Associated with Autoimmunity" was organized in Bilthoven, the Netherlands, from May 10–12, 2000. This meeting was a follow-up of a workshop on Linking Environmental Agents to Autoimmune Diseases held 1–3 September 1998 at NIEHS in Research Triangle Park, North Carolina (Cooper et al., 1999; Selgrade et al., 1999), in which two main conclusions were:

1. Develop research tools needed to explore links between environmental agents and autoimmune disease, and
2. Conduct hypothesis-driven research in occupationally exposed groups and/or in experimental animals.

A final recommendation of the workshop was that interactions between specialties should be encouraged and funding should be targeted to integrated studies that use multidisciplinary approaches to improve overall knowledge of the hazard, mechanisms of action and human health consequences associated with environmental agents and autoimmune disease.

The aim of the meeting in Bilthoven was:

1. To decide on the optimal methodologies to assess autoimmunity and autoimmune diseases associated with chemical or environmental exposures in the human population, and
2. To set up collaborative epidemiological studies on the association of exposure to hexachlorobenzene, ultraviolet radiation, silica and other agents with autoimmunity in the human population.

Research strategy and methodology

While actual epidemiological studies should be disease-specific, in order to understand the public health problem of autoimmunity and to convince funding agencies

of the need for research, it is important to consider the prevalence and incidence of these disorders as a group. There is only one recent study attempting to estimate the population burden of selected autoimmune diseases in the United States, which demonstrated that autoimmune diseases as a group were a significant source of morbidity and health problems (Jacobson, 1997). The documentation of the autoimmune conditions would be improved if they were included as part of the World Health Organization Global Burden of Disease study.

Obstacles to conducting epidemiological studies on autoimmune diseases include the poorly understood nature of the impact of immunological diseases on public health, and the current lack of funding partly due to the lack of knowledge regarding the underlying mechanisms of these diseases.

Autoimmune diseases frequently affect people at a younger age than other chronic diseases, and disability-adjusted life years (DALY) is one measure of the societal impact of autoimmune diseases that needs to be further documented. Use of this measure would quantify the substantial burden that autoimmune diseases place both on the quality of life and on health care systems due to the long period of illness and resulting disabilities. Additionally, almost all autoimmune diseases disproportionately affect women and some autoimmune diseases (e.g. SLE, scleroderma) disproportionately affect minority populations (e.g. Afro-Americans and Afro-Caribbeans). These have historically been underrepresented in research.

Development of a research strategy

A detailed research strategy which includes a description of specific aims and objectives of the studies should be developed. This should be in the form of a strategic plan which would be used by investigators world wide to co-ordinate and organise studies. A systematic ranking of research priorities will be required as part of this plan.

A recommendation from a workgroup similar to the one convened here is needed to develop specific goals and objectives for a detailed research strategy.

Classification

There is a need to develop standardized classification criteria which can be used in international studies of autoimmune diseases. While for many of these diseases the American College of Rheumatology (ACR) criteria can be used, certain disorders have a variety of classification criteria. An international consensus is needed to standardize disease classification criteria. This would be an appropriate activity for WHO and

other international organisations. In the absence of standardized classification criteria, international investigators should arrive at a consensus for case definitions for their specific studies. Criteria used as case definitions for research purposes should include a spectrum of disease; for example the condition should fulfil one, two, or more criteria for a specific diagnosis. Another option would be to specify positive test results, such as a positive anti-double stranded DNA as an intermediate marker, rather than the disease.

Descriptive epidemiology

Descriptive epidemiology (person, place, and time) is not currently available for many autoimmune diseases and should be developed. Other basic questions to be addressed include: 1) is the current incidence of autoimmune diseases increasing or decreasing, and how are these trends distributed, 2) what are the societal costs of these diseases, and 3) what are the individual costs.

The descriptive epidemiology should be population-based, thus in many areas the populations should be surveyed for baseline data. Patterns and trends in autoimmune diseases and autoimmune indices should be documented

National or regional disease registries can be useful for both descriptive and analytical epidemiology. In certain instances, it may be feasible to add questions on autoimmune conditions or specific laboratory tests to existing cohort studies or national health surveys. These may provide information on predictive significance of autoimmunity for incidences of autoimmune diseases. A second approach to learn more about disease patterns would be to use existing health care system data or hospital discharge data. A third approach would be to use samples from repositories of blood samples to assess the predictive significance of autoimmune indices. Some sources of these samples include the Hoorn Study in the Netherlands, the Framingham Study in the United States, the ALSPEC Study in the UK, and the Euro Diabetes Study.

