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Resistance to HgCl₂-Induced Autoimmunity in Haplotype-Heterozygous Mice Is an Intrinsic Property of B Cells

Gregory A. Hanley,* Joel Schiffenbauer,† and Eric S. Sobel²†

Exposure to low doses of mercury chloride induces autoantibodies to the nucleolar protein fibrillarin in H-2b, but not in H-2s, mice. Surprisingly, F₁ crosses between resistant and sensitive haplotypes are resistant. Previously, we have shown that the resistance in these F₁ mice was due to coexpression of the resistant class II allele. Using adoptive transfer techniques we have examined several mechanisms by which the resistant haplotype could be down-regulating the antifibrillarin response in F₁ (s/b) mice. Similar to other autoimmune models, mercury-induced autoimmunity requires cognate MHC-restricted T cell help. The absence of autoantibody production in F₁ mice was not due to a difference in thymic education or to the absence of antifibrillarin-specific T cell help. These results suggest that the resistance is due to an intrinsic property of the haplotype-heterozygous B cells. *The Journal of Immunology, 1998, 161: 1778–1785.

Systemic lupus erythematosus is a complicated systemic autoimmune disease with a multigenic mode of inheritance interacting with a potentially complex array of generally unknown environmental factors. Many of these features are also seen in the mercury chloride model of murine autoimmunity, which offers a unique opportunity to study the reproducible interaction between genetics and a simple, inorganic, environmental agent. Susceptibility to mercury chloride in mice is characterized by splenic hyperplasia, hypergammaglobulinemia, and autoantibody production (1, 2), all of which appear as soon as 1 wk after the start of treatment (2). The principal autoantibody target is fibrillarin, a U3 ribonucleoprotein found in the nucleolus and involved in the first step of preribosomal processing (3, 4). The antifibrillarin specificity is also seen in patients with scleroderma and other connective tissue disorders (5, 6). Recently, a comparison of the specificity of the human and murine autoantibodies showed that they recognize similar, if not identical, epitopes (7) and suggested an Ag-driven response (8).

Similar to human autoimmune disease, both MHC and non-MHC genes have been shown to have a pronounced effect on susceptibility (8). As defined by the presence of antifibrillarin Ab, mice of the H-2b haplotype are high responders, while those of the H-2b and H-2d haplotypes are nonresponders (9). In contrast, non-MHC genes affect primarily Ab titers but not specificity (8). Through the use of intra-H-2 recombinants, the presence of the HgCl₂-induced antifibrillarin Ab response has been mapped specifically to the I-A region (10). Previous studies in our laboratory have shown that F₁ animals between MHC-congenic susceptible H-2b and resistant H-2b mice to be resistant to HgCl₂-induced antinucleolar Abs (ANoAs) (11). This is surprising, since the I-A molecule is codominantly expressed in the F₁ mice, and in other autoimmune models heterozygosity of class II either enhances autoimmunity or modestly affects Ab titers (12, 13). Therefore, the profound resistance to HgCl₂-induced ANoAs seen in all haplotype-heterozygous mice tested raised the possibility that resistance was mediated by a dominant gene linked to I-A b. However, additional experiments demonstrated that resistance was, in fact, caused by coexpression of the I-A b molecule itself, and that this outcome was not merely the result of lower expression of the susceptible I-A a haplotype on the otherwise responsive B cells (11).

In the present study we used adoptive transfer experiments to explore further the mechanisms by which the resistant haplotype down-regulated the HgCl₂-induced antifibrillarin Ab response. Our experiments demonstrated that resistance was due neither to I-A b-mediated alteration of T cell repertoire nor to inadequate I-A b-restricted T cell help, but was an intrinsic property of the resistant haplotype-heterozygous B cells. These results suggest the presence of a novel mechanism of regulation of a potentially autoreactive immune response.

