



Environmental factors in autoimmune diseases, February 4–5, 2003, Durham, NC, USA

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The Environmental Factors in Autoimmune diseases (AID) workshop has been organized and supported by the National Institute of Environmental Health Science (NIESH), NIH. The conference took place on February 4–5, 2003, at the Durham Marriott hotel at the Civic Center, Durham, NC, USA. Durham, a beautiful city with the renowned Duke University situated in its boundaries.

The organizing committee included P.J. Mastin (NIESH), D. Germolec (NIESH), G. Cooper (NIESH), T. Esch (NIAID), L. Gretz (NIAMD), F. Miller (NIESH), N. Rose (Johns Hopkins University) and M.J. Selgrade (OS Environmental Protection Agency).

Dr A. Sassaman (NIESH/NIH/DHHS) and Dr M.J. Selgrade (National Health and Environmental Effects Research Lab) welcomed the attendees by providing figures regarding the estimated incidence of AID among the US population, being as high as 14–22%, and stressing the point that behind any laboratory work there are faces and names of patients; who hope for a cure. They reminded the audience that this meeting on the link between environmental agents and AID follows the first such gathering organized by the NIESH, five years ago, on September 1998.

The epidemiology of autoimmune diseases was presented by Dr G. Cooper (NIESH/NIH/DHHS).

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She discussed basic descriptive epidemiology, markers of sub clinical AID, such as autoantibodies, and specifically dealt with risk factors for systemic lupus erythematosus (SLE). AID can be divided to systemic vs. organ specific. SLE and rheumatoid arthritis (RA) are the most studied systemic AID, and multiple sclerosis (MS), type I diabetes mellitus (IDDM), and thyroiditis are the most investigated organ specific AID. Female predominance is the highest (> 85%) in Sjogren's disease, SLE, systemic sclerosis, thyroid disease and primary biliary cirrhosis (PBC). RA, polymyositis and multiple sclerosis bear a median female predominance (65–75%), whereas primary systemic vasculitis and IDDM show a low female predominance (< 50%). As to racial and ethnic predominance, SLE has long been known to be more prevalent among US blacks, in whom the disease tends to have an earlier onset and a more severe form. Similarly, northern European populations are at a higher risk to develop RA. Dr Cooper also discussed the trends in the incidence of autoimmune diseases over the past 20 years, showing an increasing incidence of type I DM as opposed to a decreasing incidence of RA and JRA. Mixed data are available regarding MS, SLE and SS. There are, however, no available data regarding these trends in hypothyroidism, hyperthyroidism, Addison's disease, myasthenia gravis and type I DM in adults. Little data exist regarding the epidemiology of autoantibodies and other autoimmune markers in asymptomatic patients. The likelihood to devel-

op an autoimmune thyroid disease in an asymptomatic patient with a positive marker of thyroid peroxidase (TPO)-microsomal antibody was found to be very high. Rheumatoid factor (RF) was found to be associated with an increased risk of RA in Pima Indians. As to ANA, there are no large scale studies, which can provide data on the predictive value with regard to its relation to future development of SLE. Dr Cooper presented her recently published data (A&R 2002) on the lack of association between the risk to develop SLE and early age at menarche, HRT, oral contraceptives and pregnancy. A positive association was, however, found between this risk and the age at menopause.

Dr F. Miller (NIESH/NIH/DHHS), dealt with gene–environment interactions in autoimmune diseases, and introduced the term ‘elemental disorder’. He suggested that most AID are caused by such an elemental disorder. Dr Miller also commented on the increasing incidence and prevalence of AID. He provided the existing evidence for the genetic background to AID and discussed their possible causes. Interactions between the environmental agent and the host matrix with its physiologic response lead to the clinical syndrome. There are, however, many difficulties to analyze and separate the exact contribution of the various agents from other contributing factors. Dr Miller reviewed the idiopathic inflammatory myopathies (IIM) with its various clinical-pathologic and serologic groups in order to demonstrate the ‘elemental disorder’ theory. The proposed mechanism is that exposure to an environmental factor, on the basis of genetic risk factors will lead to the development of a specific syndrome; hence, if we know the exact genetic risk factors we will be able to recommend the elimination of the specific environmental risk factor.

