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Resistance to Xenobiotic-Induced Autoimmunity Maps to Chromosome 1

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Although evidence indicates that environmental factors play a major role in precipitating systemic autoimmunity in genetically susceptible individuals, little is known about the mechanisms involved. Certain heavy metals, such as mercury, are potent environmental immunostimulants that produce a number of immunopathologic sequelae, including lymphoproliferation, hypergammaglobulinemia, and overt systemic autoimmunity. Predisposition to such metal-induced immunopathology has been shown to be influenced by both MHC and non-MHC genes, as well as susceptibility to spontaneous lupus, in mice and other experimental animals. Among the various mouse strains examined to date, the DBA/2 appears to uniquely lack susceptibility to mercury-induced autoimmunity (HgIA), despite expressing a susceptible H-2 haplotype (H-2d). To define the genetic basis for this trait, two genome-wide scans were conducted using F2 intercrosses of the DBA/2 strain with either the SJL or NZB strains, both of which are highly susceptible to HgIA. A single major quantitative trait locus on chromosome 1, designated Hmr1, was shown to be common to both crosses and encompassed a region containing several lupus susceptibility loci. Hmr1 was linked to glomerular immune complex deposits and not autoantibody production, suggesting that DBA/2 resistance to HgIA may primarily involve the later stages of disease pathogenesis. Identification and characterization of susceptibility/resistance genes and mechanisms relevant to the immunopathogenesis of mercury-induced autoimmunity should provide important insights into the pathogenesis of autoimmunity and may reveal novel targets for intervention. The Journal of Immunology, 2001, 167: 2396–2403.

Mercury is a widely distributed environmental and industrial pollutant (1). Exposure to large doses results in acute renal tubular lesions and immunosuppression, whereas chronic administration of smaller doses can lead to the development of systemic autoimmunity (2–4). The characteristic features of mercury-induced autoimmunity (HgIA) are very similar to many of the manifestations of systemic lupus erythematosus, and include lymphocyte proliferation (5), increased levels of class II MHC expression (6, 7), hypergammaglobulinemia (8, 9), polyclonal Abs to self Ags, including anti-nuclear Abs (8, 10), immune complex glomerulonephritis (GN) (10), and necrotizing vasculitis (11). HgIA is also similar to lupus in that the disease process requires CD4+ T cells (12, 13), certain B and T cell costimulatory molecules (14), and IFN-γ (15), which strongly supports the possibility of related or identical pathogenic mechanisms.

The development of HgIA depends not only on the amount and duration of heavy metal exposure, but also on the genetic background of the exposed animal. In mice, autoantibody specificities and susceptibility to immune complex disease have been shown to depend on H-2 haplotype, with H-2b and H-2d haplotypes associated with the greatest susceptibility, H-2k intermediate susceptibility, and H-2d the lowest susceptibility (16, 17). However, because many of these comparisons are among different strains of mice, this classification may have been influenced by background genes. The anti-nucleolar Ab (ANoA) response, which is largely directed against fibrillarin (18), is one of the best-characterized manifestations of HgIA. It has been linked to H-2d (19) and, more specifically, to the class II molecule I-A∗, by analysis of H-2 congenic mice (16, 17). The ANoA response is absent in F1 hybrids (H-2b/k), and recent adoptive bone marrow transfer studies suggest that this is due to an intrinsic resistance of H-2b/k heterozygous B cells to produce ANoA rather than differences in thymic education or a lack of fibrillarin-specific T cell help (20).