Materials and Methods

Mice

C57BL/6J (B6; I-A b, Igh b), C57BL/6J.SJL (B6.SJL; I-A a, Igh b), and C57BL/6J-Igh b Thy1 b Gpi b (B6.TC; I-A a, Igh b) mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in our breeding facility. B6.SJL.Igh a mice were developed by backcrossing B6.SJL to (B6.SJL × B6.TC)F₁. FACS analysis was used to select progeny that were I-A a and Igh b. These mice were then intercrossed, the progeny that were B6.SJL.Igh a were selected using FACS, and the strain was established from a single breeding pair. Chimeras were prepared as described in Table 1. All mice were housed in American Association for Accreditation of Laboratory Animal Care-approved facilities in compliance with all applicable federal, state, and local laws.

Preparation of chimeras

Preparation of chimeras was previously described (14). Recipient mice were provided with Septra (1%, v/v)-treated water the day before irradiation. On the day before bone marrow transfer, the mice were treated with two doses of 525 rad of γ-radiation (GammaCell 40, Atomic Energy of Canada, Ottawa, Canada) separated by 3 to 4 h. The transfer involved i.v. reconstitution with a total of 10⁷ bone marrow cells from age- and sex-matched donors. Bone marrow cells were depleted of mature T cells by...
incubation at 4°C for 30 min with a mixture of anti-mouse T cell serum (Cedarlane Laboratories, Hornby, Canada), 172-4 (rat IgM anti-CD4) (15), and 31M (rat IgM anti-CD90.2) (16) followed by treatment with C at 37°C for 1 h (Low-Tox Guinea Pig C, Cedarlane Laboratories). To prevent graft rejection, the B6.SJL mice receiving (B6.SJL × B6.TC)F₁ cells were given 0.1 mg i.p. of MmT1 (mouse IgG2a anti-CD90.2) (17) at the time of transfer. 172-4 and 31M were obtained from Dr. David Harris (University of Arizona, Tucson, AZ) and prepared from overgrown cell culture supernatant that was affinity purified on a protein G column.

In vivo treatments

HgCl₂ (Sigma, St. Louis, MO) was prepared in sterile, pyrogen-free PBS. Mice were injected s.c. at a dose of 1.5 mg/kg three times weekly after graft acceptance was verified using flow cytometry. Mice were immunized i.p. with 100 μg of human IgG (HGG);² of a second i.p. injection of 100 μg of HGG in sterile, pyrogen-free PBS.

Flow cytometry

Approximately 5 to 6 wk after bone marrow transfer, 200 μl of tail vein blood was collected into heparinized tubes. PBMCs were isolated using Lympholyte M (Cedarlane Laboratories) density gradients. The cells were then collected into PBS supplemented with 3% FCS and 0.1% NaN₃. For nonspecific ANoA, the slides were incubated for 30 min at room temperature with FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch). IgMα and IgMβ were minor modifications of previously described procedures (14). For nonallotype-specific ANoA, the slides were incubated for 30 min at room temperature with FITC-conjugated goat anti-mouse IgG2a (Nordic, Capistrano Beach, CA) and prepared from overgrown cell culture supernatant that was affinity purified on a protein G column.

Table I. Composition and characteristics of bone marrow chimeras

<table>
<thead>
<tr>
<th>Group</th>
<th>Donor 1</th>
<th>Recipient</th>
<th>Donor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain</td>
<td>Class II</td>
<td>Allotype</td>
</tr>
<tr>
<td>I</td>
<td>B6.SJL</td>
<td>s</td>
<td>b</td>
</tr>
<tr>
<td>II</td>
<td>B6.SJL</td>
<td>s</td>
<td>b</td>
</tr>
<tr>
<td>III</td>
<td>B6.SJL</td>
<td>s</td>
<td>b</td>
</tr>
<tr>
<td>IV</td>
<td>B6.SJL</td>
<td>s</td>
<td>b</td>
</tr>
</tbody>
</table>

³ (B6.SJL × B6.TC)F₁.

