# Low-Dose Exposure to Inorganic Mercury Accelerates Disease and Mortality in Acquired Murine Lupus

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**Inorganic mercury (iHg) is known to induce autoimmune disease in susceptible rodent strains. Additionally, in inbred strains of mice prone to autoimmune disease, iHg can accelerate and exacerbate disease manifestations. Despite these well-known links between iHg and autoimmunity in animal models, no association between iHg alone and autoimmune disease in humans has been documented. However, it is possible that low-level iHg exposure can interact with disease triggers to enhance disease expression or susceptibility. To address whether exposure to iHg can alter the course of subsequent acquired autoimmune disease, we used a murine model of acquired autoimmunity, lupus-like chronic graft-versus-host disease (GVHD), in which autoimmunity is induced using normal, nonautoimmune prone donor and $F_1$ recipient mice resistant to Hg-induced autoimmunity. Our results indicate that a 2-week exposure to low-dose iHg (20 or 200 μg/kg every other day) to donor and host mice ending 1 week before GVHD induction can significantly worsen parameters of disease severity, resulting in premature mortality. iHg pretreatment clearly worsened chronic lupus-like disease, rather than GVHD worsening iHg immunotoxicity. These results are consistent with the hypothesis that low-level, nontoxic iHg preexposure may interact with other risk factors, genetic or acquired, to promote subsequent autoimmune disease development.** *Key words:* **autoimmunity, glomerulonephritis, graft-versus-host disease, lupus, mercury, T cells.** *Environ Health Perspect* **111:1273–1277 (2003). doi:10.1289/ehp.6064 available via** *http://dx.doi.org/* **[Online 1 April 2003]**

Exposures to mercury compounds are widespread in the U.S. population and throughout the world (Mahaffey and Mergler 1998; National Research Council 2000). Although public health concerns about mercury exposures have generally focused on neurodevelopmental toxicity, mercury, particularly inorganic mercury (iHg), has been extensively studied in animal models for its immunotoxic properties, which include both autoimmunity and immunosuppression. The autoimmune effects of iHg in susceptible rodent strains include induction of specific autoantibodies, polyclonal activation of T and B cells, increased serum immunoglobulin G1 (IgG1) and IgE, cytokine dysregulation, and an immune complex glomerulonephritis (Bigazzi 1994; Griem and Gleichmann 1995; Mathieson 1992; Moszczynski 1997; Pollard and Hultman 1997).

However, despite considerable study, no associations have been found between mercury exposure and autoimmune disease in humans, even in highly exposed workers (Moszczynski 1997; Sweet and Zelikoff 2001; Vimercati et al. 2001). Experimental research in animal models of Hg-induced autoimmunity (HgIA) may suggest the reason for this. In mice, genotype influences not only disease susceptibility but also the specific autoantibodies produced. One of the genes involved in disease susceptibility is within the major histocompatibility complex (MHC) region. As a result, H-$2^s$ mice (SJL, B10.S, A.SW) are highly susceptible to HgIA induction to include production of anti-nucleolar and anti-fibrillarin antibodies, whereas mice from other MHC genotypes, for example, CBA (H-$2^k$), C57Bl/10 (H-$2^b$), and DBA/2 (H-$2^d$), are resistant to disease induction (Hultman et al. 1992, 1993, 1996; Hultman and Enestrom 1987, 1992; Pietsch et al. 1992; Warfvinge et al. 1995). Thus, the overt expression of HgIA requires interactions with predisposing factors such as genetic susceptibility; however, recent studies indicate that Hg can also exacerbate ongoing autoimmune disease. Studies in spontaneously autoimmune-prone strains of mice have reported greater autoimmune responses to iHg in (NZB × NZW) $F_1$ mice compared with nonautoimmune SJL or BALB/c mice (al-Balaghi et al. 1996) and also reported that iHg treatment greatly increased renal immune complex deposits in young NZB × NZW $F_1$ mice (Abedi-Valugerdi et al. 1997). Moreover, in the lupus-prone BXSB mouse, Pollard et al. (2001) found that iHg accelerated systemic autoimmunity, including cellular and humoral features of disease.

Taken together, the above studies indicate that iHg can interact with genetic factors to either directly induce autoimmunity or accelerate disease in autoimmune-prone animals. It is not clear whether iHg can interact with nongenetic factors to either increase susceptibility to autoimmune disease or accelerate disease course. In an animal model of acquired autoimmune disease, experimental autoimmune myocarditis, iHg pretreatment was reported to exacerbate disease, including cardiomyopathy and mortality, after infection with coxsackie B virus (Ilback et al. 1996; South et al. 2001); however, the doses used were relatively high and by themselves caused mortality (South et al. 2001).

