Mercury-Induced Autoimmunity in Mice

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We have studied the effect of gender, genetics, and toxicokinetics on immune parameters in mercury-induced autoimmunity in mice. Data strongly suggest that the mechanism for mercury-induced autoimmunity involves modification of the autoantigen fibrillarin by mercury followed by a T-cell–dependent immune response driven by the modified fibrillarin. Mice with different H-2 haplotypes were treated with 203HgCl2 in a dose of 0.5–16 mg Hg/L drinking water for 10 weeks. Whole-body accumulation and renal accumulation of mercury were assessed. Serum autoantibodies were used to evaluate the autoimmune response, and serum immunoglobulin E (IgE) to study effects on T-helper cells of type 2. Strains with a susceptible H-2 haplotype developed autoantibodies to the nuclear protein fibrillarin (AFA) in a dose-dependent pattern within 2 weeks. The substantially lower whole-body and organ mercury level needed to induce AFA in the susceptible A.SW strain compared with the H-2 congenic B10.S strain demonstrates that genetic factors outside the H-2 region modify the autoimmune response. Mouse strains without the susceptible haplotype did not develop any autoimmune reaction irrespective of dose and organ deposition of mercury. In susceptible mouse strains, males and females had different thresholds for induction of autoimmune reactions. In susceptible strains, serum IgE increased dose dependently and reached a maximum after 1–2.5 weeks. A susceptible H-2 haplotype is therefore a prerequisite for the autoimmune response. Mercury exposure will modulate the response, qualitatively through the existence of dose-related thresholds for autoimmune response and quantitatively as increasing doses cause increasing autoimmune response. Further, gender and non–H-2 genes modulate both the induction and subsequent development of AFA. Induction of IgE seems not to be mechanistically linked to the AFA response. Key words: autoantibodies, autoimmunity, H-2 genes, mercury, mice, toxicokinetics. Environ Health Perspect 110(suppl 5):877–881 (2002), http://ehpnet1.niehs.nih.gov/docs/2002/suppl-5/877-881nielsen/abstract.html

Autoimmune diseases apply to cases where the autoimmune process contributes to the pathogenesis of the disease. These diseases are among the major medical problems in many countries and include such diseases as rheumatoid arthritis, systemic lupus erythematosus, and type 1 diabetes. A recent review on the epidemiology and significance of autoimmune diseases in health care suggests that about 20% of the human population suffers from at least one autoimmune disease (1). Although autoimmune diseases to some extent aggregate in families, indicating the importance of genetics, autoimmune diseases have a multifactorial etiology in which different environmental factors seem to be superimposed upon a genetically complex susceptibility (2). A characteristic of environmentally induced autoimmune diseases is that the autoimmune reactions develop in only a fraction of similarly exposed individuals (3). Pertinent questions in environmentally induced autoimmune conditions are a) how environmental factors interact with genetic susceptibility, b) to what extent the dose of the exogenous factor will affect the autoimmune response quantitatively, and c) whether thresholds exist for induction of the autoimmune reactions.

Dominant among the genetic associations with autoimmune disease in humans is linkage with the major histocompatibility complex (MHC) demonstrated through a close linkage to particular HLA (human leukocyte antigen) haplotypes. Well-characterized experimental models exist in rats as well as mice, where gold or inorganic divalent mercury is used to induce an autoimmune response in susceptible animals. As in humans, a major factor regulating the susceptibility resides in the murine MHC (H-2) genes.

In this article we summarize the present knowledge on the toxicological mechanisms for induction of autoimmune response in an experimental model and draw parallels to the human situation. Further, we present the experimental model used to study the influence of genes, gender, and kinetics together with experimental data with specific reference to autoimmunity and mercury kinetics.