Analytical epidemiology

Cohort studies: Historical cohort studies could be re-evaluated for indicators of autoimmune disease. Mortality and the development of symptoms and disease could be retrospectively determined. Other studies which are in development, such as the longitudinal childhood study in the United States, should be approached to include questions on autoimmune disease and laboratory measures as well.

Highly exposed populations, such as workers, or specific disasters such as the dioxin episode in Seveso, Italy, are examples of cohorts which should be target-

ed for study. "Hot spots" like these for disease and exposure need to be identified.

Exposure-based cohort studies, including occupational studies, should also be followed up for incidence of: changes in autoimmune indices, onset of clinical disease, adverse reproductive outcomes, and shifts in the male:female ratio of births.

Case-control studies: Case-control studies of persons with specific diseases or autoimmune indices are needed both for purposes of hypothesis generating and hypothesis testing. These studies need to include adequate sample sizes to examine risk factors in both men and women. The rarity of some of these conditions will require multicenter or multinational studies.

Short-term studies of "natural experiments", such as variation in ultraviolet light exposure (perhaps from phototherapy) and of biological exposures (such as immunization) could be useful. Disease outbreaks or poisoning episodes are other instances that will provide useful information and potential new cohorts for long term follow up.

For both case control studies and retrospective cohort studies we should point out that in many circumstances the major weaknesses include absence of environmental monitoring data, thus creating difficulty in tracing past exposures and absence of base line data usable as a reference.

Methods of assessing environmental exposures and autoimmunity or autoimmune diseases

There are several issues to consider in evaluating assessment methods for studies of exposure and autoimmune disease. One issue is whether a prospective or retrospective approach will be used for data collection. A second one is the size of the study. Methods appropriate for a study including 100 people will not be appropriate for 100,000 people. A third is the urgency and time frame within which the study must be performed. For example, a study undertaken in response to a natural or accidental environmental catastrophe (earthquake, industrial explosion, etc.) will have to use a different exposure assessment methodology than a study of a long term, low intensity exposure. The appropriateness and relative advantages and disadvantages of direct measures of a specific exposure in the environment (benzene in the water), biomarkers of exposure (serum levels of PCB), and questionnaire-based estimates of exposure should be weighed. Points to consider in relation to the choice of exposure measure are, e. g., the length of time since the exposure of interest has occurred, and the pharmacokinetics and pharmacodynamics of the agents of interest, presence of environmental monitoring data, presence of biomarker

for the chemicals of concern, and availability of reference data to assess whether exposure exceeded background levels. As with evaluation of carcinogenic exposures, there may be a latent period between the triggering event (initiation, for example, breaking of self tolerance or exposure of cryptic antigens) and the development of disease. On the other hand, the agent under assessment might be involved in clinical manifestation of the disease (promotion).

Exposure assessments

If appropriate records exist pertaining to the exposure of interest, they could be used quickly and relatively inexpensively to conduct studies relating to exposure and autoimmune changes and disease. Potential sources of information include the national or government-maintained records of ambient air levels of particulates, ozone, radioactivity, and exhaust compounds, soil composition maps (for silica assessment), and water contaminants. In some situations, geographic information systems may provide an information base.

A distinction should be made between self-reported information obtained by administered questionnaires (a form given to the subjects to fill out) and data collected by structured interview. In general, structured interviews will provide data of higher quality on occupational exposures. Interview-based questionnaires can be tailored for specific hypotheses and study designs. Although it may be appropriate to use the same questionnaires in different circumstances, previously developed questionnaires often need to be adapted. For example, a questionnaire focusing on short term recent exposures may be inappropriate if the exposure of interest is one of a chronic nature. There has been considerable work developing interview-based assessment methodologies for occupational exposures. An example of a useful approach is the occupational assessment process that combines a structured interview involving the review by a industrial hygienist and an appropriate follow-up.

For some environmental exposures, such as silica, there are no known specific and validated biomarkers of exposure. However, for other environmental agents, such as metals or ultraviolet radiation, there are accurate and specific biomarkers of exposure, such as bone, blood, and urinary lead levels and skin and urine levels of urocanic acid. The measurement of exposure to other compounds, such as organochlorines and organophosphates, may be complicated by the presence of numerous metabolites, fat storage, etc. Referral laboratories, such as the Centers for Disease Control and Prevention, have standardized laboratory methods to assess these biomarkers on a large scale.

