Lupus-Prone Mice as Models to Study Xenobiotic-Induced Acceleration of Systemic Autoimmunity

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The linkage between xenobiotic exposures and autoimmune diseases remains to be clearly defined. However, recent studies have raised the possibility that both genetic and environmental factors act synergistically at several stages or checkpoints to influence disease pathogenesis in susceptible populations. These observations predict that individuals susceptible to spontaneous autoimmunity should be more susceptible following xenobiotic exposure by virtue of the presence of predisposing background genes. To test this possibility, mouse strains with differing genetic susceptibility to murine lupus were examined for acceleration of autoimmune features characteristic of spontaneous systemic autoimmune disease following exposure to the immunostimulatory metals nickel and mercury. Although NiCl 2 exposure did not exacerbate autoimmunity, HgCl 2 significantly accelerated systemic disease in a strain-dependent manner. Mercury-exposed (NZB × NZW) F1 mice had accelerated lymphoid hyperplasia, hypergammaglobulinemia, autoantibodies, and immune complex deposits. Mercury also exacerbated immunopathologic manifestations in MRL+/+ and MRL−/− mice. However, there was less disease acceleration in MRL−/− mice compared with MRL+/+ mice, likely due to the fact that environmental factors are less critical for disease induction when there is strong genetic susceptibility. Non-major histocompatibility complex genes also contributed to mercury-exacerbated disease, as the nonautoimmune AKR mice, which are H-2 identical with the MRL, showed less immunopathology than either the MRL−/− or MRL+/+ strains. This study demonstrates that genetic susceptibility to spontaneous systemic autoimmunity can be a predisposing factor for HgCl 2 -induced exacerbation of autoimmunity. Such genetic predisposition may have to be considered when assessing the immunotoxicity of xenobiotics. Additional comparative studies using autoimmune-prone and nonautoimmune mice strains with different genetic backgrounds will help determine the contribution that xenobiotic exposure makes in rendering susceptible populations susceptible to autoimmune diseases. Key words: animal model, autoimmunity, lupus, mercury, nickel, xenobiotic. — Environ Health Perspect 107(suppl 5): 729-735 (1999).

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Analysis of the role of xenobiotics in autoimmunity, particularly in humans, is hampered by identification of potentially sensitive populations. Epidemiologic studies have revealed associations between xenobiotic exposure and certain systemic autoimmune diseases (1,2) but do not identify susceptible individuals. Conversely, although it is appreciated that systemic autoimmune diseases are under multigenic control (3), identification of the genetic elements involved and whether they respond to environmental exposures is in its infancy. However, recent studies have raised the possibility that both genetic and environmental factors act synergistically at several stages or checkpoints of disease pathogenesis (3). This exacerbating role for environmental agents has been suggested by a number of studies that have shown that xenobiotics can accelerate the onset of autoimmunity in animal models (4-7). These studies predict that individuals susceptible to spontaneous autoimmunity should be more susceptible following xenobiotic exposure by virtue of the presence of predisposing background genes. Such accelerated disease would be expected to more closely resemble the immunologic features of idiopathic autoimmunity rather than that induced by xenobiotic exposure in a nonautoimmune-prone host.

The availability of animal models of systemic autoimmune disease provides a valuable resource with which to study the responses of a population sensitive to xenobiotic-induced acceleration of autoimmunity. Among the best-known animal models of human systemic autoimmune disease are those mouse strains that spontaneously develop features of systemic lupus erythematosus (SLE) (3,8,9). Each of the lupus-prone strains has been derived from different genetic backgrounds, which results in both quantitative and qualitative differences in disease expression. In some cases specific features of autoimmune disease have been ascribed to individual genetic defects in individual strains (9). Lupus-prone strains thus provide unique genetic backgrounds for the study of xenobiotic influences on the constellation of immunologic features that comprise systemic autoimmunity. The strains used in this study include (NZB × NZW) F1 (NZBW F1) (H-2 d ) female mice, which due to the influence of sex hormones develop systemic autoimmunity earlier in life than their male counterparts (3,8). Also examined were MRL (H-2 5 ) mice, which consist of two substrains, MRL−/− and MRL+/+ (3,8). In the MRL−/− autoimmunity occurs very early in life and is associated with massive lymphoproliferation due to abnormal Fas (CD95) expression. In contrast, the MRL+/+ substrain, which has normal Fas expression, develops less severe systemic autoimmune disease, with onset considerably later in life than the MRL−/−. In order to compare the influence of non-major histocompatibility complex (MHC) genes on xenobiotic acceleration of autoimmunity, the nonautoimmune strain AKR (H-2 5 ) from which the MRL substrains derive their H-2 was also tested.