Indirect immunofluorescence

Sera from mice collected 5 wk after the initiation of HgCl₂ were tested for the presence of ANoA by indirect immunofluorescence using commercially prepared mouse frozen kidney slides (Sanofi, Chaska, MN). The slides were incubated with sera diluted 1/50 in PBS for 30 min at room temperature. For nonallotype-specific ANoA, the slides were incubated for 30 min at room temperature with FITC-conjugated goat anti-mouse IgG (Fc fragment specific; Jackson ImmunoResearch) diluted 1/50 in PBS. For the allotype-specific ANoA determination, slides were incubated with rabbit anti-mouse IgG2a or IgG2aβ (Nordic, Cspistrano Beach, CA) followed by FITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch). These reagents had been pretitrated by ELISA against an allotype-non-specific rabbit anti-IgG2a Ab (Nordic) to produce equivalent sensitivities. Antibody titer was defined by the formula: equivalent dilution factor = (dilution of the test serum) / (dilution of a standard reference serum) x 10⁴.

Results

MHC-restricted T cell help was required to produce ANoAs

Exposure to HgCl₂ induces a wide range of physiologic effects in mice, including the release of large amounts of cytokines, especially the Th2 cytokine IL-4. It was therefore possible that the antifibrillarin response could result from non-MHC-restricted interactions in genetically susceptible mice. To test this possibility, B6.TC mice were reconstituted with T cell-depleted B6.SJL marrow (group I). Because intrathymic positive selection of T cells is mediated by the radioresistant thymic cortical epithelial cells (24), the animals would be tolerant to both haplotypes. Taniguichi et al. (26) also showed that tolerance is mediated by bone marrow-derived dendritic cells, although thymic epithelial cells can make a significant contribution (25); therefore, in these mice all CD4⁺ T cells would be positively selected to interact with the host I-Aβ and not the donor I-Aα haplotype. Therefore, the B6.SJL-derived B cells would not be expected to receive MHC-restricted help. In contrast, central tolerance is mediated by bone marrow-derived dendritic cells, although thymic epithelial cells can make a significant contribution (25); therefore, the animals would be tolerant to both haplotypes. Taniguichi et al. (26) also showed that tolerance is mediated by bone marrow-derived cells. Transplantation of I-E⁻ transgenic donor bone marrow into an I-E⁻ host mouse resulted in the deletion of B6.11 CD4⁺ T cells. Flow cytometric analysis of PBL showed that all the B cells were of donor B6.SJL origin. Surprisingly, despite the presence of these susceptible B cells and the massive production of cytokines following HgCl₂ administration, none of the animals developed ANoAs (Table II). The addition of B6.TC cells in the inoculum did not alter the outcome (group II). In marked contrast, syngeneically reconstituted B6.SJL mice (group III) responded
Table II. MHC-restricted T cell help was required to produce ANoAs

<table>
<thead>
<tr>
<th>Group</th>
<th>Donor</th>
<th>Host</th>
<th>n(^{a})</th>
<th>ANoA Positive (%)(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>B6.SJL</td>
<td>B6.TC</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>B6.SJL and B6.TC</td>
<td>B6.TC</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>B6.SJL</td>
<td>B6.SJL</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>IV</td>
<td>B6.SJL and B6.TC</td>
<td>B6.SJL</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^{a}\) Number of mice.  
\(^{b}\) Fraction of positive mice.

Table III. ANoA response in chimeric (B6.SJL \(\times\) B6.TC)\(F_{1}\) host mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Source of Donor Bone Marrow</th>
<th>n(^{a})</th>
<th>ANoA Positive (%)(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>B6.SJL</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>VII</td>
<td>B6.TC</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>VIII</td>
<td>B6.SJL and B6.TC</td>
<td>16</td>
<td>44</td>
</tr>
<tr>
<td>IX</td>
<td>(B6.SJL (\times) B6.TC)(F_{1})</td>
<td>17</td>
<td>12</td>
</tr>
</tbody>
</table>

\(^{a}\) Number of mice.  
\(^{b}\) Fraction of positive mice.

well to mercury chloride, demonstrating that lack of responsiveness was not an artifact of radiation. The addition of B6.TC cells (group IV) to the inoculum was unable to prevent the autoimmune response. Syngeneically reconstituted B6.TC mice (group V) failed to respond (data not shown). We therefore conclude that the antifibrillarin specificity is mediated by I-A\(^{a}\)-restricted T cell help.