Health effects

Self-reported diagnosis of most autoimmune diseases may be useful as a first step in case ascertainment. However, diagnoses need to be validated by medical record review or standardized clinical evaluation.

Population-based health data systems that may aid in case ascertainment are available in some countries. For example, Sweden, Norway, and Denmark have extensive data bases that have been used in many epidemiological studies. In the United Kingdom, General Practitioners Research Databases contain information based upon general practitioners diagnoses that may provide another source of information. Similar systems are not available in the United States on a national level; however, the managed health care systems, such as Kaiser Permanente and Mayo Clinic, provide access to large linked data bases that may include lab and outpatient data. Specific registries are available in both the United States and Europe for end stage renal disease.

Self-administered or interview based questionnaires may be used to obtain information on symptoms, health profile, and quality of life for prognosis or follow-up. Standardized and validated instruments are also available to assess depression and other psychosomatic parameters. Because of the possible role of stress in immune dysfunction, it may be useful to assess stressful life events using standardized approaches.

There are repositories for biological specimens that could be useful for studies of autoimmune changes. For example, the National Health and Nutrition Examination Survey III in the USA collected blood on a representative sample from 30,000 people from the United States population from 1988–1992. Standardized methods were used to ascertain numerous hematological parameters and many biomarkers of exposure (for example, lead). Sera were stored and could be used for assessment of autoantibody profiles and hormone levels (such as thyroid hormones). Analogous stores of samples have been archived in other countries too.

Laboratory measures of health outcomes

Laboratory methods in addition to the detailed history and comprehensive physical examination are widely used in providing clinical diagnosis of autoimmune diseases. Autoantibody detection is one of the most useful serological tests in the diagnosis of systemic and organ-specific autoimmune diseases. Similar laboratory methodology can be used to detect autoimmune responses following acute or chronic exposure to environmental agents. It is necessary to have a broad panel of assays that will detect a variety of abnormalities

associated with induction of autoimmunity. If there is no information like, e.g., preliminary experimental data on clinical symptoms, it should indicate the type of autoimmune effects such as systemic musculoskeletal or organ-specific actions that are related to environmental agent exposure. This extended laboratory methodology panel will provide minimization of false negative results in screening for autoimmune effects in epidemiological studies. Positive results in laboratory testing do not make a diagnosis or predict the subsequent development of autoimmune disease. This screening panel should be done in conjunction with clinical evaluation. If abnormalities are detected by the screening procedure, then further, more specific testing should be done to aid in the diagnosis of possible autoimmune disease.

Only methods well validated for their intra- and inter-laboratory reproducibility and sensitivity/specificity should be used in large epidemiological studies.

Commercial testing kits for detection of autoantibodies will be useful in such studies.

The following screening protocol is recommended:

General laboratory tests

(These tests will provide basic information about health abnormalities).

- Complete blood count (white and red blood cell (RCB) counts, differential leukocyte counts, thrombocyte counts, hemoglobin concentration, haematocrit, red cell indices) will provide information on hematological status and inflammatory conditions.
- Urinalysis (glucose, protein, hemoglobin by dipstick; if positive, specimen should be centrifuged and the pellet examined for RBCs and casts) should be done to detect kidney dysfunction and/or diabetes.
- Clinical chemistry: ALT (alanine transaminase) and AST (aspartate aminotransferase) as markers of liver damage, CPK (creatinine kinase) for muscle damage, creatinine for kidney dysfunction. C-reactive protein will point at an acute phase response (inflammation).
- T3/T4 or TSH will indicate thyroid dysfunction.

Immunological laboratory tests

(These tests will provide more specific information about immune dysregulation and autoimmune reactions).

- Immunoglobulin levels (IgG, IgA, IgM) should be used for detection of polyclonal stimulation. For example polyclonal elevations of IgG levels can be a characteristic of SLE or Sjögren's syndrome. IgE

and/or subclasses of IgG should be determined as an indication of changes in the Th1/Th2 balance.