Mechanisms in Heavy-Metal Induction of Autoimmunity

Heavy metals, most notably mercury, interact with the immune system in several ways. Provided that the dose of mercury is adequate, most murine strains will develop lymphoproliferation. This seems to manifest as a polyclonal activation of T cells and then B cells (4, 5). A few strains, notably those with H-2 haplotypes J and Q, develop an autoimmune response predominantly against nuclear antigens, with the 34-kDa protein fibrillarin as the common target (6). Interestingly, some patients with systemic scleroderma have an autoantibody response restricted to fibrillarin (7). The mercury-induced antifibrillarin antibody (AFA) response is not only H-2 restricted but also T-cell dependent (8), and the antinuclear monoclonal antibodies from mercury-treated mice show evidence of somatic mutations (9). Taken together, these data strongly suggest that the AFA response is antigen driven. The nature of the antigen is not known with certainty, and it could be either a molecule mimicking fibrillarin or sequences thereof, or fibrillarin itself. Fibrillarin not only binds to mercury (10) but also is sensitive to novel proteolytic cleavage during mercury-induced necrotic cell death (11). Some evidence suggests that this cleavage might result in cryptic immunogens that are recognized as “nonself” by the T cells and result in cognate help to B-cell clones recognizing native fibrillarin. After some time the T cells seem to be able to recognize native fibrillarin, possibly by the process of intramolecular spreading (12).

An Experimental Model to Study Autoimmune Response Induced by Mercury in Mice

Animals

The use of inbred and genetically well-characterized strains of mice allows specific studies of the influence of the H-2 haplotypes as well as background (non–H-2) genes. We used eight mouse strains (Table 1), allowing comparisons between the autoimmune response in H-2-congenic strains with different background genes (A.SW, B10.S, SJL) as well as between strains with different H-2 haplotypes but identical background genes (B10). A.SW mice were purchased from Bommice Breeding and Research Centre, Ry, Denmark; other strains were purchased from Harlan Ltd., Oxon, United Kingdom. Male and female mice were 10–14 weeks old when included in experiments. None of the animals experienced any adverse effects before sacrifice and weight gain was similar in exposed and control animals. All experiments were performed in accordance with the Swedish Animal Experimentation Act.

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**Mercury Exposure**

The experimental groups were exposed to mercuric chloride in the drinking water for 10 weeks before sacrifice. The mercury was labeled with 203Hg to allow whole-body counting during the experimental period as well as easy determination of renal accumulation of mercury after sacrifice. The experimental model to study kinetics of mercury has been described in detail in Nielsen (13). Briefly, drinking-water consumption was measured and used to calculate mercury intake, and whole-body retention (WBR) of mercury was measured at regular intervals in live animals throughout the experimental period by use of a whole-body counter (Nal well crystal) connected to a multichannel analyzer (Series 35 Plus; Canberra Industries Inc., Meriden, CT, USA). To adjust for counting efficiency and radioactive decay, a standard of known intensity (1 mL of drinking water) was used at all counting sessions. After 10 weeks, all animals were killed by cervical dislocation, and relevant organs were excised and counted in a Searle 1195R (Searle Analytical Inc., Des Plaines, IL, USA) gamma counter. Blood was obtained from the tail vein after 2.5 weeks and from the thoracic cavity at sacrifice. The study period was 10 weeks to assure that the WBR of mercury had reached steady state and to allow time for a maximal autoimmune response to develop. Dose, and thereby WBR and organ deposition of mercury, was varied by changing the concentration of mercuric chloride in the drinking water) was used at all counting sessions. After 10 weeks, all animals were killed by cervical dislocation, and relevant organs were excised and counted in a Searle 1195R (Searle Analytical Inc., Des Plaines, IL, USA) gamma counter. Blood was obtained from the tail vein after 2.5 weeks and from the thoracic cavity at sacrifice. The study period was 10 weeks to assure that the WBR of mercury had reached steady state and to allow time for a maximal autoimmune response to develop. Dose, and thereby WBR and organ deposition of mercury, was varied by changing the concentration of mercuric chloride in the drinking water. In this article, we present data on mice exposed through the drinking water to mercury concentrations between 0.5 mg/L and 16 mg/L.

**Antinuclear Antibodies**

The presence, pattern, and titer of serum antinuclear antibodies (ANAs) of the immunoglobulin G (IgG) class were determined by indirect immunofluorescence using mouse myeloma protein of the IgG isotype (Sigma Chemical Company, St. Louis, MO, USA) and anti-C3c antibodies (Organon-Technica, West Chester, PA, USA). The titers were determined by serial dilution of the antibodies to 1:5,120.

**Serum IgE**

Serum IgE concentrations were determined by enzyme-linked immunosorbent assay as described before (16). Briefly, microtiter plates were coated with rat anti-mouse IgE (Southern Biotechnology), followed by blocking and incubation with diluted serum. Bound IgE was detected by horseradish peroxidase–conjugated goat anti-mouse IgE (Nordic Immunological Lab, Tilburg, The Netherlands), and the IgE concentrations were derived from a standard curve using mouse myeloma protein of the IgE isotype (Sigma Chemical Company, St. Louis, MO, USA).