Absence of ANoA production in haplotype-heterozygous mice was not due to a difference in thymic education

Although the above experiments showed the importance of T-B cell collaboration, it is possible that I-A\(^{b}\) expression in the host and/or donor affected thymic education and therefore eliminated a population of responsive T cells. This led us to investigate the responses in (B6.SJL \(\times\) B6.TC)\(F_{1}\) hosts. When (B6.SJL \(\times\) B6.TC)\(F_{1}\) mice were used as hosts and reconstituted with B6.SJL (group VI), all (\(n = 12\)) responded to HgCl\(_2\). When the same hosts were used but the donor changed to B6.TC (group VII), none (of six) of the mice produced ANoAs (Table III). Therefore, the donor haplotype determined whether autoantibodies are produced when developing cells were positively selected on both haplotypes.

Allogeneic mixed chimeras developed ANoA titers in response to HgCl\(_2\)

In the rat model of mercury chloride-induced autoimmunity, allogeneic mixed microchimerism has been able to induce a state of tolerance (27). In contrast, other models have shown the potential for epitope spreading once tolerance is broken (28, 29). Therefore, it was of interest to determine whether the coexistence of B cells sensitive and resistant to the effects of HgCl\(_2\) could influence each other.

To test these possibilities, we lethally irradiated (B6.SJL \(\times\) B6.TC)\(F_{1}\) mice and transferred T cell-depleted bone marrow from the following: B6.SJL alone (group VI), B6.TC alone (group VII), a combination of B6.TC and B6.SJL (group VIII), and (B6.SJL \(\times\) B6.TC)\(F_{1}\) alone (group IX). In all four groups, T cells would be positively selected by either I-A\(^{a}\) or I-A\(^{b}\). After confirmation of mixed chimerism in group VIII (see below), the mice were treated with HgCl\(_2\) and the results are shown in Table III. As expected, (B6.SJL \(\times\) B6.TC)\(F_{1}\) mice reconstituted with only B6.SJL bone marrow responded readily to HgCl\(_2\) while mice given only resistant B6.TC bone marrow failed to develop an ANoA Ab titer. In contrast, syngeneic reconstitution with resistant (B6.SJL \(\times\) B6.TC)\(F_{1}\) bone marrow resulted in a very poor ANoA response. These results essentially duplicated our experience in analogous nonchimeric HgCl\(_2\)-treated B6.SJL, B6.TC, and (B6.SJL \(\times\) B6.TC)\(F_{1}\) mice (11) and again demonstrated that radiation did not affect this outcome.

Strikingly, mice given a combination of resistant and susceptible bone marrow had an intermediate result, with nearly 50% of the mice responding (Table III). By allotype-specific ANoA, all the responders produced exclusively b allotype autoantibody and therefore were of donor B6.SJL origin (data not shown). This is not due to an intrinsic property of the a allotype, for the a allotype B6.SJL-Igh\(^{a}\) strain responded equally well to mercury (data not shown). Thus, the presence of ANoA in these mice resulted from the loss of tolerance to fibrillarin in I-A\(^{a}\) B cells.

Lack of ANoA in nonresponsive mixed chimeras was unrelated to B cell composition

Of interest, the response rate of 44% in the mixed chimeras (group VIII) was significantly different from either the 100% seen in group VI (\(p < 0.02\), by \(z\)-test for proportions) or the 12% seen in group IX (\(p < 0.04\)) and suggested that the presence of resistant class II could exert a negative influence even when not coexpressed on the cells otherwise capable of responding. To evaluate for the possibility that this effect was merely due to dilution of potentially susceptible I-A\(^{a}\)-bearing B cells by resistant I-A\(^{b}\) B cells, we compared the B cell makeup of the positive and negative mice. The results are shown in Figure 1. The median percentage of I-A\(^{a}\)-expressing B cells was 58% in those mice that failed to produce ANoAs, while it was 60% in those mice that produced ANoAs (Fig. 1A). Even mice that had as few as 38% of their B cells from the sensitive parent were able to produce autoantibodies. Comparable results were seen when analyzed by B cell allotype (Fig. 1B). Therefore, these data suggest that the decreased responsiveness in the presence of resistant B cells was not just a dilutional effect.