There is also considerable evidence implicating non-MHC genes in susceptibility to HgIA. Among H-2d haplotype strains, for example, BALB/c mice are highly susceptible to both lymphoproliferation and immune complex GN, B10.D2 mice are susceptible to lymphoproliferation, but develop less severe immune complex GN than BALB/c mice, while DBA/2 mice are reportedly resistant to both lymphoproliferation and GN (16, 17, 21). Other studies have also recently demonstrated that lupus-prone strains are particularly sensitive to the induction of systemic autoimmunity following exposure to mercury (22, 23). Among the various strains, the DBA/2 background appears to be the most resistant to HgIA, because it is the only background expressing a susceptible H-2 that does not develop autoimmunity and, of 22 strains, it alone did not develop hypertrophy of poptic lymph nodes following HgCl2 injection (21). Although the basis for this resistance is not known, DBA/2 mice have reduced in vitro mercury-induced activation of CD4+ T cells, but not CD8+ T cells compared with BALB/c mice (12), suggesting that the initiation of the autoimmune response may be compromised. However, this lack of susceptibility to HgIA does...
not appear to be due to a generalized resistance to autoimmunity, because the DBA/2 strain is highly susceptible to syngeneic graft-vs-host disease induced by cyclosporin A (24) and adoptive transfer of DBA/2 spleen cells into (C57BL/6 × DBA/2)F1, mice is a commonly studied model of chronic graft-vs-host disease that includes the development of immune-mediated GN (25, 26). Definition of the basis for HgIA resistance will undoubtedly provide insights into the pathogenesis of HgIA, which will also most likely be relevant to spontaneous lupus. Furthermore, because the DBA/2 strain is not known to be immune deficient, this information may also suggest disease interventions that will have minimal effects on the immune response to pathogens.

Given the complexity of heavy metal interaction with cellular and subcellular components of the immune system and the large number of molecules that may be directly or indirectly affected, we initiated genetic studies to define the DBA/2 genes responsible for resistance to HgIA through a genetics approach. This strategy has the advantage of not requiring previous knowledge of the genes or mechanisms, and direct examination of the relationship of genetic alterations predisposing to HgIA and spontaneous lupus by comparing the locations of quantitative trait loci (QTL) and eventually the specific genetic alterations. Genome-wide searches were performed using two F2 intercrosses involving the resistant DBA/2 to either the SJL or NZB strains, both HgIA susceptible. These studies identified a single locus on chromosome 1 present in both sets of crosses. Hmr1 is particularly interesting because it confers resistance to HgIA in nonautoimmune (SJL) and autoimmune (NZB) backgrounds and is derived from a background strain (DBA/2) that is resistant to HgIA, but otherwise not immune compromised.

Materials and Methods

Mice

DBA/2, SJL/J, NZB/B1ScCr, (SJL × DBA/2)F1 (SDF1), and SDF2 mice were obtained from The Scripps Research Institute animal facility and were maintained under specific pathogen-free conditions. Exposure to mercury consisted of twice per week injections from 6 wk of age with 40 μg HgCl2 in 100 μl PBS s.c. for 4 wk, as previously described (15). H-2 haplotypes were determined by the D17Mit16 or Tnf microsatellite markers (Research Genetics, Huntsville, AL); both are located 0.5 cm from the I-A β gene.

Serology

Antinuclear Abs. Indirect immunofluorescence was performed as described previously (18). Briefly, HEP-2 cells on slides (Bion Enterprises, Park Ridge, IL) were incubated with 2-fold serial dilutions of serum starting from 1/100, followed by a 1/100 dilution of FITC-conjugated goat anti-mouse IgG + IgM Abs (Caltag Laboratories, Burlingame, CA). Anti-nuclear Ab patterns and titers were assessed under blinded conditions. An anti-chromatin Ab that could detect a specific fluorescence (end-point titer). Positive titers

<1:40 were considered background. Vessel wall IgG deposits were graded on a 0–4+ scale (15). Slides were examined under blind conditions.

Mapping. Construction of linkage maps spanning all autosomal chromosomes and PCR typing of mice were performed as previously described (29). A complete list of polymorphic markers used in this study can be obtained from the corresponding author. QTL were identified using QTL Cartographer version 1.14 (http://statgen.ncsu.edu/qtlcart) with maps constructed by Mapmaker3 (http://waldno.umd.edu/tpf/distribution/software/mapmaker3) (30). Likelihood ratios (LR) were calculated using the LRmapqtl program. Composite interval mapping was performed using model 6 of the Zmapqtl program with options set at 2-cM intervals, 10-cM window size, and five background parameters. The experimental significance level for each trait was determined by analyzing 1000 random shuffling permutations of the actual phenotype data.

Statistics

Data were analyzed by unpaired t test, Fisher’s exact test, or χ² (StatView; Abacus Concepts, Berkeley, CA), as indicated in the legends. Values of p <0.05 were considered significant.