Dr M. Pollard (The Scripps Research Institute) discussed the immunology and genetics of murine mercury-induced autoimmunity and mercury as a disease accelerator. The major target which is attacked by mercury is the fibrillarin gene. Fibrillarin is a 34 KD protein component of the small nuclear ribosome. Some mice strains develop such a disease, whereas others do not. Thus the incidence of autoantibodies differs in different strains,

and the gene deficiency or a single-gene-knockout will also affect the autoantibody profile. Mice with this AID demonstrate an increase in IL-4, IgE and antibodies to IgG1—a Th2 type reaction. Interferon- γ is known to have a major role in mercury induced autoimmunity, and its suppression will suppress the disease. It should be noted that there are no reports of human mercury induced autoimmunity.

Dr N. Rose (Johns Hopkins Center of Autoimmune diseases) discussed infection, environment and autoimmune diseases. Dr Rose introduced the topic of the interaction between the genetic predisposition and the environmental factors leading to AID, using the model of Coxsackie virus induced autoimmune myocarditis. This virus which is a main reason of heart failure in patients younger than 40 years, has high affinity to heart tissue, but in most patients the disease is an acute and self-limited disease. However, in the minority of the patients, as well as in the ASW mice strain the disease takes a different course. Following the early stage of the infection with the virus, the disease continues and changes its nature. The mice start to develop autoantibodies against heart myosin. The development of these autoantibodies is a marker for an on-going disease. The ASW mice model is a susceptible strain for the development of this autoimmune myocarditis. Immunization of these mice with the cardiac myosin, in the absence of the virus will lead to the development of this autoimmune myocarditis. Thus, it is a classical T-cell dependent AID. Even immunization with a peptide drawn from the heart myosin will cause a similar disease. It was later noted that environmental factors have an impact on disease induction. When LPS was added to the myosin, even un-susceptible strains, developed the myocarditis. LPS promotes cytokine induction such as IL-1, IL-12 and TNF- α . Furthermore, LPS blockage was found to inhibit myocarditis development in susceptible strains, stressing again the importance of cytokines, such as IL-1 and IL-18 and TNF. Early complement blockage will also decrease the extent of the disease. The model provides therefore, data supporting the interaction of environmental factors with the genetic pre-disposition by either inducing or suppressing the tendency to develop AID.

Dr V. Rider (Pittsburg State University) presented the issue of sexual dimorphism in autoimmunity- and the role of environmental factors. The potential role of estrogen in SLE has been extensively investigated. The male female ratio in USA patients is 1:10. It has been shown by Lahita that there is an increased frequency of SLE in males bearing XXY chromosomes. Furthermore, abnormal estradiol metabolism in females with SLE as well as alleviation of SLE symptoms (in some) using hormonal therapy has been shown.

Estradiol increases phosphatase and free phosphatase activity in T cells from SLE patients in comparison to a non-SLE control group. Calcineurin is a final end product of T-cell activation by antibodies, via the CD40 ligand which is expressed on T cells. In vitro studies have shown an increased expression of calcineurin mRNA when T cells from SLE patients were exposed to estrogen. Estrogen increases the expression of CD40 ligand in T cells from SLE female patients in a dose dependent fashion, an effect which is not seen in male patients and controls without SLE. Females with SLE seem to have an over sensitivity to estrogen, compared to healthy females or male lupus patients.

Examples of influences of proteins and not genes in autoimmune diabetes were presented by Dr D. Faustman (Massachusetts General Hospital). The role of environmental factors in IDDM is more than 50%, as opposed to type II DM which is not an AID. A specific environment even without any genetic pre-disposition may lead to the development of IDDM. NOD mice are a known murine model for spontaneous development of autoimmune DM. Disease symptoms onset (splenitis) develop at 6 weeks in-vitro and 10 weeks in-vivo. Any influences on the proteins, which are post genetic code and may have an impact on the genetic penetrance, would be environmental.

Proteins represent marked differences between monozygotic identical twins. Serum autoantibodies are an example, thus even in the case of the same genetic background, there might be different products. Differences observed in monozygotic twins with IDDM include differences in lymphoid cells, which may precede the disease in as much as 8 years differences and defects in antigen presenta-

tion in NFkB, and in cytokine balance. The author concluded that 'proteins have a value' and that proteins and cells have remarkable similarities between AID. Thus, treatment of protein defects may represent new targets for disease termination and attacking of autoimmune diseases.