**Autoimmunity and Genetics**

The adverse immune reactions that develop in rodents in response to mercury exposure are controlled by multiple genes, with genes residing in the MHC region being the most important. In the mouse, susceptibility to induction of AFAs is linked to the mouse MHC region (H-2), with the and haplotypes conferring susceptibility and most other haplotypes conferring resistance.

We have studied the induction of AFAs and renal IgG deposits after 10 weeks of drinking water exposure to mercuric chloride in eight strains of mice representing four different haplotypes (Table 1). If susceptibility to induction of an autoimmune response were linked solely to a certain haplotype, mice with other H-2 haplotypes would not develop autoimmunity and strains with the H-2 haplotype would demonstrate identical autoimmune responses.

Using four intra–H-2 recombinant strains, we could demonstrate that the H-2 haplotype, as expected, but also the H-2 haplotype conferred susceptibility to induction of AFA after exposure to mercuric chloride (Tables 2, 3). Further, strains with the H-2 and H-2 haplotypes did not develop any autoimmune response (Tables 2, 3) despite an exposure to mercuric chloride 3 times higher and consequently 3 times higher WBR and renal mercury accumulation (Table 3). Because of the lack of expression of the E genes in the susceptible H-2 haplotype, these loci cannot be important for susceptibility. In addition, the mere presence of the gene in the K locus was not associated with susceptibility (H-2). Therefore, either the A or the S, D, or L loci harbor the susceptibility to induction of autoimmunity. Because and genes in the three latter loci did not affect susceptibility (H-2), it can be concluded with reasonable certainty that the susceptibility to induction of AFA with mercury resides in the A loci. This tallies with the fact that the A loci belong to the classic immune response (I response).

A comparison of ANA, renal IgG deposits, and renal mercury accumulation among three strains of mice sharing the H-2 haplotype revealed that factors outside the H-2 region affected the autoimmune response as well as mercury kinetics. Thus, ASW and SJL strains both had almost complete AFA response (90–100%), high AFA titers, and similar renal IgG deposits (Table 2). The B10.S strain, on the other hand, had significantly lower AFA titer and IgG deposits compared with the

| Table 1. H-2 configurations in eight mouse strains. |
| --- |
| | H-2b haplotype | H-2b loci<sup>*4</sup> |
| **Strain** | **K**<sup>s</sup> | **A<sub>p</sub>**<sup>s</sup> | **A<sub>n</sub>**<sup>s</sup> | **E<sub>p</sub>**<sup>s</sup> | **E<sub>n</sub>**<sup>s</sup> | **S** | **D** | **L** |
| A.SW | s | s | s | s | s | s | s | s | s |
| A.TL | s | s | s | s | s | s | s | s | s |
| B10.S | s | s | s | s | s | s | s | s | s |
| B10.TL | s | s | s | s | s | s | s | s | s |
| B10.S(Rfl) | s | s | s | s | s | s | s | s | s |
| B10.D2 | s | s | s | s | s | s | s | s | s |
| DBA | s | s | s | s | s | s | s | s | s |
| SJL | s | s | s | s | s | s | s | s | s |

<sup>*4</sup>Designation of the H-2 haplotypes and loci according to Klein et al. (29). No expression of E molecules on the cell surface.

| Table 2. AFA, renal IgG deposits, and renal mercury accumulation in female mice exposed to 5 mg/L mercuric chloride in the drinking water for 10 weeks. |
| --- |
| **Strain** | **H-2b haplotype** | **Renal IgG deposits (reciprocal titer)** | **AFA** | **Titer in AFA-positive mice (reciprocal titer)** | **Positive response (%)** | **Renal Hg accumulation (µg Hg/g wet wt.)** |
| A.SW | s | 804 | 3,840 | 100 | 24.9 |
| B10.S | s | 712 | 1,440 | 90 | 27.7 |
| DBA | s | 107 | 213 | 70 | 14.9 |
| A.TL | s | 24 | 0 | 0 | 22.8 |

All numbers are given as medians (n = 8).
A.SW and SJL strains (Table 2; Mann-Whitney test, p < 0.05). Supporting evidence for the influence of the toxicokinetics of mercury on autoimmune response in this experimental model is that renal mercury deposition (and WBR, data not shown) was twice as high in A.SW and SJL mice compared with renal mercury deposition in B10.S mice (Table 2).