Results

Susceptibility of SJL, DBA/2, SDF1, and SDF2 mice to HgIA

To identify loci that influence susceptibility to HgIA, the SJL (susceptible) and DBA/2 (resistant) strains were selected for analysis. Of particular interest was the identification of DBA/2 genes that confer resistance to mercury-induced autoimmune disease. Fig. 1 and Table I summarize the major autoimmune manifestations elicited in the parental strains, F1 hybrid, and F2 intercrosses following a 4-wk exposure to HgCl2. Consistent with previous observations (31, 32), both autoantibody responses and end organ deposits of immune complexes were markedly reduced or not detectable in the DBA/2 mice, whereas SJL mice developed typical features of HgIA (p < 0.05, Table I).

The hybrid SDF1 responses were also assessed (Table I and Fig. 1). From the autoantibody profiles, it appeared that both dominant and recessive modes of inheritance were applicable, depending on specificity. The lack of ANoA response in SDF1 mice is consistent with previous studies suggesting that anti-chromatin Ab response of equal magnitude to the SJL mice suggests recessive transmission. Alternatively, the anti-chromatin Ab and ANoA responses may indicate recessive and additive dominant transmission of susceptibility genes from the SJL background. Based on these findings, F2 intercrosses were used to map QTLs, because this approach permits analysis of all combinations of alleles from both parental strains. Fig. 1 and Table I show the incidence and severity of disease manifestations among the SDF2 mice. Average SDF2 responses were generally between the two parental strains. The incidence of kidney vessel IgG (15 mice) and C3 (4 mice) deposits in the SDF2 mice was insufficient for performing mapping studies.

Loci linked to HgIA traits in SDF2 mice

Genome-wide mapping for autoantibody production and glomerular deposits was performed on 211 SDF2 mice using 96 markers spanning roughly 90% of the autosomal genome (~1300 cm). The incidence of vessel deposits was too low in the SDF2 mice for analysis. Three QTL linked to mercury-induced traits were identified on chromosomes 1, 7, and 17. The locus on chromosome 17, defined by the Tnf marker within the H-2 complex, was linked very tightly to ANoA with a LR of 96, a value substantially higher than the genome-wide calculated significance level of 12.4, for χ² = 0.05 (experimentwise significance level was determined by analyzing for each trait, 1000 permutations of the actual data after random shuffling). In fact, none of the H-2, and only a small fraction (5.6%) of H-2 α heterozygous SDF2 mice had ANoAs (Table II), consistent with previous studies suggesting that anti-brillarin Ab production requires the I-As haplotype (17, 20), or
possibly, that the H-2d haplotype suppresses the response. In addition, the tight linkage of ANoA to the H-2 s haplotype despite the diverse F2 backgrounds indicates that production of ANoA is unlikely to be greatly influenced by non-MHC DBA/2 genes. Furthermore, the H-2 (Tnf) region was not linked to glomerular deposits (p > 0.05), nor were there significant differences in glomerular deposits of IgG and C3 in mice with or without ANoAs or anti-chromatin Abs (p > 0.05, data not shown). The H-2 region was also linked to IgG anti-chromatin Ab production (LR 12.8, experimentwise significance level p<0.05 at LR of 12.4).

In contrast to the chromosome 17 locus, the other two QTL identified were linked solely to later stages of disease pathogenesis. The chromosome 1 locus was linked to glomerular deposits of both IgG (D1 Mit15, LR 10.4, genome-wide permutation significance level α = 0.05 at LR of 13.1, nominal p value = 0.001) and C3 (D1Nds1, LR 16.2, α = 0.05 at LR of 12.5), whereas the acromeric chromosome 7 QTL was weakly linked only to IgG glomerular deposits (D7 Mit57, LR 9.4, α = 0.05 at LR of 13.1, nominal p value = 0.002). In all instances, resistance to disease mapped to the DBA/2 genome. To better define the location of these QTL, composite interval mapping was then performed, as shown in Fig. 2. This method combines interval mapping and multiple regression to adjust for the presence of other QTL. The chromosome 1 locus, designated Hmr1 (heavy metal resistance 1), was mapped to the middle-distal portion of the chromosome and the chromosome 7 locus to the proximal region. However, the LR of Hmr1 using composite interval mapping did not quite reach genome-wide significance (16.1 and 16.7, respectively); therefore, a second set of mice was analyzed in an attempt to verify this locus.