During the afternoon session, NIESH grantees, dealing with environment/infection/gene interactions in autoimmune diseases, presented their studies.

A new potential mechanism contributing to mercury induced autoimmunity was attenuation of activation induced cell death, which was presented by Dr M. McCabe (University of Rochester). Environmental factors may contribute to apoptosis or cell accumulation, like in malignancies or in autoimmune diseases. Mercury attenuated death receptor mediates growth inhibition. Dr McCabe has shown that mercury prevents CD95 induced apoptosis, and increases the number of autoreactive T cells. Mercury inhibits the CD95 signalling pathway, and binding sites on cell receptors, thus leading to cell death. Mercury probably fosters improper clustering of CD95. The increase in the autoreactive T cells which were shown by exposure to mercury can be attributed to some factors: metal altered self, molecular mimicry, autoantigens modification and autoantigens secretion after cell death. Cell death is probably related to caspases and mercury has been shown to prevent the cleavage of caspase-8.

The genetic and environmental impact of IL-2 and Fas-L in autoimmune diseases was discussed by Dr Shyr-Te Ju (Medical college of Virginia). Highly active T-cells induce FAS and FAS-L and thus apoptosis. The phenotype of the Mrl/Mpj-lpr/lpr mice model, which have a defect in the Fas expression, expressed by spontaneous lymphadenopathy and splenomegaly, abnormal TCR cells, autoantibodies, glomerulonephritis and fertility problems. The phenotype of IL-2 knockout mice includes impaired T cell response to mitogens, poor perforin and FAS-L mediated cytotoxicity, increased serum IgG1 levels, splenomegaly, lymphadenopathy, hemolytic anemia, anti-DNA antibodies (in some strains), low birth weight, short life span, sterility and ulcerative colitis. The double mutant mice (DKO) do not develop however,

glomerulonephritis, inflammatory bowel disease, anti-DNA antibodies and hemolytic anemia as the others, and their body weight is normal or high. The data presented thus suggest that FAS defect would induce AID and that IL-2 is required for autoreactive lymphocyte expression and lpr phenotype.

Dr P. Fraser (Harvard Medical School) dealt with the glutathione *S*-transferase genetic polymorphism, as a pre-disposition for environmental risk of DNA damage, autoantibodies and lupus. The proposed mechanisms for oxidative environmental stress in SLE is via photosensitivity and sun exposure, (UVB light >UVA), harming keratinocytes, and promoting anti Ro antigens and via organic solvents, which affect hematopoietic cells. This oxidative stress has two phases, the first via drug metabolizing enzymes like cyp450, the second via anti-oxidants like the glutathione *S*-transferase (GST). Dr Fraser presented the population based Carolina Lupus study, which included 243 cases and 298 controls. The study summarized the potential risk factors for lupus examining the GST polymorphisms in lupus. The authors did not find any effect of GST and an increased risk of lupus, nor did they find any effect of occupational sun exposure and an increased risk for lupus (*J. Rheumatol.* 2003;30,276). They did, however, find a gene–environment association, i.e. sun exposure on a genetic polymorphism of the GST was associated with a high risk of SLE.

No association was found between sun exposures and an increased risk of developing anti Ro antibodies. However, again a gene–environment association was found, i.e. sun exposures on top of a genetic polymorphism of GST was linked with a risk of developing anti Ro antibodies. The study bares however, some limitations, as the dosimetry of the sun exposure, sun reflectance, estimations of the racial impact, dietary antioxidants and the impact of environmental estrogens. A recent community study in Boston failed to find any connection between organic solvent occupational exposure and SLE.

Dr L. Morel (University of Florida) presented his data on congenic strains as a tool to analyze gene–environment interactions in SLE. Sixty-five percent of human AID loci fall into 18 clusters in

the human genome. The panel of congenic strains represents a useful environmental agent in the study of polygenic diseases. Estrogens (E2) were found to have a minimal effect on lymphocyte subtypes and activation, as well as on autoimmune responses. No effect was found on the renal pathology and a possible interaction between E2 and SLE1 with the enhancement of dermatitis in the B6 strain. In the Pristane study when pristane was injected, pristane did not enhance the effects of a specific SLE locus. Thus, no evidence of gene–environment interactions between the SLE1, SLE2 and SLE3 genes and estrogens or pristane. Environmental agents may thus require a polygenic background to have a significant effect, or may act on other loci not yet tested.