- Autoantibodies: (by immunofluorescence on Hep2 cells). If ANA by immunofluorescence is positive, specificity of the antinuclear antibodies in the serum should be determined. ANA specificities associated with the development of systemic autoimmune diseases are: autoantibodies against double-stranded DNA, nucleosomes, histones, Ro/SS-A, La/SS-B, U1-RNP, Sm, DNA-Topoisomerase I (Scl-70), and centromere protein.
- Anti-neutrophil cytoplasmic antibodies (ANCA) by ELISA for myeloperoxidase ANCA and Proteinase-3 ANCA or by immunofluorescence on normal neutrophils. ANCA are markers of Wegener's granulomatosis (cANCA) or vasculitis (pANCA).
- Rheumatoid factor by ELISA or, if ELISA testing is not available, by latex agglutination can be determined. Rheumatoid factor, refers only to the IgM antibody which binds aggregated IgG as its antigen. It might be associated with RA.
- Organ-specific autoantibodies – anti-thyroid (anti-thyroid peroxidase) for detection of thyroid specific autoimmunity. Other organ specific autoantibodies may also be selected if organ – specific autoimmune reactions are expected.

Interpretation of the tests for autoantibodies will depend on the class and titre of the antibody and the age and sex of the test subject. Autoantibodies can be found in normal, healthy individuals, especially elderly females.

Serum samples should be taken as soon as possible, and at intervals of 3 months and 1 year, to detect changes over time. Frozen samples should be stored for later analysis. Tests would require one tube of peripheral blood collected into EDTA and three tubes collected into serum separator tubes. The urine sample should be obtained freshly.

Frozen banked serum samples from previous studies can be analyzed for immunoglobulin levels, autoantibodies, and serum chemistry analyses. Note that IgM antibodies may precipitate when samples are frozen and that IgE levels can only be compared to other samples frozen for a similar period of time.

Blood spots on filter paper can be obtained for DNA tests (HLA haplotype and gene polymorphism analysis).

Optional assays

FACS Analysis (B and T lymphocytes, NK cells and genetic or cell activation markers such as CD25 and HLA-DR); Soluble Interleukin 2 receptors (IL2R) as a marker of T-cell activation.

Environmental exposures that may influence autoimmunity

Silica

Crystalline silica, or quartz, is an abundant mineral found in sand, rock, and soil. High level exposure to respirable silica dust can cause chronic inflammation and fibrosis in the lung and other organs. Studies of occupational groups with high-level silica exposures (e. g. miners) have shown increased rates of autoimmune diseases compared to the expected rates in the general population. Experimental studies show that silica can act as an adjuvant and non-specifically enhance the immune response. This is one mechanism by which silica may be involved in the development of autoimmune disease.

For the following discussions we focus on SiO₂, as other derivatives or forms of silica, such as silicones, have different properties. There have been a number of epidemiological studies examining the relationship between exposure to silica and autoimmune disease and strong associations have been made between SLE, rheumatoid arthritis, ANCA associated vasculitis and glomerulonephritis, and scleroderma. Weaker links, primarily reported as case studies or case series, have been suggested for Sjogren's, Goodpasture's syndrome and poly/dermatomyositis.

A significant number of questions remain with regard to the pathophysiology, etiology, mechanisms and multiplicity of effects following silica exposure. For example, silicosis patients appear to be at increased risk for the development of specific autoimmune diseases (e. g., scleroderma), but it is not known if silicosis is a prerequisite for the development of autoimmune disease. In addition, in a number of silica exposed individuals, autoimmune disease develops prior to or without overt manifestations of silicosis. To date a majority of studies have used disease as an endpoint, and systemic examinations of changes in autoimmune parameters in silica exposed individuals are lacking. Thus we have little information on what types of immunologic changes occur, what is the persistence of these changes, how these changes relate to the progression and development of autoimmune disease, and whether these changes relate to the dose of silica received. While a number of epidemiological studies have been conducted in groups of individuals exposed to crystalline silica, little is known regarding associations between autoimmune changes or disease, and other forms of silica or other compounds which cause fibrogenic lung disease, such as asbestos.

Investigations to date have shown associations between silica exposure and kidney disease, both glomerular and tubular dysfunction. Additional studies

regarding the effect of silica on kidney disease are needed to elucidate the role of immune versus non-immune mechanisms in causing kidney disease and dysfunction. These studies are needed in populations with silica exposure as well as with the diagnosis of silicosis.