Xenobiotics selected for study were two metals, mercury and nickel, known to produce distinctly different immunologic responses. Mercury induces autoimmunity in mice (10) and rats (11) and an immune-mediated kidney disease in humans (12). Mercury-induced autoimmunity (HgIA) in rodents is associated with three major pathologic sequelae—lymphoproliferation, hypergammaglobulinemia, and the development of autoimmunity—manifested as autoantibody production and immune complex disease (10,11). Elicitation of these pathologic features is dependent on genetic background, with lymphadenopathy occurring in most strains; autoimmunity is more restricted, being controlled in large part by the MHC (10). HgIA was initially thought

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to be a CD4⁺ T-helper (Th)2 response because of increased levels of predominantly interleukin (IL)-4, and IgG1 autoantibodies after HgCl₂ exposure (13). However all features of systemic autoimmune (hypergammaglobulinemia, autoantibodies, immune complex deposits) are now known to be dependent upon the Th1 cytokine interferon (IFN)-γ (14).

In contrast, exposure to nickel is associated with allergic rather than autoimmune reactions. Nickel elicits increases in nitric oxide production (15), activation of nuclear factor-kB (16), and delayed-type hypersensitivity (DTH) reactions in humans (17) and rodents (18). Nickel-induced DTH is characterized by the presence of IFN-γ-producing Th1 and Th0 CD4⁺ T cells (19).

In this study, particular emphasis was placed on the potential of these xenobiotics to exacerbate the natural course of systemic autoimmune disease in lupus-prone mice as revealed by lymphadenopathy, elevations of serum immunoglobulin, appearance of autoantibodies, and immune complex deposits. Although NiCl₂ exposure had little effect on autoimmunity, mercury significantly accelerated systemic disease in a strain-dependent manner. Thus, mercury exposure exacerbated almost all features of autoimmunity in NZBWF₁ mice. Mercury also exacerbated immunopathologic features in both MRL substrains, although less effect on the humoral response was observed in mice with the lpr mutation. Non-MHC and non-lpr genes contributed to the mercury-exacerbated disease in the MRL, as H-2 identical nonautoimmune AKR mice showed less immunopathology. These studies suggest that autoimmune-prone mice are useful models for nonhuman studies on the effects of xenobiotics on sensitive populations.

### Materials and Methods

#### Mice

Female NZBWF₁, (H-2d/z), MRL-Fas⁺/⁺ (MRL/lpr) (H-2k), MRL/Fas⁺/⁺ (MRL+/+) (H-2k), and AKR/J (H-2k) mice were obtained from The Scripps Research Institute Animal Colony (La Jolla, CA) and maintained under specific pathogen-free conditions. All experimental procedures using animals followed the guidelines set down in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (20).

#### Treatment of Mice

Groups of up to eight mice (4 weeks of age) were injected subcutaneously twice per week for 4 weeks with 100 μL phosphate-buffered saline (PBS) containing 40 μg HgCl₂, 40 μg NiCl₂, or PBS alone. Mice were bled before the first injection and sacrificed on day 30; serum was stored at −70°C until use. Autopsies were performed as previously described (21). Samples of kidney and spleen were harvested for analysis of immune complex deposition and histology (see “Tissue Complex Deposits” and “Light Microscopy”).