B cells of both B6.TC and B6.SJL origin were functional in group VIII mixed chimeras

It was possible that the presence of a and b allotype B cells in the mixed chimeras influenced the development of ANoAs through Ag-nonspecific mechanisms. For example, in some combinations, allotype-specific suppression of immune responses has been seen (30). This possibility was minimized by the use of allotype-heterozygous host mice. However, to assess further the functionality of B cells of both B6.SJL and B6.TC origin, allotype-specific total IgM and IgG2a ELISAs were performed. In addition, the mice were assayed for their responses to immunization with a T cell-dependent Ag, HGG. HGG was used to determine to what degree the immune system remained intact after chimerism and to determine whether those B cells that failed to respond to fibrillarin were still functional. As shown in Figure 2, total IgM and IgG2a of both allotypes were present. Interestingly, despite the predominance of IgM of B6.SJL origin, IgG2a was much better balanced between the two donors. Moreover, there was a good IgG2a response to a T cell-dependent Ag by B cells of both B6.SJL and B6.TC origins (Fig. 3A), and both ANoA-positive and -negative mice responded equally well (data not shown).
Lack of HgCl2-induced ANoA response by haplotype-heterozygous mice was not due to the absence of antifibrillarin-specific I-A\(^{s}\)-restricted T cell help

To determine whether the intermediate response seen in the mixed chimeras and in HgCl2-resistant (B6.SJL×B6.TC)F\(_1\) mice was due to a lack of I-A\(^{a}\)-restricted T cell help, we reconstituted (B6.SJL×B6.TC)F\(_1\) mice with a combination of syngeneic (B6.SJL×B6.TC)F\(_1\) and B6.SJL bone marrow (group X). Forty-five percent (5 of 11) of these mice produced ANoAs upon HgCl2 treatment. The fact that some of the mice responded meant that T cells were available in which tolerance to fibrillarin was broken. Allotypic analysis revealed that all the autoantibodies were of the b allotype. It seems likely that they arose from the B6.SJL donor, since B cells from (B6.SJL×B6.TC)F\(_1\) mice would have produced autoantibodies of either a or b allotype, and no a allotype autoantibodies were detected even at a 1/10 dilution (data not shown). Thus, despite the presence of T cells that provided help in an I-A\(^{a}\)-restricted fashion, those B cells that coexpressed both haplotypes did not produce autoantibodies.

B cell composition was not a determining factor in responsiveness

Figure 4 shows the B cell makeup of the B6.SJL×(B6.SJL×B6.TC)F\(_1\) chimeras. Flow cytometric analysis of class II expression showed these mice to be very well balanced (I-A\(^{sb}\) range, 33–54%). The predominance of IgM of the b allotype (61–82%) was expected, considering that approximately one-half of the B cells from the (B6.SJL×B6.TC)F\(_1\) donor would express IgM of the b allotype. Similar to the parental into (B6.SJL×B6.TC)F\(_1\) mice, no correlation was noted between B cell makeup and responsiveness (Fig. 4).

**FIGURE 1.** Immunofluorescence on PBL of B6.SJL×B6.TC→(B6.SJL×B6.TC)F\(_1\) chimeras. Data are presented as a percentage of the cells expressing class II (A) or IgM (B). Individual mice are represented by ANoA status (X, positive; O, negative) in response to HgCl2 treatment.

**FIGURE 2.** Allotype-specific total IgM and IgG2a ELISA data of B6.SJL×B6.TC→(B6.SJL×B6.TC)F\(_1\) chimeras. Data are presented as a percentage of the total isotype of IgM (A) and IgG2a (B) or as absolute values (C and D). Individual mice are represented by ANoA status (X, positive; O, negative) in response to HgCl2 treatment.
Both donors provided functional B cells

The apparent lack of response from the a allotype B cells led us to examine whether these B cells were functionally equivalent. Figure 5 shows that IgM and IgG2a of both allotypes were produced, although the composition was somewhat skewed to the b allotype. Again, this is an expected finding, since the cells from the (B6.SJL × B6.TC)F₁ mice would produce either a or b allotype. Similarly, B cells of the a allotype responded equally well when the response to a T cell-dependent Ag (HGG) was analyzed in an allotypic fashion (Fig. 3B). A comparison between positive and negative mice revealed no significant differences. Taken together, we conclude that the inability of haplotype-heterozygous mice to respond to HgCl₂ with an ANoA response is an intrinsic property of the B cells.