Autoimmunity and Toxicokinetics

As visualized above, the autoimmune response depends on factors not solely related to the H-2 haplotype. These factors may, if genetically based, impose different strain-specific susceptibilities on the animals. Inorganic mercury is used to induce the autoimmune response in this experimental model, and the toxicokinetics of inorganic mercury is known to vary among mouse strains (1,5).

Inorganic mercury has a whole-body elimination half-time of less than 10 days in mice after oral exposures. After drinking water exposure is initiated, mercury WBR increases and is expected, based on the elimination half-time, to reach steady state in less than 5 weeks. If the autoimmune response in susceptible mice depends solely on the amount of mercury accumulated in the animal as a whole or specifically in target organs, the exposure time, dose (concentration of mercury in the drinking water), and WBR of mercury should all affect the autoimmune response.

Further, because mercury accumulation in the mice will not steadily increase with increasing exposure time but settle at a steady-state level where absorption equals elimination, we should theoretically be able to find strain-specific thresholds for induction of autoimmune response in the susceptible mouse strains. We therefore studied the influence of exposure time and dose (WBR) on the autoimmune response.

Exposure Time

The IgE concentration reached a maximum response 1 week after exposure to 1 mg of mercuric chloride/L was initiated, followed by a slow decline during the next 3 weeks back to the preexposure level (Figure 1). Concomitant with the declining IgE concentration, the AFA response began to increase. Data on AFA titers from weeks 3 and 4 (Figure 1) together with data from week 10 (Table 4) indicate that the AFA titer in serum increases steadily during the exposure period. However, not only did the AFA titer raise with prolonged exposure time, but so did the fraction of animals with AFA (Table 4). Similar patterns for development of positive AFA response with increasing exposure time were seen in male A.SW mice as well as in female B10.S mice (Table 4). These findings are in agreement with previous data demonstrating induction of AFA in all A.SW mice after 2 weeks of subcutaneous injections of higher mercury doses (5,17). Because 50% of the final steady-state level of mercury is reached shortly after treatment is initiated (18), there are sufficient conditions for an interaction between mercury and fibrillarin during the first days after onset of treatment. That a rapid interaction takes place is also indicated by the appearance of AFA in serum after 2 weeks, during which time T-cell recognition of the modified fibrillarin (peptide) (19) followed by activation and maturation of the B cells to Ig production, must take place. These findings show that the critical phase of the AFA induction occurs during the first weeks of treatment over a wide dose range and after oral as well as subcutaneous exposure.

Interestingly, the very lowest doses of mercury giving rise to AFA did not cause AFA until after 10 weeks (Table 4). It is likely that such low doses during the first days give rise to mercury levels that are too low to reach the threshold for modification of fibrillarin (binding and/or proteolysis) because 50% of the final steady-state level of mercury was reached after 2 days but 75% after only 2–3 weeks.

A rapid and strong increase of serum IgE is a hallmark of mercury-induced autoimmunity both in rats (19) and mice (20), and serum IgE has been considered a relevant marker for mercury-induced interleukin-4 and therefore, indirectly, for T-helper type 2 (Th2)-cell activation in H-2 mice. Mercury-induced autoimmunity in mice was, until a few years ago, considered to be a Th2-dependent condition, but a number of recent findings have shown that this may not be the case (21). Supporting this finding, the threshold for induction of serum IgE was generally higher than for the induction of AFA, with