#### Detection of Serum Antibody

Antibodies nuclear antigens (ANA) were detected using HEP-2 cell slides (Bion Enterprises, Park Ridge, IL) as previously described (22). Prior to assay, sera were diluted 100-fold in PBS containing 0.1% bovine gamma globulin (BGG), 0.5% bovine serum albumin (BSA), 0.001% gelatin, and 0.05% Tween 20. Goat anti-mouse IgG-fluorescein isothiocyanate (FITC) (Caltag Laboratories, South San Francisco, CA) diluted 100-fold in PBS containing 0.5% BGG, 0.1% BSA, and 0.05% Tween 20 was used as detecting reagent. Anti-fibrillarin (nucleolar) monoclonal antibody 72B9 (23) was used as control. Intensity of fluorescence was graded on a scale of 0–4+, with 1+ considered positive.

Antichromatin antibodies were detected by enzyme-linked immunosorbent assay (ELISA) as previously described (24). Sera were diluted 100-fold prior to assay, and chromatin-bound antibodies detected with horseradish peroxidase (HRP)-conjugated goat antimouse IgG (Caltag Laboratories) diluted 2,000-fold. Antichromatin monoclonal antibody 1D12 was used as positive control (25).

#### Serum Immunoglobulin Quantitation

Serum IgG, IgG₁, and IgG₂a levels were quantified by ELISA, essentially as previously described (26). ELISA plates were coated with 200 μL of 2 μg/mL goat antimouse kappa light chain antibody (Caltag Laboratories) in PBS, and incubated overnight at 4°C. Plates were postcoated for 1 hr with 0.1% gelatin in PBS, followed by three washes with PBS–0.05% Tween 20. Sera were diluted in serum diluent (26), and incubated at room temperature while shaking for 2.5 hr, followed by three washes with PBS–0.05% Tween 20. 1/40 HRP-conjugated goat antimouse IgG, IgG₁, or IgG₂a antibodies (Caltag Laboratories) diluted in anti-immunoglobulin diluent (26) were added before incubation with shaking for 90 min. After three washes with PBS–0.05% Tween 20 and four washes with PBS, 2,2'-azinobis(3-ethylbenzthiazolesulfonic acid) (ABTS) substrate solution was added and optical density read at 405 nm (OD₄₀₅).

Standard curves were generated by serial dilutions of polyclonal mouse reference serum containing predetermined levels of immunoglobulin isotypes and subclasses (The Binding Site, Birmingham, UK). Serum IgG, IgG₁, and IgG₂a concentrations were quantified by converting the average OD₄₀₅ of duplicate wells to immunoglobulin concentrations by use of standard curves. Only serum dilutions that gave OD₄₀₅ values falling within the linear portion of the standard curve were used to calculate immunoglobulin levels.

#### Tissue Immune Complex Deposits

Sections 2- to 3-mm thick of the kidney and spleen were snap frozen in isopentane-CO₂ and prepared for direct immunofluorescence as previously described (27). Cryostat sections 4- to 5-μm thick were fixed in ethanol and incubated with serial dilutions of FITC-conjugated goat antibodies to IgG (γ-chain specific) or C₃ (Southern Biotechnology Associates, Birmingham, AL). The endpoint titer of the immune complex deposits was defined as the highest dilution of antibody at which specific fluorescence could be detected and was expressed as the reciprocal titer. A score of 0 was recorded when no specific fluorescence was detected at a dilution of 1/40. The presence of granular deposits in small and medium-size arteries was also examined. All histology slides were examined without knowledge of treatment given or other results.

#### Light Microscopy

A 2- to 3-mm thick section of kidney was immersed in Histochoice fixative (Amresco, Solon, IL), embedded in paraplast, and cut into 1- to 2-μm sections that were stained with periodic acid Schiffs reagent and with periodic acid silver methenamine. Slides were then examined by light microscopy without knowledge of treatment given or other results. The types of glomerular alteration were determined and the degree of endocapillary cell hyperplasia was scored for each animal as follows: 0 = normal, 0.5 = just detectable alteration, 1 = slight, 2 = moderate, 3 = strong, and 4 = maximal.