Absence of ANoA production in haplotype-heterozygous mice was not due to the presence of 1-Aβ-restricted T cells

It was possible that the nonresponsiveness of the mixed chimeric as well as the syngeneically reconstituted (B6.SJL × B6.TC)F₁ mice was due to a negative regulatory effect of 1-Aβ-restricted T cells. To test for this possibility we lethally irradiated B6.SJL mice and transferred T cell-depleted bone marrow from (B6.SJL × B6.TC)F₁ mice (group XI). To ensure no carryover of T cells

**FIGURE 3.** Allotype-specific anti-HGG ELISA data. Data are presented as a percentage of total isotype (A and B) and as absolute values (C and D) of IgG2a. For these experiments, two groups of chimeras were prepared: B6.SJL + B6.TC → (B6.SJL × B6.TC)F₁ mice (A and C); and B6.SJL + (B6.SJL × B6.TC)F₁→(B6.SJL × B6.TC)F₁ mice (B and D). Individual mice are represented by ANoA status (X, positive; O, negative) in response to HgCl₂ treatment.

**FIGURE 4.** Immunofluorescence on PBL of B6.SJL + (B6.SJL × B6.TC)F₁→(B6.SJL × B6.TC)F₁ chimeras. Data are presented as the percentage of cells expressing class II (A) or IgM (B). Individual mice are represented by ANoA status (X, positive; O, negative) in response to HgCl₂ treatment.
educated in an (B6.SJL × B6.TC)F₁ mouse we treated the recipients at the time of transfer with 0.1 mg i.p. of MmT1 (mouse IgG2a anti-CD90.2) (17). The T cells in these mice are unable to provide help through I-Ab, since they developed in an I-A s-expressing host. Flow cytometric analysis confirmed that all the B cells in these mice expressed both haplotypes. Interestingly, none (0 of 11) of the mice produced ANoAs following HgCl₂ treatment. The resistance of these mice could not have been due to negative influences by I-Ab-restricted T cells.

Discussion

HgCl₂ treatment in mice induces a wide range of physiologic responses, including marked increases in IL-4 production (31, 32). The requirement for T cells in this model was shown by the fact that nude mice on the H-2s background fail to develop autoantibodies (33). Splenic CD4⁺ T cells of HgCl₂-treated H-2s mice have been shown to have a strong increase in IL-4 mRNA, whereas those of H-2d mice showed only a weak increase (34). Interestingly, treatment of H-2s mice with anti-IL-4 mAb did not prevent ANoA production, although it changed the isotype profile of the autoantibodies (32). Despite the possibility of noncognate help via cytokines, we have shown that MHC-restricted T cell help was required to produce ANoAs. The B cells were not merely responding to cytokines in the environment, but required specific MHC-restricted signals from T cells.

Cognate interactions are also required in several other models of autoimmune disease. Double parental-into-F₁ chimeras were used in the graft-vs-host model to show that autoantibodies were derived nearly entirely from B cells receiving direct alloreactive T cell help (18). Bone marrow chimeras were also used to show that autoantibody production in lpr mice was restricted to those B cells that received T cell help (35). Thus, in three very different murine models of autoimmunity, in vivo autoantibody responses were MHC restricted.