The second part of this day, in the afternoon session, was opened with a lecture dealing with the environmental triggers of autoimmune thyroiditis. Dr C.L. Burek (Johns Hopkins Hospital) discussed the effect of dietary iodine on the increased incidence of murine autoimmune thyroiditis. Iodine is immunogenic and so is the iodinated thyroglobulin. In humans with a pre-disposition for developing thyroiditis, when the iodine concentration in the nutrients increases; disease onset is earlier and its progression is more severe. Reduced iodine intake decreases this effect whose mechanism is unknown. Obviously host factors are related to the effect including the target thyroid tissue, APC presentation, MHC-TCR recognition and the cytokine response.

The NOD H2 mice animal model develops spontaneous thyroiditis when fed with iodine, and does not develop diabetes mellitus. The autoantibody level is in correlation with the thyroid lesions and the cellular infiltration to the thyroid tissue. The infiltrating cells are mainly CD4+ lymphocytes, but also few CD8+ cells, macrophages as well as B220, B cells. NK cells infiltrate the thyroid as well, and secreted ICAM-1 is increased. NKT cell line transfers disease only to mice pretreated with iodine. Iodine has thus multiple effects on autoantigens, thyroid tissue and on immunological cells, but it is not the only trigger; and infections, chemicals and radiation are other potential triggers for thyroiditis. These may have an additive effect on top of the effect of iodine.

Dr E. Gershwin (University of California, Davis) presented the issue of xenobiotics and the induction of primary biliary cirrhosis (PBC). PBC is an autoimmune disease where the small biliary ducts are destroyed. The majority of the patients were women; the median age of onset is 35–45 years, though the disease can occur at any age. Anti-mitochondrial antibodies (AMA), produced by antigen spreading are highly specific in this disease with the pyruvate-dehydrogenase (PDH) being the major autoantigen. The finding of AMA in an asymptomatic patient correlates with a very high probability for developing PBC within 15 years. AMA is present in more than 95% of the PBC patients at the time of presentation. The disease etiology is unclear. Dr Gershwin set forward the hypothesis that xenobiotic exposure to chemicals such as lipoic acid lysine and its mimics is related to the pathogenesis of PBC. Sera of PBC patients reacted with 18 different chemicals even better than with the lipoic acid. One hundred percent of rabbits injected with BSA, and one of these chemicals such as Bromohexanoic acid (a compound found in variable concentrations in some soaps) developed AMA within one month. Thus, rabbit sera recognize rabbit mitochondrial proteins. To date these animals do not develop PBC, though a liver function abnormality is seen.

The final lecture on the first day of the meeting was by Dr E. Sobel (University of Florida) on the accelerated development of lupus in (NZB×NZW)F1 mice by an environmental toxicant-Chlordecone. This is a chlorinated pesticide, which bares estrogenic effects. Oophorectomized mice exposed to chlordecone were found to have a decreased survival rate in a dose response reaction. Moreover, an accelerated rate of renal disease with immune complex formation was noted as well. Similarly to animals treated with estradiol, those treated with chlordecone showed an increased expression of CD69. In non-autoimmune mice strains such as in the Balb/c strain, all experiments with exposure to the chlordecone showed negative results.

The first day of the workshop ended with a two hour reception and poster session, all dealing with environmental factors in autoimmune diseases as well as with potential treatments. Some animal

models were presented expressing different autoimmune diseases or baring a pre-disposition for environmental effects on the progress of disease. Potential treatments were also offered.

The second day of the workshop opened with a short review by Dr J. Nyland (Johns Hopkins Hospital) dealing with the current progress in research on environmental triggers of autoimmune diseases. She reviewed the literature since the previous meeting in 1998, on the potential effects of mercury, silver, gold, selenium, organic compounds, pesticides and stress, on AID. It is the authors' impression that there is a skewing of research to animal models for certain xenobiotics with an obvious lack of epidemiological studies.