#### Statistical Analysis

Groups were compared by single-factor ANOVA, Mann-Whitney U test, or Fisher’s exact test, as appropriate. Comparisons are of HgCl₂-treated mice with PBS- and NiCl₂-treated animals unless described otherwise. p ≤ 0.05 was considered significant.

#### Results

Effect of HgCl₂ and NiCl₂ on Features of Autoimmunity in NZBWF₁ Mice

Mercury-exposed NZBWF₁ mice had elevated serum IgG, IgG₁, and IgG₂a compared to PBS- and NiCl₂-exposed animals (Table 1, Figure 1). Antibody to nuclear antigens (ANA) (Table 1) of a predominantly dense
fine to homogenous nuclear speckling of interphase cells and metaphase chromosomes was found in all NZBWF1 mice exposed to HgCl2. All pretreatment sera as well as PBS- and NiCl2-treated mice showed less frequent ANA responses. Levels of antichromatin antibodies (Table 1, Figure 1) in HgCl2-exposed NZBWF1 mice were markedly elevated above those found in either NiCl2- or PBS-exposed animals.

Exposure of NZBWF1 mice to mercury increased organ wet weight of spleen and cervical lymph nodes but not mesenteric lymph nodes when compared to PBS- and NiCl2-treated animals (Table 2). All HgCl2-treated mice developed very high titers of IgG and C3 deposits in glomeruli (Table 2). Granular IgG deposits in the glomeruli were localized to the capillary loops in 3/8 mice, restricted to the mesangium in one mouse, and present along the capillary loops as well as in the mesangium in the remaining four mice. Granular deposits of C3 were found in the mesangium in 6/8 animals, whereas 2/8 mice showed C3 deposits along the capillary loops as well as in the mesangium. Renal and splenic vessel walls showed granular deposits with high titers of IgG and C3. In contrast, mice treated with NiCl2 or PBS had much lower titers of IgG in the glomerular mesangium in 2/8 and 3/8 mice, respectively, without any deposits along the capillary loops. The titers of C3 in the glomeruli were also significantly lower in these mice. None of the NiCl2- or PBS-treated animals showed deposits of IgG or C3 in the renal or splenic vessel walls. Histologically there was only slight endocapillary cell hyperplasia in the glomeruli, which did not differ among the three treatment groups, probably because of the young age of mice at sacrifice. There were no alterations in the glomerular basement membrane among the three treatment groups.

**Effect of HgCl2 and NiCl2 on Features of Autoimmunity in MRL/lpr Mice**

Metal exposure did not lead to elevations of immunoglobulin in MRL/lpr mice, as all three treatment groups developed hypergammaglobulinemia during the experimental period (Table 3, Figure 2). Metal- and saline-exposed MRL/lpr mice showed no significant differences in ANA and antichromatin antibody at the end of the treatment period; each of the groups developed antichromatin antibodies during the exposure period (Table 3, Figure 3).

Mercury-exposed MRL/lpr mice had larger mesenteric lymph nodes than PBS- or NiCl2-treated animals but showed no difference in cervical node or spleen weights (Table 4). Mercury treatment increased the incidence of glomerular, mesangial IgG deposits in all MRL/lpr mice compared with NiCl2 and only 2/8 PBS-treated animals (Table 4). Mercury significantly increased the titers of mesangial IgG and C3 deposits (Table 4). No vessel wall deposits were seen in any of the animals. There was a mild endocapillary cell hyperplasia in the glomeruli, which tended to be more pronounced in the HgCl2-treated group, but this did not reach statistical significance.

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*Table 1. Immunoglobulin levels and autoantibodies in NZBWF1 mice following metal exposure. a,b*