When the difference in responsiveness between B6.SJL→ B6.TC and B6.SJL→B6.SJL mice was examined, it became apparent that the possibility existed for significant differences in the T cell repertoire. Glimcher et al. (36) generated chimeric animals expressing MHC class II molecules on either bone marrow-derived or thymic stromal cells by using a strain of MHC class II-deficient mice. CD4⁺ T cells developed only where class II MHC molecules were expressed on radioresistant thymic cells. Similar results regarding positive selection were obtained using bone marrow chimeras to investigate T-B collaboration in the lpr model of autoimmunity (35). In our model it was possible that lack of expression of I-A⁺ on the radioresistant thymic population eliminated responsive T cells. The use of (B6.SJL × B6.TC)F₁ mice as hosts allowed for positive selection on both I-A⁺ and I-A⁻ and led us to conclude that responsiveness is dependent on the donor haplotype when T cells for both haplotypes are positively selected.

Mixed chimerism has been shown to prevent autoimmunity in several systems. Nonobese diabetic mice were protected from diabetes when they were made chimeric with diabetes-resistant bone marrow (37, 38). It appeared that the autoimmune potential of the NOD cells was restrained. However, when the amount of resistant cells was decreased, a low incidence of insulinitis was seen. Using the rat model of mercury-induced autoimmunity, Delaney et al. (27) showed that the presence of resistant bone marrow cells (microchimerism) converted an otherwise sensitive rat strain to a resistant one. If similar to the rat model, it would be expected that the presence of immune competent cells of resistant origin would prevent B6.SJL-derived B cells from responding to HgCl₂ with an antifibrillarin response. Our experiments differed somewhat in that we used a resistant (B6.SJL × B6.TC)F₁ mouse that had been confused with a combination of resistant and sensitive bone marrow. However, B6.SJL alone as the bone marrow donor completely restored the ability to produce ANoAs upon treatment with
HgCl₂. Despite containing a significant amount of resistant cells, we still obtained an intermediate response.

The presence of autoantibodies could play a role in spreading autoimmunity from a dominant epitope to previously cryptic epitopes (39). Presumably, binding of Ab can alter Ag processing, revealing new epitopes. Several other investigators have shown that it is possible to break T cell tolerance to self Ags by coimmunizing mice with self and foreign Ags that, in turn, generate cross-reactive B cells that can elicit an autoimmune T cell response to previously cryptic self determinants on the autoantigen (28, 29, 40, 41). These forms of epitope spreading do not appear to be occurring in our model. The production of autoantibodies by the B6.SJL B cells in the B6.SJL + (B6.SJL × B6.TC)F₁ → (B6.SJL × B6.TC)F₁ and the B6.SJL + B6.TC → (B6.SJL × B6.TC)F₁ mice did not result in the loss of tolerance to fibrillarin by previously resistant B cells.

Several other differences exist between our model and that used by Delaney (27). To induce chimerism without myeloablation, the rats were transiently treated with the immunosuppressive agent FK506. Some protection from the manifestations of HgCl₂-induced autoimmune disease was seen in the rats receiving FK506 alone. The researchers acknowledged that the results suggest that transient immunosuppression was an important component of the protection. We used a complete myeloablation procedure that allowed us to forego any immunosuppressive treatment. Another important difference lies in the fact that in the rat model regulatory T cells appear that render the rats resistant to additional HgCl₂ injections and can confer resistance to naive rats (42). Regulatory T cells have not been identified in the mouse model, and the animals do not become resistant to further treatment.

The intermediate response seen in group VIII mice was a surprising finding. It was possible that the ANoA-negative mice failed to respond because the number of potentially responsive B cells of I-A⁺ origin had been reduced by the presence of resistant B cells of I-Aᵇ origin. This mechanism appears unlikely, inasmuch as the B cell population of the chimeras had no relationship to the development of an ANoA response. Another Ag-nonspecific mechanism that could have accounted for the lack of response involves allele suppression. For example, in studying chronic graft-vs-host disease, Morris et al. found that in allotype-heterozygous recipients, the autoantibodies were preferentially made by those host cells that expressed the donor allele, whereas those host B cells that expressed nondonor allotype were relatively suppressed. In allotype-homzygous recipients, the donor cells frequently suppressed the host allotype completely (30). To minimize the possibility that this phenomenon could be occurring in our mice, we used allotype-heterozygous mice as hosts. Allotypic analysis of spontaneous as well as Ag-specific Abs demonstrated good participation by both allotypes. Therefore, allotype suppression was not a factor in our mixed chimeras.