A number of general aspects which should be considered for silica as well as for the other studies which examine the association between environmental/occupational exposures and autoimmune diseases include: 1) The importance of dose (intensity, cumulative, duration) and route of exposure; 2) The role of other factors such as age, genetics, gender, lifestyle and health status; 3) Co-exposure to additional chemicals. 4) It is imperative that clear diagnostic criteria are used for the definition of autoimmune diseases throughout these types of studies. There is significant concern that we may be underestimating the numbers of individuals with autoimmune changes because we evaluate on the basis of end stage disease rather than on immunologic alterations. A graded system, such as is used with scleroderma (Masi, 1980) or SLE (Hochberg, 1997), as well as laboratory measures, is useful to define earlier manifestations of autoimmune disease. 5) The rarity of individual autoimmune diseases require large cohorts to be studied. Because the numbers in individual studies may be limited, the combination of cohorts with similar exposures, and the use of similar measures of exposure and health assessment should be encouraged.

Proposed studies for silica

Prospective cohort studies of occupationally exposed populations are strongly encouraged. Examples of highly exposed cohorts may be found in Eastern Europe, Japan, China and South Africa. Such populations would include uranium and gold miners, ceramic workers, and sandblasters. In addition, an attempt should be made to target groups outside the traditional "dusty" trades, who may be exposed to significant, but lower levels of silica, such as farmers. These studies should be conducted as a two tier process and include both small studies in specific populations, and larger, more extensive studies, which may make use of follow up of existing silica exposed cohorts and/or silicosis registries. These studies should make use of screening questionnaires for exposure and health assessment as well as collection of serum and urine samples to examine the prevalence of autoimmune changes. These studies could also use banked serum from exposed populations to look for autoantibodies. In addition, case-control studies for target autoimmune disorders are recommended. Particularly for the more rare diseases and smaller cohorts it will be necessary to make the appropriate power calculations to en-

sure that adequate numbers of cases and controls are included to arrive at reasonable estimates of relative risk.

Hexachlorobenzene (HCB)

Hexachlorobenzene (C_6Cl_6) is a lipophilic agent that is highly stable in the ecosystem. It was initially used as a fungicide but, because of its persistence in the environment and the severe human effects that occurred as a consequence of exposure in Turkey, fungicidal use was banned in most countries in the 1970's. The chemical still enters the ecosystem through the chemical industry in unknown amounts as a by-product of the manufacture of many compounds. In some processes it occurs in relatively large amounts, for example in the production of tri- and perchloroethylene. It is known that hexachloroethane use in the secondary aluminium industry results in the production of 0.5 % HCB as a by-product. The extent of the distribution of HCB made under these circumstances is unknown at present but recognized as a risk. It has been found in many human tissues in varying concentrations and is estimated to have a half-life of at least seven years. The lipid solubility, stability and lack of metabolism have made it a priority chemical for many nations. As HCB has a very long half life, blood concentrations constitute a good marker of exposure.

Proposed studies for hexachlorobenzene

It was agreed that hexachlorobenzene deserves more investigation in the areas of immunity, autoimmunity and autoimmune diseases. This was based on existing animal study results that indicate hexachlorobenzene-induced immune dysfunction, as well as some human cohort studies that raise concerns of possible autoimmune involvement. There are some areas with recent high exposure levels and some cohorts with very high exposures and other information that suggests there may be current industrial exposure of a high degree. Areas of interest include Catalonia (Spain), and certain regions in Turkey, south-eastern Brazil and Canada.

It was also noted that other important clinical outcomes have been identified in previous studies that are not considered to be autoimmune but should nevertheless be followed, such as cancer and reproductive effects as well as porphyria cutanea tarda (PCT).

For epidemiological studies it was recommended that studies include male and female subjects.

The discussion focused on the methodological difficulties associated with the strong possibility of any control group having substantial background exposure to hexachlorobenzene. This prompted a recommendation for the use of a "nested case control study". In this

instance a broad group of subjects from the appropriate populations would be assessed. For example, from a large population group, measures of immune function would be taken. In the Turkish population group, those with historic evidence of exposure to HCB would be identified by a previous clinical documentation of PCT. This (nested) group would then be compared to the larger group in terms of the frequency of autoimmune indices. The historic presence of porphyria would serve as a surrogate for the historical high exposure to HCB. In addition, because of the transgenerational exposure possibility, inclusion of children of mothers with PCT in the Turkish study would make an interesting exposure subgroup for analysis.

For a highly exposed population of south-eastern, Brazil, it was recommended that a survey be undertaken. The current serum HCB levels would be measured in a population group living in the region and these levels would be correlated with the autoimmune indices and clinical examination for autoimmune disease.