| Strain (sex) | No. | Treatment | IgG (mg/mL) | IgG1 (mg/mL) | IgG2a (mg/mL) | ANA (pos/no.) | Antichromatin (OD405) |
|--------------|-----|-----------|-------------|--------------|--------------|---------------|----------------------|
| NZBWF1 (female) | 8 | Pre-PBS | 1.44 ± 0.97 | 0.20 ± 0.08 | 0.47 ± 0.22 | 0/8 | 0.02 ± 0.01 |
| | 8 | Post-PBS | 2.64 ± 0.94 | 0.30 ± 0.08 | 1.50 ± 0.50 | 0/8 | 0.06 ± 0.05 |
| | 8 | Pre-NiCl2 | 1.69 ± 0.65 | 0.28 ± 0.06 | 0.74 ± 0.34 | 0/8 | 0.04 ± 0.02 |
| | 8 | Post-NiCl2 | 3.17 ± 1.38 | 0.30 ± 0.06 | 2.06 ± 0.86 | 1/8 | 0.04 ± 0.03 |
| | 8 | Pre-HgCl2 | 1.56 ± 0.05 | 0.41 ± 0.11 | 0.96 ± 0.25 | 0/8 | 0.06 ± 0.04 |
| | 8 | Post-HgCl2 | 10.58 ± 2.35 | 3.06 ± 1.54 | 8.10 ± 1.70 | 0/8 | 1.27 ± 0.65 |

**Abbreviations:** OD405, optical density read at 405 nm; PBS, phosphate-buffered saline; pos/no., positive/number of animals. *p-values are from comparison of mercury-treated group with PBS- and nickel-treated groups. *p < 0.0002; **p < 0.0001. Data are expressed as mean ± standard deviation.

*Figure 1. Hypergammaglobulinemia and antichromatin antibodies in NZBWF1 mice after exposure to mercury. OD405, optical density read at 405 nm; PBS, phosphate-buffered saline. Female NZBWF1 mice were injected with HgCl2, NiCl2, or PBS for 4 weeks as described in "Materials and Methods." Serum IgG, IgG1, and IgG2a (left panels) were determined by enzyme-linked immunosorbent assay (ELISA) before (c) and after (e) metal exposure. IgG antichromatin antibodies (right panel) were determined by ELISA before (c) and after (e) metal exposure. See Table 1 for statistical analysis.*

*Table 2. Pathologic changes in NZBWF1 mice following metal exposure. a,b,c*

| Strain (sex) | No. | Treatment | Organ wet weight (mg) | Kidney immunopathology | Spleen immunopathology |
|--------------|-----|-----------|-----------------------|------------------------|-----------------------|
|              |     |           | Spleen | Cervical LN | Mesenteric LN | Glomerular IgG | Glomerular C3 | Vessel IgG | Vessel C3 | Vessel IgG | Vessel C3 |
| NZBWF1 (female) | 8 | PBS | 87 ± 3 | 32 ± 2 | 107 ± 5 | 25 ± 37 | 480 ± 171 | 0 | 0 | 0 | 0 |
| | 8 | NiCl2 | 89 ± 3 | 29 ± 3 | 107 ± 8 | 20 ± 37 | 330 ± 151 | 0 | 0 | 0 | 0 |
| | 8 | HgCl2 | 129 ± 5 | 70 ± 5 | 97 ± 6 | 1760 ± 662 | 1440 ± 452 | 1600 ± 393 | 1160 ± 339 | 1280 ± 593 | 1400 ± 746 |

PBS, phosphate-buffered saline. *p-values are from comparison of mercury-treated group with PBS- and nickel-treated groups. *p < 0.001; **p < 0.0001. Data are expressed as mean ± standard deviation. *IgG and C3 titers are expressed as reciprocal titers.
Table 3. Immunoglobulin levels and autoantibodies in MRL mice following metal exposure.a,b