| Strain | Sex (M/F) | Dose (mg/L) | Titer in AFA-positive mice (mean ± SD) | Positive response (%) | Renal Hg accumulation (µg Hg/g wet wt.) |
|--------|-----------|-------------|--------------------------------------|-----------------------|----------------------------------------|
|        |           |             | AFA                                  |                       |                                        |
|        |           |             | 2.5-Week exposure                     | 10-Week Exposure      |
| B10.S  | F         | 0           | 0                                    | 0                     | 0                                      |
|        |           | 0.5         | 0                                    | 0                     | 0                                      |
|        |           | 1.0         | 0                                    | 0                     | 0                                      |
|        |           | 2.0         | 0                                    | 0                     | 0                                      |
|        |           | 4.0         | 0                                    | 0                     | 0                                      |
|        | M         | 0           | 0                                    | 0                     | 0                                      |
|        |           | 0.5         | 0                                    | 0                     | 0                                      |
|        |           | 1.0         | 0                                    | 0                     | 0                                      |
|        |           | 2.0         | 0                                    | 0                     | 0                                      |
|        |           | 4.0         | 0                                    | 0                     | 0                                      |
| B10.S  | F         | 0           | 0                                    | 0                     | 0                                      |
|        |           | 0.5         | 0                                    | 0                     | 0.05                                   |
|        |           | 1.0         | 0                                    | 0                     | 0.02                                   |
|        |           | 2.0         | 0                                    | 0                     | 0.03                                   |
|        |           | 4.0         | 0                                    | 0                     | 0.02                                   |
|        | M         | 0           | 0                                    | 0                     | 0                                      |
|        |           | 0.5         | 0                                    | 0                     | 0                                      |
|        |           | 1.0         | 0                                    | 0                     | 0                                      |
|        |           | 2.0         | 0                                    | 0                     | 0                                      |
|        |           | 4.0         | 0                                    | 0                     | 0                                      |

Table 3. AFA and renal mercury accumulation in female mice with different H-2 haplotypes but identical background genes exposed for 10 weeks to mercuric chloride.

| Strain | H-2 haplotype | Dose (mg/L) | Titer in AFA-positive mice (reciprocal titer) | Positive response (%) | Renal Hg accumulation (µg Hg/g wet wt.) |
|--------|---------------|-------------|-----------------------------------------------|-----------------------|----------------------------------------|
| B10.S  | s             | 5           | 128                                           | 70                    | 4.7                                    |
| B10.S(9R) | t4          | 5           | 140                                           | 40                    | 4.2                                    |
| B10.TL | t1           | 16          | 0                                             | 0                     | 12.4                                   |
| B10.D2 | d             | 16          | 0                                             | 0                     | 13.4                                   |

All numbers are given as medians (n = 8).

Table 4. AFA and WBR in male and female mice after drinking water exposure to mercuric chloride.

| Strain | Sex (M/F) | Dose (mg/L) | Titer in AFA-positive mice (mean ± SD) | Positive response (%) | Titer in AFA-positive mice (mean ± SD) | Positive response (%) | WBR (µg Hg) |
|--------|-----------|-------------|--------------------------------------|-----------------------|--------------------------------------|-----------------------|-------------|
| A.SW   | M         | 0           | 0                                    | 0                     | 0                                    | 0                     | 0           |
|        |           | 0.5         | 0                                    | 0                     | 0                                    | 0                     | 0.85        |
|        |           | 1.0         | 0                                    | 0.25                  | 1.890 ± 1.667                       | 100                   | 1.76        |
|        |           | 2.0         | 0                                    | 0                      | 4.980 ± 2.735                       | 100                   | 4.08        |
|        | M         | 0           | 0                                    | 0.25                  | 5.200 ± 7.127                       | 100                   | 1.19        |
|        |           | 0.5         | 0                                    | 0                      | 1.688 ± 2.176                       | 62.5                  | 2.14        |
|        |           | 2.0         | 0                                    | 0                      | 2.600 ± 1.875                       | 100                   | 5.24        |
|        |           | 4.0         | 0                                    | 0.25                  | 5.440 ± 2.136                       | 100                   | 16.10       |
| B10.S  | M         | 0           | 0                                    | 0                      | 0                                    | 0                     | 0           |
|        |           | 0.5         | 0                                    | 0                      | 0                                    | 0                     | 0.36        |
|        |           | 1.0         | 0                                    | 0                      | 0                                    | 0                     | 0.92        |
|        |           | 2.0         | 0                                    | 0                      | 800 ± 679                           | 25                    | 3.93        |
|        |           | 4.0         | 0                                    | 0                      | 335 ± 265                           | 100                   | 9.65        |
|        |           | 8.0         | 0                                    | 0                      | 3.320 ± 1.926                       | 100                   | 15.70       |
|        | M         | 0           | 0                                    | 0                      | 0                                    | 0                     | 0           |
|        |           | 0.5         | 0                                    | 0                      | 0                                    | 0                     | 0.39        |
|        |           | 1.0         | 0                                    | 0                      | 0                                    | 0                     | 1.13        |
|        |           | 2.0         | 0                                    | 0                      | 800 ± 679                           | 25                    | 4.45        |
|        |           | 4.0         | 0                                    | 0                      | 880 ± 1.055                         | 75                    | 9.58        |
|        |           | 8.0         | 0                                    | 0                      | 1.822 ± 1.776                       | 87.5                  | 21.70       |

Abbreviations: M, male; F, female. *Reciprocal titer.