In all cases the design would include detailed clinical questionnaires that would ask the same questions in all countries in a similar fashion. There should be a formal clinical examination by an expert clinician in autoimmune disease. In addition to the recommended measures of immune dysfunction, it was recommended that anti-cardiolipin and anti-phospholipid antigens should be added. Also, because of the genetic susceptibility issues in this area of investigation, HLA typing and gene polymorphism studies of appropriate subjects should be considered.

Because of the work that has already been published on HCB, other outcome measures are important, including cancer (ovarian, hepatic) and reproductive outcomes (abortion, sex ratio).

For those involved in research on this chemical there is an interest and commitment to continue these investigations. For such an international study involving different cohorts, a steering committee approach is recommended with one member from each country represented. The steering committee should cover the following disciplines: epidemiology, clinical medicine, toxicology, industrial hygiene, and analytical chemistry.

UV-radiation and autoimmune diseases

UV radiation is the major cause of non-melanoma skin cancer. In addition, exposure to UV is immunosuppressive and studies with experimental animals and biopsy-proven skin cancer patients have indicated that the immune suppression induced by UV exposure contributes to skin cancer induction. It is well established that exposure to UV radiation in animals impairs resistance to viral, bacterial, and parasitic infections. It has been

proposed that a deficient immune response through persistence of infection and inflammation can be involved in development of autoimmune conditions. This possibility is supported by the observation that immune deficiency syndromes are often associated with autoimmune abnormalities. UV exposure is a potent immune modulator and in light of the widespread exposure to UVR, we suggest that UVR may influence the induction of autoimmunity. This is corroborated by the effects of UVR on SLE.

One of the earliest indicators of the carcinogenic effect of UVR was the latitude gradient of the effect. More skin cancer was noted in fair-skinned populations living closer to the equator. Preliminary studies have suggested that a latitude gradient also exists for the incidence of rheumatoid arthritis, insulin dependent diabetes mellitus, multiple sclerosis, and atopic eczema, although not all of these disorders may have an autoimmune basis. Although there is good evidence of the effect of UV on skin cancer induction and immune function in humans, little is known about the effects of UVR on autoimmunity and autoimmune disease. There are no well designed epidemiological studies to document the relationship of UV radiation on incidence of autoimmunity in humans, and to the best of our knowledge, no animal models. We suggest that there is a need for further studies to determine the role of environmental exposure to solar radiation in the induction of autoimmunity.

It is recognized that some autoimmune disorders (RA, MS) are T-helper 1 (Th1) – associated autoimmune disorders, while others (thrombocytopenia, Goodpasture syndrome, SLE) are T-helper 2 (Th2) – associated. Because UV preferentially suppresses Th1 function, UV exposure may have differential effects on these two different classes of autoimmune disorders.

There is therefore a need for comprehensive epidemiological studies focused on better characterization of exposure and health outcome, rather than solely reporting of examples of exacerbation. To explain current limited evidence there is also a need of validation studies by means of experimental animal studies.

Design of studies linking UVR exposure to autoimmunity

For this issue several designs are possible: ecological studies, migration studies, case-control studies and cohort studies with individual exposure assessment (retrospective questionnaires, diaries, personal dosimetry).

Ecological studies: Studies on latitude gradients, as have been performed for skin-cancer, and geographic differences in patterns of childhood infection. Possible confounders are HLA and other genetic differences.

Migration studies: Such studies may provide information on whether groups of migrants to a country at a higher or lower latitude get the same incidence of autoimmune disease as people of the country to which they migrated (for example Scottish people migrating to Australia). A possible difficulty is the recruitment of a sufficiently large study group to reach adequate power. This may be overcome by using autoimmune changes (e.g. disease-specific autoantibodies) rather than autoimmune disease as outcomes.

Case-control studies: These are most suitable for in depth epidemiological studies, in which individual exposure to both UVR and other possible risk factors (both short-term and life-time) can be estimated. This is appropriate as autoimmune disease is a rare event, when considered at the population level. This may be achieved by coupling the desired study to an already existing study by which a cohort of individuals with well defined autoimmune disease had been identified, for instance by applying the questionnaire on UVR exposure developed at RIVM to examine links between UVR exposure and infectious diseases, to the ongoing study in Arizona aimed at finding relationships between pesticide exposure and SLE.