| Strain (sex) | No. | Treatment | IgG (mg/mL) | IgG1 (mg/mL) | IgG2a (mg/mL) | ANA (pos/no.) | Anti-chromatin (OD₄₅₀) |
|-------------|-----|-----------|-------------|--------------|--------------|--------------|----------------------|
| MRL/+/+ (female) | 8 | Pre-PBS | 2.71 ± 2.10 | 1.03 ± 0.67 | 3.45 ± 2.74 | 0/8 | 0.02 ± 0.05 |
| | 8 | Post-PBS | 9.58 ± 4.44 | 3.66 ± 1.25 | 6.06 ± 2.85 | 4/8 | 0.35 ± 0.43 |
| | 8 | Pre-NiCl₂ | 2.11 ± 0.71 | 0.62 ± 0.45 | 1.88 ± 0.83 | 1/8 | 0.01 ± 0.01 |
| | 8 | Post-NiCl₂ | 10.41 ± 3.17 | 3.45 ± 1.02 | 4.48 ± 1.56 | 8/8 | 0.70 ± 0.46 |
| | 8 | Pre-HgCl₂ | 1.30 ± 0.33 | 0.79 ± 0.16 | 1.25 ± 0.54 | 1/8 | 0.03 ± 0.01 |
| | 8 | Post-HgCl₂ | 8.65 ± 3.86 | 4.07 ± 2.53 | 4.62 ± 1.46 | 7/8 | 0.56 ± 0.43 |
| MRL/+/+ (female) | 8 | Pre-PBS | 2.93 ± 4.32 | 1.17 ± 2.15 | 1.45 ± 2.47 | 5/8 | 0.10 ± 0.15 |
| | 8 | Post-PBS | 4.06 ± 2.71 | 1.45 ± 1.40 | 2.01 ± 2.04 | 7/8 | 0.17 ± 0.16 |
| | 8 | Pre-NiCl₂ | 1.71 ± 1.14 | 0.46 ± 0.27 | 0.64 ± 0.31 | 6/8 | 0.57 ± 1.20 |
| | 8 | Post-NiCl₂ | 3.92 ± 2.43 | 1.29 ± 0.86 | 1.81 ± 1.05 | 7/8 | 0.48 ± 0.48 |
| | 8 | Pre-HgCl₂ | 1.44 ± 0.69 | 0.41 ± 0.14 | 0.72 ± 0.44 | 4/8 | 0.38 ± 0.64 |
| | 8 | Post-HgCl₂ | 7.12 ± 1.75 | 3.47 ± 0.87 | 3.68 ± 1.84 | 6/8 | 1.88 ± 1.71* |

Abbreviations: OD₄₅₀, optical density read at 405 nm; PBS, phosphate-buffered saline; pos/no., positive/number of animals. *p-values are from comparison of mercury-treated group with PBS- and nickel-treated groups. *p < 0.05; †p < 0.02; *p < 0.005. Data are expressed as mean ± standard deviation.

**Figure 2.** Hypergammaglobulinemia in MRL and AKR mice after exposure to mercury. PBS, phosphate-buffered saline. Female MRL/+, MRL/+ and AKR mice were injected with HgCl₂, NiCl₂, or PBS for 4 weeks as described in "Materials and Methods." Serum IgG, IgG1, and IgG2a were determined by enzyme-linked immunosorbent assay (ELISA) before (○) and after (●) metal exposure. See Tables 3 and 5 for statistical analysis.

**Effect of HgCl₂ and NiCl₂ on Features of Autoimmunity in MRL/+ Mice**

Mercury-exposed MRL/+ mice had elevated serum IgG and IgG1 compared to PBS- and NiCl₂-exposed animals, whereas IgG2a levels were elevated compared to NiCl₂-treated mice (Table 3, Figure 2). A variety of ANA patterns were found in MRL/+ mice before and after the various treatments (data not shown). Mercury treatment of MRL/+ mice resulted in elevated levels of antichromatin antibodies, which were significantly different from PBS- and NiCl₂-treated animals (Table 3, Figure 3).

Mercury-exposed MRL/+ mice had larger cervical nodes than their control counterparts, but spleen weights were not increased and mesenteric nodes were smaller (Table 4). All MRL/+ mice treated with HgCl₂ showed glomerular and mesangial IgG deposits, whereas only 5/8 NiCl₂-treated mice and 3/8 PBS-treated mice showed such deposits (Table 4). Renal vessel wall deposits of IgG were seen in 7/8 and C3 in 2/8 of the HgCl₂-treated MRL/+ mice. Only one
animal had deposits of Ig and C3 in splenic vessel walls. Vessel wall deposits were not found in either the kidney or spleen of PBS- and NiCl₂-treated animals. Compared with PBS-treated animals, there was significantly increased focal/segmental hyperplasia of endocapillary cells in the mercury group (0.59 ± 0.23 vs 1.25 ± 0.13; p < 0.001).