**Mercury susceptibility of NZB mice and crosses**

For the second mapping study, DBA/2 mice were crossed with NZB mice partly based on our recent finding that lupus-susceptible strains are more sensitive to HgIA (22, 23), and the possibility that if Hmr1 or other DBA/2 gene (or genes) is capable of suppressing disease in these strains, they are more likely to be significant and possibly relevant to the pathogenesis of spontaneous disease. NZB mice were specifically selected because they are the only lupus-prone strain expressing the same H-2d haplotype as the DBA/2. Furthermore, NZB susceptibility loci promoting spontaneous disease have been mapped by us (29) and others (33–36).

In preliminary experiments, female NZB, DBA/2, and (NZB × DBA/2)F1 (BDF1) mice were exposed to the standard regimen of HgCl2 at an age before the development of spontaneous disease (6 wk), and then examined for immunopathology (Table III and Fig. 3). NZB mice developed significantly greater amounts of IgG and C3 deposits in glomeruli and vessels (p < 0.05) than both the DBA/2 mice and control NZB mice given PBS alone (NZB-PBS). Thus, similar to other autoimmune strains, NZB mice appear highly susceptible to HgIA. In contrast, BDF1 hybrids exhibited low susceptibility to mercury with little or no vessel deposits and
intermediate amounts of glomerular IgG material (Table III and Fig. 3), suggesting dominant transmission of DBA/2 resistance.

Loci modifying susceptibility/resistance to HgIA in BDF$_2$ intercross mice

For mapping of mercury-related QTLs, 282 BDF$_2$ mice were examined for susceptibility to HgIA. The distributions of individual mice for glomerular and vessel deposits, and levels of autoantibodies are shown in Fig. 3, and the results are summarized in Table III. Genome-wide analysis was performed with 98 markers encompassing all autosomal chromosomes and ~90% coverage, including all regions previously linked to spontaneous lupus in the NZB strain (29, 37). Glomerular IgG deposits mapped very strongly to chromosome 1 (D1 Mit111, LR 80, NZB 1093 ± 95, BDF$_2$ 695 ± 60). No other QTLs were identified that were at the α = 0.05 level for genome-wide significance. Composite interval mapping demonstrated that the NZB chromosome 1 locus (~80–101.2 cM, Mouse Genome Informatics; The Jackson Laboratory, Bar Harbor, ME) overlapped with Hmr1 (~66.8–93.2 cM) (Fig. 4). Composite interval mapping also revealed an additional locus on chromosome 4 (LR 18.2, Fig. 4) linked to HgIA resistance in the DBA/2 background (DBA/2 483 ± 124, NZB 862 ± 111, BDF$_2$ 623 ± 68). The proximal chromosome 7 region was also weakly linked to glomerular IgG deposits (D7 Mit178, LR 8.1, nominal $p = 0.005$, Fig. 4). Mapping of the other traits did not identify any other QTL that passed all autosomal chromosomes and experimentwise significance levels for experimentwise significance levels for

Discussion

In this study, genome-wide mapping of two independent crosses was used to define the genetic resistance of DBA/2 mice to HgIA. In the SDF$_2$ cross, glomerular deposits of IgG and/or complement mapped to chromosome 1 (designated Hmr1), and more weakly to proximal chromosome 7. In the BDF$_2$ cross, glomerular deposits of IgG similarly mapped to chromosome 1 and weakly to chromosome 7, but also to chromosome 4. Thus, resistance of the DBA/2 appears to be multigenic and largely due to the effects of Hmr1, and to a lesser extent, to a locus on chromosome 7.