Cohort studies: Such studies are desirable as follow-up of people over the course of many years may elucidate risk factors for the induction of autoimmune disease. There are 3 weak points:

1. Power analysis will probably show that the number of participants required is very high as individual autoimmune disease is a rare event.
2. The costs, as a consequence, may be very high.
3. Maintaining complete cohorts often proves quite difficult.

The contrast between the exposed and non-exposed group may be enhanced by focussing attention on highly exposed subgroups like outdoor workers, and comparing them with indoor workers. Contrast may be further enhanced by looking at different cohorts at different latitudes.

Questionnaires

Standard questionnaires that have been used to establish sun exposure in other populations (i.e., skin cancer patients) may be adapted and modified for these studies. In addition, standard questionnaires that have been used to investigate other autoimmune diseases such as lupus may be adapted and modified to cover exposure to UV. An important problem encountered when designing such questionnaires is our uncertainty about the duration and time between exposure and the development of outcome.

As in questionnaires designed for skin cancer lifetime exposure is considered by episode (decades, 5 year periods), these skin cancer questionnaires may be helpful for elucidating the time-window that is relevant for UVR associated autoimmune phenomena.

Laboratory measures of exposure

For UVR-studies we may add the use of personal dosimetry and presence of cis-UCA in skin and urine (non-invasive) for exposure assessment and validation of the questionnaire in a sample of participants. Duration of exposure should be considered in investigating UV exposure.

Groups to be studied

Information on characteristics of exposed groups is essential when designing the study and selecting the study group. A potential source may be skin cancer registries, in which cancer can be considered as a surrogate marker for high UV exposure. One established cohort that is defined with respect to the occurrence of autoimmune diseases is a cohort selected to study the prevalence of lupus, and pesticides exposure in Nogales, Arizona. A study group that may be well defined with respect to UVR exposure may be found within an occupational setting, such as farmers or construction workers, who may be exposed to UV for 8 hours per day over many years. Another possibility, that is likely to be rather expensive, yet could be very powerful, would be to follow up children for the risk of developing autoimmune diseases.

Conclusions

A prime conclusion drawn by the participants of the meeting was that there is insufficient knowledge about the mechanisms of autoimmune diseases. Autoimmune diseases comprise a broad group of illnesses in which dysfunction or damage to different body systems are produced by humoral and cellular reactions of the immune system against components of the body ("self"). The clinical diseases include rheumatoid arthritis, scleroderma, systemic lupus erythematosus (SLE), myasthenia gravis, several types of vasculitis, and other conditions, and the autoimmune reactions are represented by many types of antibodies, (e.g. antinuclear antibodies, anti-neutrophil cytoplasmic antibodies), and autoreactive lymphocytes.

Clinical, laboratory and epidemiological evidence show the significance of autoimmunity in the causation of diseases of major importance in the community.

There are indications of the role of certain chemicals in the environment and at work in causing or maintaining autoimmune illnesses, or in exciting autoimmune responses not always associated with a clinical disorder. The importance of studying environmental causes of diseases and autoimmunity lies in the identification and prevention of risks to the public health, and in improving our knowledge of basic mechanisms of health and disease.

The workshop focused on epidemiological and laboratory techniques to study autoimmune diseases in various populations and on ways in which the illnesses and autoimmune responses could be related to environmental exposures. Particular attention was paid to the most efficient means of performing such studies. The frequency of the disorders, the recognized difficulty of assessing relevant environmental exposure over long periods, or well before the onset of the corresponding disease, and the need to investigate numerous immunological factors, mean that the epidemiological work might be complex and costly.

The conclusion drawn from the discussions on genetic and other laboratory measures was that at present there are no methods available that predict the development of autoimmune diseases.

Most attention was paid to three examples, based on prior experience in the laboratory and in the clinic of the links to autoimmunity and autoimmune diseases, namely silica, UV radiation and hexachlorobenzene. It was recognized that other, widely distributed environmental exposures deserve study as they may also be associated with autoimmune disorders, notably certain heavy metals, pesticides and solvents. It is likely that further associations between environmental factors and immunological disorders will be recognized, possibly resulting in other types of disease, so this aspect of public health protection must be kept under review.

Recommendations

1. Much more information is needed about the pathogenic mechanisms of autoimmune diseases. Clinical studies as well as animal models need to be further advanced, with a concentration on genetic risk factors and molecular aspects of pathogenesis.
2. We need to know more about the specific effects of environmental agents on the immune system. Experimental studies in laboratory animals, as well as epidemiological studies may extend our knowledge base.
3. As the immune system is likely to be most vulnerable to effects of exogenous agents while developing and maturing, attention should be placed on perinatal exposures or exposures at young age.