Effect of HgCl₂ and NiCl₂ on Features of Autoimmunity in AKR Mice

AKR mice also developed hypergammaglobulinemia with increases in IgG and IgG2a following HgCl₂ exposure (Table 5). Mercury-treated AKR mice developed ANA but only low levels of antichromatin antibodies (Table 5). Compared to PBS controls, mercury-exposed AKR mice had significant increases of IgG (51 ± 55 vs 274 ± 78; p < 0.0001) and C3 (34 ± 28 vs 120 ± 52; p < 0.005) deposits in the mesangium of the kidney glomeruli. There were no deposits in the kidney vessels or spleen of AKR mice and no histologic abnormalities by light microscopy.

Discussion

The exacerbation by mercury of systemic autoimmunity in lupus-prone mice identifies a potential model for the study of xenobiotic effects on autoimmune-sensitive populations. The autoimmune features and the extent to which they were accelerated differed between the strains tested, suggesting that genetic background influenced the response to xenobiotic exposure. Much of the disease acceleration appeared to be due to non-MHC lupus-predisposing genes, as an MHC-matched nonautoimmune strain had less severe disease manifestations. Strain-dependent responses, as well as the importance of non-MHC genes in disease severity, suggest that xenobiotic exposure led to acceleration of idiopathic disease beyond the induction of HgIA. Acceleration of autoimmunity was dependent on the specific immunostimulant; another metal that can activate the immune system, nickel, had little effect.

The most pronounced response to mercury exposure was observed in female NZBWF₁ mice, considered to be the prototypic murine model for human lupus (11). This strain exhibited lymphadenopathy in the spleen and the cervical lymph nodes, which drain the mercury injection site. NZBWF₁ mice also had pronounced hypergammaglobulinemia. Most striking was the marked elevation in antichromatin autoantibodies—an autoantibody response characteristic of murine lupus (28) and idiopathic (29) and drug-related human lupus (30) but found less frequently in murine HgIA (31). Extensive tissue deposits of immunoglobulin and complement, particularly in the vessels of the kidney, are consistent with the progressive glomerulonephritis of idiopathic disease in NZBWF₁ mice (32). The higher titer of glomerular deposits in the NZBWF₁ compared to the MRL substrains is also consistent with the idiopathic picture in these strains (32). These studies with mercury, and those with other exogenous agents (4,7), suggest that the NZBWF₁ strain may be particularly suited to studies on xenobiotic-induced exacerbation of systemic autoimmunity.
The MRL/lpr has been used extensively in studies on autoimmunity, primarily to understand the significance of the lpr gene but also because its accelerated disease and early mortality facilitate \textit{in vivo} studies. However, the long life of the MRL/lpr+ offers the advantage of examining the effects of long-term xenobiotic exposures on the expression of autoimmunity. Previous studies have suggested that autoimmunity in MRL mice is either accelerated (33,34) or unaffected (33) by xenobiotic exposure. Both MRL substrains were examined in this study to determine if the accelerated disease of the MRL/lpr, and the delayed disease of the MRL/lpr+, would be similarly affected by exposure to metals. The presence of the lpr gene, which is associated with elevations of IgG and ANA as early as 2 months of age (32,35), appeared to be the predominant influence on immunoglobulin and autoantibody levels, as neither metal led to increases above those of the PBS controls. In contrast, mercury-exposed MRL/lpr+ mice showed evidence of both accelerated hypergammaglobulinemia and elevated antichromatin antibodies compared to nickel- or PBS-treated groups. Interestingly, both MRL substrains had increased glomerular deposits of IgG compared with those in control mice, and MRL/lpr+ mice showed more severe immunopathology with IgG and C3 deposits in kidney vessels as well as hyperplasia of endocapillary cells. The increased glomerular disease in lpr mice exposed to HgCl2 despite similar levels of immunoglobulins and autoantibodies as PBS and NiCl2 controls suggests that HgCl2 may also promote disease by mechanisms beyond autoantibody production, possibly related to autoantibody specificity or effector pathways involved in end-organ damage.