Figure 1. Serum IgE (µg/mL) and AFA reciprocal titers in female A.SW mice exposed to 1 mg mercuric chloride/L drinking water for 4 weeks. Results are means, and bars denote SEM (n = 7).
relatively little variation between the A.SW and B10.S strains and between genders (22). Taken together, these findings strongly indicate that the mechanisms for inducing AFA and IgE are mechanistically unrelated. However, both AFA and IgE may be seen as important markers during the early development of autoimmunity reactions after exposure to mercury.

**Dose–Response Relations and Thresholds for Induction of AFA**

The data above indicate that a certain autoimmune response may be induced by either a short exposure to a high mercury dose or a more prolonged exposure to a lower mercury dose. This finding suggests that the autoimmune response in susceptible mice is a function of the product of dose and exposure time. However, because 75–100% of the steady-state WBR of mercury is reached within 2–3 weeks of exposure (18,22), there is no simple linear relationship between WBR and induction of autoimmune response. Previous data have indicated that kidney accumulation of mercury was associated with autoimmune response in different mouse strains (23), but the absence of data on renal accumulation of mercury after 2.5 weeks does not allow further conclusions at present.

In an experiment with two susceptible [A.SW and B10.S (haplotype H-2d)] and one resistant [A.TL (haplotype H-2t)] mouse strains an autoimmune response could not be induced in the resistant strain despite a heavy mercury load (Table 5). As evident from Table 5, renal accumulation of mercury in A.TL mice given 16 mg/L for 10 weeks is 8–10 times higher than in A.SW or B10.S mice with 100% autoimmune response. Thus, increasing the dose/WBR/target organ deposition will not override the prerequisite for a susceptible haplotype. It is also evident from the female A.SW mice given 5 mg/L and 16 mg/L, respectively, that an apparent maximal autoimmune response exists despite increasing the dose and thereby renal mercury accumulation close to 10-fold (Table 5).

At certain dose levels we do not observe any induction of an autoimmune response even after 10 weeks of mercury exposure to the susceptible mouse strain B10.S (Table 4). Thus, data indicate that thresholds exist below which autoimmune reactions will not develop despite a susceptible haplotype. This threshold varies among susceptible mouse strains with identical H-2 haplotype (Figure 2, Table 4), which implies that factors residing outside the H-2 region will also influence the autoimmune response.

Apparently, a susceptible haplotype is a prerequisite for induction of an autoimmune response, but thresholds as well as the quantitative autoimmune response also depend on the genotype outside the H-2 region as well as degree of environmental exposure.

**Autoimmunity and Gender**

Autoimmune diseases occur with different frequencies in males and females. The female: male ratio for systemic lupus erythematosus and Sjögren syndrome is close to 9:1 in humans, and rheumatoid arthritis has an incidence in women 3 times higher than in men (24). The reason for the observed differences in incidences of autoimmune diseases between men and women is not clear. However, sex hormones can modify an immune response, and women generally produce more antibodies than do men after an immune response (24). An experimental model to study autoimmunity should preferably demonstrate a similar pattern of gender differences in mice.

The influence of gender on the autoimmune response was studied in two susceptible mouse strains. In A.SW mice, females generally had higher AFA titers than did males at similar dose levels (Table 4). The one exception was at the lowest dose (0.5 mg/L) where one male mouse had an extremely high AFA titer (10,240), a titer that was not observed in any other mouse regardless of dose level. Results similar to those in A.SW mice were obtained in B10.S mice, although titers were lower. The female mice not only had higher titers but also seemed to respond at lower doses than males, and the fraction of mice with positive autoimmune response (AFA titer) apparently reached 100% at lower dose levels in females than in males (Table 4).