Interestingly, Hmr1 overlaps a previously identified locus on the NZB background, Lbw7 or nba2, that has been linked to the spontaneous development of autoantibodies, splenomegaly, and/or GN in several different crosses of the NZB with nonautoimmune and autoimmune background strains (29, 34, 35, 38). However, mapping studies have suggested that the major contribution of Lbw7 or nba2 is enhancement of autoantibody production, while Hmr1 was not linked to this trait in either cross. Nevertheless, in the case of the BDF$_2$ cross, linkage to chromosome 1 may be partly due to the presence of the NZB Lbw7 allele if, in fact, this allele can promote susceptibility to HgIA. Further dissection of this region with interval-specific congenic sublines will address this issue.

The Hmr1 interval on chromosome 1 is particularly interesting, because it overlaps with other lupus-predisposing loci in addition to the NZB locus mentioned previously, as well as with several genes that, when altered, either enhance or reduce susceptibility to lupus-like disease. The other predisposing loci include Slel (from the NZM2420 and NZW backgrounds (39–41) and Bxs3 from the BXSB strain (42). Strikingly, all of the highly susceptible lupus-prone strains except for the MRL-Fas$^{+/+}$ have loci that overlap with the Hmr1 interval. Genes associated with predisposition to the development of lupus include Fast (fas ligand) (43), Sapr (serum amyloid P-component) (44), Fcgr2b (FcγRIIB) (45), C2 (CD21/CD35) (46, 47), and Pipre (CD45) (48), whereas deficiency of FcRγ (FcR γ-chain) (49) results in resistance to

Table II. Influence of H-2 haplotype on HgIA

<table>
<thead>
<tr>
<th>MHC</th>
<th>No. Mice</th>
<th>ANoA*</th>
<th>Glom. IgG</th>
<th>Glom. C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2$^a$</td>
<td>51</td>
<td>82.4%</td>
<td>29.4%</td>
<td>35.3%</td>
</tr>
<tr>
<td>H-2$^d$</td>
<td>52</td>
<td>0%</td>
<td>19.2%</td>
<td>44.2%</td>
</tr>
<tr>
<td>H-2$^{ad}$</td>
<td>108</td>
<td>5.6%</td>
<td>23.1%</td>
<td>38.0%</td>
</tr>
</tbody>
</table>

*Percentage of mice with ANoA scores ≥ 1+ are shown. Three H-2$^a$, three H-2$^d$, and no H-2$^{ad}$ haplotype mice had trace positive ANoA scores. p < 0.0001 for ANoA; p > 0.05 for glomerular IgG and C3 deposits ($\chi^2$ test).

FIGURE 2. Loci linked to glomerular deposits in SDF$_2$ mice. Composite interval maps of LR test (LRT) results are shown for glomerular IgG (solid line) and C3 (dashed line) deposits. Horizontal lines showing experimentwise significance levels for α = 0.1 and 0.05 were 15.3 (thin, dashed) and 17.7 (thin, solid) for IgG deposits, and 14.6 (thick, dashed) and 16.7 for C3 deposits. Markers for chromosome 1 (from acrocnome: D1Mit66, D1Mit212, D1Mit306, D1Nds1, D1Mit265, D1Mit15, D1Mit36, D1Mit17) and chromosome 7 (D7Mit57, D7Mit23, D7Mit227, D7Mit211, D7Nds1, D7Mit323, D7Mit314) are indicated by solid triangles. D1Mit15 and D1Mit36 are closely situated and appear as a single triangle.

Table I. Manifestations of HgIA in SJL, DBA/2, SDF1, and SDF2 mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>ANoA</th>
<th>ANoA score</th>
<th>Anti-Chromatin</th>
<th>Glom IgG</th>
<th>Glom IgM</th>
<th>Glom C3</th>
<th>Kid Ves IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBA/2</td>
<td>0%</td>
<td>0</td>
<td>0.005 ± 0.002</td>
<td>0</td>
<td>320 ± 56</td>
<td>80 ± 14</td>
<td>0</td>
</tr>
<tr>
<td>SJL</td>
<td>90%</td>
<td>1.7 ± 0.3</td>
<td>0.264 ± 0.148</td>
<td>180 ± 87</td>
<td>620 ± 166</td>
<td>190 ± 32</td>
<td>160 ± 84</td>
</tr>
<tr>
<td>SDF1</td>
<td>0%</td>
<td>0</td>
<td>0.98 ± 0.116</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SDF2</td>
<td>23%</td>
<td>0.5 ± 0.1</td>
<td>0.035 ± 0.010</td>
<td>69 ± 13</td>
<td>501 ± 23</td>
<td>226 ± 13</td>
<td>33 ± 12</td>
</tr>
</tbody>
</table>