The morning session was concluded by Dr T. Esch (DAIT/ NIAID/NIH/DHHS), summarizing the autoimmune diseases coordinating committee research plan, recently published (and available over the NIH web site), dealing with the search of biomarkers, measures for developing an efficient data base, integrating the epidemiology data with the clinical trials and improving the multidisciplinary approach with formation of consortia for various major research projects. The highlights of the research plan include:

- The disease burden.
- Etiology and genetics.
- Identification of environmental triggers.
- Establishing mechanistic relationships.
- Biomarkers in diagnosis and progression of disease.
- Immunologic studies studying appropriate animal models and mechanisms.
- 'Think globally – act locally'.

The morning session was concluded with 6 breakout sessions—workshop groups each dealing with a major issue, summarizing it with proposals for improving the progress of research in that specific issue. The six sub-groups dealt each with one of the following: gene–environment interactions, altered antigens, immune modulation, signal transduction, translational research in systemic autoimmune diseases and in organ specific diseases.

Summaries of the breakout sessions were presented in the afternoon session. The main conclu-

sions of the whole group were that we should all work to create and improve a better data base, work for more collaboration and integrate multi-disciplinary specialties in the research on environmental factors in AID, in order to have the best means to achieve large-scale studies, which will provide us with the proper answers to our outstanding and unanswered questions.

Dr N. Rose delivered the concluding remarks of the very successful meeting, reminding all that our future research should always be based on population—epidemiological studies on the one hand and mechanistic research on the other. He stressed the importance of uncovering the environmental factors affecting and accelerating autoimmune diseases. In this way, we will be able to progress and avoid exposure to the environmental agents and in this way hopefully prevent AID. Excellent exam-

ples for a prototype of a disease with an environmental factor overcoming is Coeliac Disease, where avoiding ingestion of gluten prevents both the clinical and immunological features of the disease. If we find which environmental factor plays a role in a disease, we will then hopefully be able to prevent the disease even if there is a genetic background. Obviously, there is still a long way to go and collaborations are needed.

The workshop ended with a great hope expressed by all from this successful meeting, hoping that collaborations will be formed between the attendees and their research programs, a database on environmental factors in AID will be formed, and that the next meeting dealing with environmental factors in autoimmune diseases will take place within 1–2 years.

The World of Autoimmunity; Literature Synopsis

Autoantibodies against T-cell costimulatory molecules

The presence of autoantibodies directed towards surface molecules on lymphocytes in dogs with various autoimmune diseases was investigated by Khatlani et al. (*J Immunother* 2003;26:12). Anti-CTLA-4 antibodies were found in 31.8% of those with rheumatoid arthritis, in 20% of those with systemic lupus erythematosus, 12.5% of those with pemphigus, in none of the healthy donors and of those with immune-mediated hemolytic anemia. On the other hand, anti-CD28 autoantibodies were not found in either of these populations.

Beneficial effects of thymectomy in early-onset myasthenia gravis

The possible beneficial effect of thymectomy on the course of myasthenia gravis (MG) has been studied by Romi et al. (*Eur Neurol* 2003;49:210). 34 thymectomized and 18 non-thymectomized MG patients were included and followed-up for 5, 10, 15 and 20 consecutive years. In all follow-up points, MG severity was higher in the non-thymectomized group compared with the thymectomized MG patients. Remissions occurred in 21 of 34 patients who underwent thymectomy as opposed to only 4 of 18 patients who were not thymectomized. The presence of high or low anti-acetylcholine receptor autoantibody titer was not associated with thymectomy outcome.

Carotid artery intima-media thickness in antiphospholipid syndrome

The extend of carotid artery intima-media thickness (IMT) was compared between 28 patients having primary antiphospholipid syndrome and 28 controls (*Medina et al., Ann Rheum Dis* 2003;62:607). IMT was found in 23 of 28 patients but only in 7 of 28 controls. A decrease in the arterial lumen diameter was also detected in 11 of 28 patients with primary antiphospholipid syndrome who did not exhibit carotid atherosclerotic plaque, but in fibrosis in G-EAT.

The classical risk factors were not associated with IMT in this cohort. Patients having carotid IMT had more vascular disease than patients without IMT, and these diseases included stroke, myocardial infarction, and mesenteric thrombosis. The authors suggest that IMT in antiphospholipid patients might be associated with stroke.