Mercury is used as chemical inducer of an autoimmune response, and mercury kinetics in males and females is known to be different (19). Thus, at similar dose levels, males tend to accumulate slightly more mercury than do females (19). To exclude the influence of this gender-related difference in mercury kinetics from our study on the influence of gender on autoimmune response, we therefore chose to illustrate the autoimmune response as a function of the absorbed and retained whole-body mercury deposition rather than a function of the mercury dose. When the fraction of mice with a positive AFA response is illustrated as a function of the WBR of mercury at steady state, the lower threshold for induction of AFA in female mice compared with that in males is clearly observed (Figure 2). Further, not only was the sensitivity higher (lower threshold for induction of AFA) but also the responsivity (lower WBR to reach 100% AFA response), in females than in males. Thus, in both strains, response curves for females were shifted to the left compared with similar curves for male mice (Figure 2).

The present study is in very good agreement with clinical experience from humans demonstrating a clear effect of gender, with females being more susceptible. However, the quantitative difference in degree of response between male and female mice in this study is not even close to the differences observed in some human diseases such as systemic lupus erythematosus, although the female: male incidence ratio in B10.S mice at the lower mercury doses comes close to 3, matching that of rheumatoid arthritis in humans. Although the larger incidences observed in females may be explained in terms of hormones, the reason for the large variations in ratios between males and females in different diseases is presently unresolved in humans.

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**Table 5.** Autoimmune response (AFA) and renal accumulation of mercury in female mice exposed to increasing doses of mercuric chloride in drinking water.

| Strain | H-2 haplotype | Dose (µg/L) | Titer in AFA-positive mice (reciprocal titer) | Positive response (%) | Renal Hg (µg Hg/g wet wt.) |
|--------|---------------|-------------|---------------------------------------------|-----------------------|-----------------------------|
| A.TL   | t1            | 1           | 0                                           | 0                     | 3.9                         |
|        |               | 5           | 0                                           | 0                     | 13.0                        |
|        |               | 16          | 0                                           | 0                     | 129.7                       |
| A.SW   | s             | 1           | 240                                         | 50                    | 1.1                         |
|        |               | 5           | 7,552                                       | 100                   | 12.1                        |
|        |               | 16          | 8,320                                       | 100                   | 115.8                       |
| B10.S  | s             | 1           | 128                                         | 70                    | 4.7                         |
|        |               | 5           | 1,280                                       | 100                   | 15.1                        |

All numbers are given as means (n = 8).

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**Figure 2.** The fraction of mice with AFA presented as a function of the whole-body retention of mercury (µg Hg) after 16 weeks of drinking water exposure. Each point represents eight mice given between 0 and 8 mg mercuric chloride/L.
Conclusion
On the basis of our recent studies on the influence of toxicokinetics on adverse immune reactions in a murine model, we conclude the following:
- Genetically determined susceptibility is linked to the murine H-2 haplotype, and a susceptible haplotype is a prerequisite for an autoimmune response expressed as antifibrillarin antibodies.
- Because haplotypes H-2\(t\) and H-2\(s\) confer susceptibility to mercury-induced autoimmune response to a comparable extent, whereas H-2\(t\) causes resistance, our data suggest that susceptibility may be restricted to the A\(_{d}\) and A\(_{p}\) loci in H-2.
- Different quantitative autoimmune responses were observed among susceptible mouse strains with identical H-2 haplotype. We conclude that induction and development of AFA may be modulated by mercury toxicokinetics, but non-H-2 genes may also modulate this response independent of kinetics.
- AFA and IgE are both important markers for adverse immune reactions after exposure to mercuric chloride, but the responses are probably mechanistically unrelated.
- Thresholds exist below which no autoimmune response is observed even after prolonged exposure. At low mercury exposures, autoimmune response is not observed within the first weeks but develops gradually. This observation is probably caused by mercury accumulation in whole body and target organs along with increased exposure time.
- Increasing the mercury exposure causes an increased target organ deposition and an increased autoimmune response. However, an apparent maximal autoimmune response exists that increased mercury exposure will not change. The quantitative response is strain specific and demonstrates the importance of toxicokinetic differences among mouse strains for induction of autoimmunity.
- The autoimmune response depends on gender. Female mice have a higher sensitivity (lower threshold for induction of AFA) as well as a higher responsibility (lower WBR to reach 100% autoimmune response) than male mice.
- The experimental model for induction of autoimmune responses demonstrates good agreement with observations from human autoimmune diseases.

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