The MHC is a major contributor to both idiopathic lupus (3) and HgIA (31,36). It could therefore be argued that exacerbation of autoimmunity in lupus-prone mice is primarily due to an effect on H-2 genes and not acceleration of idiopathic disease per se. To examine this possibility, AKR mice, from which the MRL derives its H-2 genes, were exposed to mercury. Although the AKR possesses the H-2b haplotype, which confers susceptibility to HgIA (31), the degree of autoimmunity elicited differed from that of MRL mice. AKR mice had predominant elevations of IgG2a, whereas MRL/lpr+ mice showed increased IgG1. HgCl2-exposed MRL/lpr+ mice had more severe disease, with higher titers of antichromatin antibodies, increased deposits of IgG and C3 in kidney vessels, and histologic glomerular changes. This comparison between autoimmune-prone strains and an H-2 identical nonautoimmune strain demonstrates the influences of both non-MHC and non-lpr genes on mercury-accelerated systemic autoimmunity in mice predisposed to spontaneous disease. Furthermore, the data demonstrate how an environmental agent, mercury, can act synergistically on individuals with intermediate susceptibility (NZBWF1, and MRL/lpr+) to accelerate disease, whereas there was less effect on MRL/lpr mice with the highest susceptibility. This is consistent with the threshold liability model (9) and the potential importance of environmental factors in influencing disease induction, particularly in individuals with intermediate susceptibility.

Mercury, in various forms, elicits systemic autoimmunity in a number of nonautoimmune mouse strains (10). Susceptibility to HgIA is genetically controlled, as this xenobiotic is able to elicit differing degrees of autoimmunity depending upon the genetic background of the host (10,27). The immunologic features of HgIA, which include lymphadenopathy, hypergammaglobulinemia, autoantibodies, and immune complex disease, resemble features of SLE. Even so, there are significant differences between murine models of HgIA and idiopathic SLE. HgIA is transient, with disease features resolving with time once exposure to mercury ceases (27). In idiopathic SLE, disease is progressive, often leading to death (8,9). At the height of disease HgIA is associated with increased IL-4 and IgG1 autoantibodies (13). In contrast, murine models of idiopathic SLE have autoantibodies of IgG2a and IgG2b isotypes (28) and elevations in IL-1 and IFN-γ (9).

Several mechanisms have been postulated to account for mercury’s autoimmune-promoting effects, including generation of cryptic T-cell determinants (37) or production of T cells reacting against self-class II (38). The finding that mercury did not accelerate humoral responses in the presence of the lpr gene raises the possibility that mercury, like the lpr defect, may act through inhibition of apoptosis. This would be consistent with the polyclonal lymphoid hyperplasia and immunoglobulin increases found in the MRL/lpr+ after mercury treatment. Mercury could possibly prevent apoptosis by interacting with the sulfhydryl moiety of cysteine in the active site of one or more caspases (39) that are involved in the signaling and effector pathways responsible for induction of apoptotic cell death.

Comparison between the MRL and AKR strains revealed the importance of studies with genetically related nonautoimmune strains in dissecting the contribution of xenobiotic exposure to acceleration of idiopathic disease. Additional studies contrasting the genetically related autoimmune-prone BXSB (H-2q) and nonautoimmune C57BL/6 (H-2q) have also shown that mercury exposure exacerbates idiopathic autoimmunity (40). The significance of such studies cannot be overstated. Human studies have shown that xenobiotic-induced disease, although exhibiting features of the idiopathic syndrome, may lack key pathologic sequelae and have clinical features not associated with idiopathic disease (2,41). Studies that seek to link xenobiotics with autoimmunity must therefore attempt to discriminate between xenobiotic-induced autoimmunity and xenobiotic-accelerated idiopathic disease. Comparative studies using genetically related autoimmune-prone and nonautoimmune mice might help determine the contribution that xenobiotic exposure makes in rendering sensitive populations susceptible to autoimmunity.

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