*Phenotypes examined were ANoA positivity (ANoA titers ≥ 1+) and score, anti-chromatin Abs, and immunofluorescence examination of kidney (Kid) for glomerular (Glom) and vessel (Ves) deposits. Serum anti-nuclear Abs were detected on HEp-2 cells by indirect immunofluorescence, autoAb levels were determined by ELISA, and endpoint titers for deposits were obtained by scoring immunofluorescence-stained cryostat sections (15). Mean ± SE are shown except for ANoA (percent positive). Deposit scores are inverse titers.

Values of $p$ for comparisons between DBA/2 and SJL mice are shown. The unpaired $t$ test was used for all phenotypes except for ANoA (Fisher’s exact test). Number of mice: 8 DBA/2, 8–20 SJL, 26 SDF1, 210–211 SDF2. Among SDF2 mice, there was only low incidence of IgG (15 mice) and C3 (4 mice) deposits in kidney vessels.

Percentage of mice with ANoA scores ≥ 1+ are shown. Three H-2$^a$, three H-2$^d$, and no H-2$^{ad}$ haplotype mice had trace positive ANoA scores. p < 0.0001 for ANoA; p > 0.05 for glomerular IgG and C3 deposits ($\chi^2$ test).
autoimmunity. Although the relationship of Hmr1 to these loci and genes remains to be determined, this study appears to add yet another gene to a region that is particularly rich in lupus-affecting genes. The concentration of such genes in this interval may account for some of the linkage of systemic lupus erythematosus traits to homologous regions on human chromosome 1 in diverse populations (50–53).

The other possible DBA/2 resistance locus on chromosome 7, which was identified in both crosses, encompasses a region that overlaps the NZW susceptibility locus, Sle2 or Lbw5 (29, 39) and the CD22 gene. Gene knockout of CD22 has been associated with lupus-like disease (54–56), and it has been recently suggested that the CD22a allele may predispose to autoimmunity because of an insertion of a B1 repetitive element in the second intron that leads to alternative splicing of the 5' untranslated region (exons 1–3) and reduced levels of CD22 in unstimulated and LPS-stimulated B cells (57). However, it is unlikely that the CD22a allele is responsible for the DBA/2 resistance, because both DBA/2 and NZB...

FIGURE 3. Manifestations of HgIA among NZB, DBA/2, BDF1, and BDF2 mice. Column scattergraphs of glomerular and kidney vessel deposits of IgG and C3 are shown in the top four panels. See Table III for a summary of results and additional details. Autoantibody levels (bottom panel) show the distribution of individual animal responses for the DBF2 mice only. CG, IgG anti-chromatin Ab; CM, IgM anti-chromatin Ab; SG, IgG ssDNA Ab; SM, IgM ssDNA Ab (OD units are in log scale).

Table III. Immunopathology of NZB, DBA/2, BDF1, and BDF2 mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Glom IgG</th>
<th>Glom C3</th>
<th>Kid Ves IgG</th>
<th>Kid Ves C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBA/2</td>
<td>0</td>
<td>80 ± 14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NZB</td>
<td>1760 ± 234</td>
<td>660 ± 183</td>
<td>2.5 ± 0.19</td>
<td>2.6 ± 0.18</td>
</tr>
<tr>
<td>BDF1</td>
<td>560 ± 193</td>
<td>93 ± 41</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BDF2</td>
<td>603 ± 40</td>
<td>951 ± 41</td>
<td>0.52 ± 0.06</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>NZBpbs</td>
<td>120 ± 56</td>
<td>194 ± 46</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>p*</td>
<td>&lt;0.001</td>
<td>0.0068</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mean ± SE are shown. Same set of DBA/2 mice from Table I are included. Deposit results for glomeruli are inverse titers and vessel deposits are scored by intensity and degree of involvement (0–3 scale).