A more likely possibility for the poor responsiveness of haplotype-heterozygous mice was lack of specific T cell help. By substituting (B6.SJL × B6.TC)F₁ for B6.TC as the resistant donor in mixed chimeras also receiving susceptible B6.SJL bone marrow, we provided a mechanism to verify the presence of I-A⁺-restricted T cell help in individual chimeric mice. In those mice with a positive ANoA, T cells capable of helping antifibrillarin-expressing B cells of I-A⁺ origin must be present, and these activated T cells, particularly with their reduced stimulation threshold, should also be capable of interacting with antifibrillarin-expressing B cells of I-A⁺/b origin. Moreover, by using haplotype-heterozygous mice as codonors, the overall expression of the resistant I-Aᵇ haplotype was reduced in cells of donor origin. Despite this, there was no increase in the ANoA response rate. More surprisingly, in the ANoA-positive chimeras, this specificity was limited to the b allotype, indicating that the haplotype-heterozygous B cells failed to participate in this response. These results strongly suggest that lack of responsiveness is an intrinsic regulatory property of B cells.

It was possible that haplotype-mismatched class II molecules were produced in our (B6.SJL × B6.TC)F₁ mice that could have altered the T cell repertoire. The formation of these mismatched pairs has been shown to be inefficient due to their inability to compete with the matched pairs (43). Previously, working with B6 mice whose I-Aᵇ gene had been knocked out (44), we were unable to detect any I-A molecules consisting of Aₚ₉ pairing with Aₗ₅ (11). Although we cannot formally rule out the formation of a mismatched pair in our F₁ mice, we think that this is an unlikely possibility.

The presence of I-Aᵇ-restricted T cell help could have accounted for the intermediate response seen in the mixed chimeras. The (B6.SJL × B6.TC)F₁ mice that served as hosts provided an environment in which T cells would be selected to interact with I-Aᵇ expressed on the B cells from the B6.TC hosts. By transferring (B6.SJL × B6.TC)F₁ bone marrow into B6.SJL mice, there would be little potential for positive selection of T cells on I-Aᵇ. Despite the absence of these cells, there was no ANoA response to HgCl₂. This again points to an intrinsic defect in (B6.SJL × B6.TC)F₁ B cells.

One possibility for this intrinsic property is the concept of MHC-guided processing leading to dominant capture (45). This hypothesis states that when an Ag is taken up by APCs and begins unfolding, different MHC molecules can compete for determinants. Once it is bound by class II, the Ag is then trimmed down to its final size, while the remainder of the Ag, including cryptic epitopes, is discarded. An example of this phenomenon was seen in the autoimmune disease insulin-dependent diabetes mellitus (46). The response to the subdominant A⁻SDO-restricted determinant of HEL disappears when NOD mice were made transgenic by introduction of the E₄⁺. The responsiveness was restored when scission of the HEL separated this determinant from its adjoining, competitively dominant, E⁴⁻-restricted determinant. This suggested that the E₄⁻ molecule bound and protected its dominant determinant on a long peptide, while captured neighboring determinants were lost during proteolysis. In our mice, I-Aᵇ could effectively be binding fibrillarin in the (B6.SJL × B6.TC)F₁ cells, thereby preventing I-A⁻ from presenting fibrillarin and receiving T cell help.

Recently, the molecular and antigenic properties of mercury-modified fibrillarin have been examined. The exposure of fibrillarin both in vivo and in vitro caused a change in its migration under nonreducing SDS-PAGE and resulted in a loss in reactivity to autoantibodies. Mutation of the cysteines in fibrillarin resulted in a loss of mercury-induced modification. The authors concluded that unmodified fibrillarin is the B cell Ag, while the T cell Ag appears to be mercury-modified fibrillarin (47). Therefore, if our model is correct, the presence of I-Aᵇ affected the processing of Hg-modified fibrillarin in the context of I-Aᵇ and provides a unique opportunity for testing the role of Ag competition in an important environmental model of induced autoimmunity.

References