4. Study of the occurrence of autoimmune conditions, of their association with individual genotypic and phenotypic factors, and their association with environmental chemicals and other factors, is only possible if there is assured clinical diagnosis of each autoimmune disease. It is essential that rigorously standardized diagnostic criteria are employed to define each disease. Internationally accepted criteria should be used when they are available, or, if they have not yet been developed, every effort should be made to gain agreement on a single definition of each disorder. The diagnostic criteria employed in every study must be stated.
5. To capitalize on the foundations laid by laboratory, clinical and epidemiological research into the nature, processes and causes of autoimmune disorders, there is need for an interdisciplinary approach to epidemiological studies of the environmental and other causes of these disorders in human populations.
6. The main priorities for epidemiological research are as follow:
 - Systematic descriptive epidemiological data on autoimmunity and autoimmune disorders are lacking. Therefore, international data should be sought and collated on: (i) the background prevalence of autoantibodies influenced by region, race and age, (ii) the prevalence of autoimmune disorders, (iii) the population burden of disease/disability they represent, and (iv) where appropriate, the mortality attributable to these disorders.
 - To assess better the social impact of these disorders, estimates of direct and indirect economic costs should also be made.
 - There is a need to identify time-trends in the incidence of autoimmune disorders or their biomarkers. Retrospective time-trend data should be sought on auto-antibodies, using blood samples stored for other research purposes over recent decades in cohorts as they have been established in the USA, Europe, and Australia.
 - Case-control studies of autoimmune disorders should be conducted. In order to attain sufficiently large numbers of study subjects, multi-centre studies (using standardized research protocols) may be desirable. Such studies would variously test and generate hypotheses.
 - Prospective studies of cohorts exposed to presumed or suspected environmental causes of autoimmune disorders should be initiated, with particular emphasis on studying the occurrence of auto-antibodies. A specific opportunity exists to seek a coordinated, perhaps data-pooling, approach to the several HCB-exposure cohort studies. Other such opportunities (e.g. with studies of silica-exposed persons) should be sought.
7. To facilitate high quality population-based epidemiological research, the establishment of comprehensive disease-reporting registries should be encouraged. While this is desirable as a general goal, particular note should be made of the special need for systematic disease registration in relation to large scale, unusual, population exposures to putative causal agents.
8. To improve international coordination and standardization of research, especially epidemiological research, on autoimmune disorders, an international consortium of interested scientists should be established. This consortium would develop and coordinate an agreed program of research, including promoting the standardization of research methods. In recognition of their activities and achievements, leading roles could be played, for example, by scientists at RIVM, CDC, NIOSH, and NIEHS. The WHO (Geneva) might play a supportive role.
9. Situations of high exposure to putative environmental causes of autoimmune disorders should be identified, both historically and prospectively.
10. It is recommended that the laboratory methods of exposure assessment and questionnaires used in epidemiological studies be standardized.
11. Laboratory techniques used to detect and quantify autoimmunity, e.g. the type and specificity of antibodies, cell phenotype, the response of lymphocytes to antigens etc., should be carefully standardized so that consistent results can be obtained, and findings in different laboratories can be compared. Yet, it should be ensured that such standardization does not result in rigidity; a flexible approach, depending on the study requirements, needs to be taken. We strongly recommend the commercial development of testing kits that will detect autoantibodies.
12. Where appropriate and possible, the inclusion of parameters of autoimmunity and autoimmune diseases, and of parameters of relevant environmental exposures, in ongoing epidemiological studies (e.g. large cohort studies, or national cross-sectional surveys) should be encouraged.

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Note: the meeting has been followed up by initiatives, taken by the National Institute of Public Health and the Environment, Bilthoven, the Netherlands; the London School of Hygiene and Tropical Medicine, London, UK; the Centers for Disease Control and Prevention, Atlanta GA, USA; and the National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; under auspices of the International Programme of Chemical Safety at WHO, Geneva, Switzerland, to install a task force Physical and Chemical Influences on the Occurrence and Progression of Autoimmunity and Autoimmune Diseases (PCIOPAAD). The goals of this task force are:

To serve as a focal point
To develop a research agenda

To stimulate development of methodology
To promote an integrated approach
To coordinate activities
To promote multicenter approaches
To identify emerging problems
To offer sessions at international conferences

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