Values of p for unpaired t test comparisons of the DBA/2 and SJL mice are shown. Number of mice: 7–8 DBA/2, 8 NZB (HgCl2-exposed), 7 NZBpbs (PBS controls), 12 BDF1, 237–282 BDF2. Eight-week-old female mice were injected s.c. with 40 μg HgCl2 in 100 μl sterile PBS twice a week. After 1 mo, mice were sacrificed, and kidney sections were examined for glomerular and vessel deposits for IgG and C3 by direct immunofluorescence.
mice express the CD22a allele, while SJL mice express the normal CD22b allele (58).

This study demonstrates that NZB mice are highly sensitive to HgIA, similar to observations in most lupus strains or crosses, including BXSB, MRL++, and BWF1 (22, 23). Furthermore, the two significant loci identified in the BDF2 cross-mapped to previously identified NZB lupus susceptibility loci on chromosomes 1 (Lbw7 and nba2) and 4 (nba1, Sle2, and Lbw2) (29, 39, 59–61), although no linkage was observed for three other NZB loci linked to lupus traits on chromosomes 6 (29), 11 (29, 35), and 13 (34). This suggests that mercury may synergize with some, but not all, loci predisposing to spontaneous lupus. In contrast to the chromosome 1 locus (Hmr1), none of the traits in the SDF2 cross-mapped to the chromosome 4 interval (nominal p > 0.05). Thus, linkage to chromosome 4 in the BDF2 cross is more likely due to the NZB susceptibility locus (nba1, Sle2, and Lbw2) rather than a resistance locus from the DBA/2 strain. Another interesting finding was the much stronger linkage of the chromosome 1 region (Lbw7) to HgIA than the chromosome 4 interval (Lbw2), which contrasts with our previous observation in BWF2 crosses that the Lbw2 locus was much more strongly linked to GN (29). A possible explanation for the major effect of the chromosome 1 interval in the BDF2 cross may be a combined effect of the Hmr1 resistance locus from the DBA/2 and the NZB susceptibility locus.

Analysis of the SDF2 cross also showed that both the anti-fibrillarin and anti-chromatin Ab responses were linked to H-2 and, in the case of the anti-fibrillarin Abs, regardless of other background gene combinations. This is consistent with the notion that autoantibody specificity is largely determined by the MHC haplotype, presumably because of the role of MHC molecules in Ag presentation. However, anti-fibrillarin or anti-chromatin Abs or the H-2* were not associated with immune complex glomerular deposits. Although the roles of anti-fibrillarin and anti-chromatin Abs in the pathogenesis of mercury-induced immune complex disease are not known, these findings raise the possibility that other autoantibody specificities, which have yet to be identified, are also important in disease pathogenesis. Thus, although resistance of DBA/2 mice to mercury is associated with the absence of autoantibodies, it is possible that autoantibodies are present, but undetected because their specificities are not known. This is supported by the finding that BALB/c mice, which express the same H-2* as the DBA/2, develop HgIA with immune complex glomerular deposits, but do not have autoantibodies to fibrillarin or chromatin (17, 62).

Overall, these findings suggest that resistance of DBA/2 mice to HgIA involves a later stage of pathology than the production of autoantibodies, which would be consistent with our mapping studies.

Mercury is only one of a number of immunostimulatory heavy metal xenobiotics that can induce adverse immunotoxicity. Several of these, such as silver (31, 63, 64) or gold (65, 66), also promote the production of anti-fibrillarin autoantibodies in H-2* haplotype mice. Interestingly, the DBA/2 strain is also resistant to both silver- and gold-induced autoimmunity. Thus, findings related to Hmr1 resistance to mercury-induced disease may be more broadly applicable to other xenobiotic agents.

Predisposition to the adverse effects of environmental agents may be influenced by genetic variations in the population that affect both susceptibility and resistance. Herein, we have identified a locus, Hmr1, that appears to contribute to the resistance of DBA/2 mice to HgIA and that maps to a region on chromosome 1 implicated in susceptibility to spontaneous lupus. Definition of genetic alteration and mechanisms responsible for the Hmr1 phenotype should provide new insights into the relationship of environmental and genetic susceptibility in autoimmune diseases.

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