The present study directly addresses whether iHg preexposure can alter the subsequent course of autoimmunity induced by nongenetic mechanisms. To this end, we used a murine model of acquired autoimmunity, the parent-into-$F_1$ model of chronic graft-versus-host disease (GVHD) in which a lupus-like disease is induced in otherwise normal mice that are genetically resistant to the induction of HgIA, for example, DBA/2 and B6D2$F_1$ mice (Via and Shearer 1988a). Moreover, we purposely used very low doses of mercury (20 or 200 μg/kg every other day for 15 days) compared with the range commonly used in studies of Hg immunotoxicity (500–2,000 μg/kg for as long as 3 months) in order to avoid potential confounding by lethality or nonimmunologic toxicity due to iHg. Our results indicate that a brief, low-level exposure to iHg before the induction of autoimmunity can significantly worsen the subsequent course of disease.

## Materials and Methods

***Mice.*** We purchased 6- to 8-week-old female B6D2$F_1$ ($BDF_1$) and DBA/2 mice from the Jackson Laboratory (Bar Harbor, ME). All studies were conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore.

***iHg ($HgCl_2$) administration.*** DBA/2 donor and $BDF_1$ host mice were randomly assigned to pretreatment groups, weighed, and

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administered iHg dissolved in water and then diluted to make doses of 20 or 200 μg/kg (iHg20 or iHg200) in a total administered volume of 0.1 mL/10 g animal. Controls received equivolume injections of NaCl solution. NaCl or iHg was administered by subcutaneous injection every other day for 15 days (or a total of eight doses) to both donors and hosts. Animals were monitored daily for weight loss, excessive urination, or other signs of iHg toxicity. Five days after the last injection of iHg or NaCl, mice were assigned to the following experimental groups: group A, normal $F_1$ mice; group B, sham (NaCl injection) + chronic GVHD (i.e., NaCl-treated donor and host); group C, iHg20 + GVHD (20 μg/kg iHg treatment of both donor and host before GVHD induction); and group D, iHg200 + GVHD (200 μg/kg iHg treatment of both donor and host before GVHD induction). No mice treated with iHg only were included in this preliminary study because extensive experience with these treatments in our laboratory and by others (Goering et al. 2000; Hultman et al. 1993; Silbergeld et al. 2000) has demonstrated that iHg, at these doses, does not induce nephropathy, weight loss, or mortality or affect other parameters measured in this study in these mouse strains.

***Induction of GVHD.*** Single-cell suspensions of splenocytes were prepared from DBA/2 females in phosphate-buffered saline, filtered through sterile nylon mesh screen, and diluted to a concentration of $10^8$ viable cells/mL as determined by trypan blue exclusion. $F_1$ mice received $80 \times 10^6$ parental cells by intravenous administration. This dose of parental donor cells is slightly above the threshold for consistent induction of chronic GVHD (~$60–70 \times 10^6$ DBA/2 donor cells) and has been reliably used by us to induce a mild lupus-like disease (Rus et al. 1995; Shustov et al. 2000; Via and Shearer 1988a). Control mice consisted of age- and sex-matched uninjected mice receiving no iHg pretreatment.

***Observational studies.*** A single cohort of five mice/group was monitored for long-term mortality and monthly proteinuria and serum anti–single-stranded DNA (ssDNA) antibody levels as described below. Animals were inspected at least every other day. Date of death was recorded, and dead animals were promptly removed from the cages. Mice were euthanized by $CO_2$ inhalation when they became moribund. Survival data were plotted by the Kaplan-Meier method and analyzed by the log-rank test.

***Urine protein measurement.*** Proteinuria was assessed semiquantitatively using urine dip sticks (Albustix; Bayer Diagnostics, Basingstoke, UK).

***Flow cytometry studies.*** Splenocytes were first incubated with anti-murine FcγR monoclonal antibody (mAb) 2.4G2 (Unkeless 1979) for 15–20 min and then stained with saturating concentration of fluorescein isothiocyanate-conjugated, phycoerythrin-conjugated, or biotin-conjugated monoclonal antibody. Fluorochrome conjugated anti-CD4, anti-CD8, anti-B220, and anti-$H2K^b$ were purchased from Pharmingen (San Diego, CA). Three-color flow cytometry was performed using a FACScan flow cytometer (Becton-Dickinson Immunocytometry Division, San Jose, CA). Lymphocytes were gated based on forward and side scatter, and analysis was performed on 10,000 gated cells. Donor $CD4^+$ and $CD8^+$ T cells were identified as cells staining positive for the respective T-cell marker and negatively for MHC class I of the non-donor parent.

***Serologic assays.*** Serum was tested by enzyme-linked immunosorbent assay for the presence of anti-ssDNA IgG antibodies as previously described (Via et al. 2001). Optical density was determined at 405 nm. Sera from MRL/lpr mice were assayed as a standard and arbitrary units calculated using a value of 1,000 units/mL for pooled undiluted MRL/lpr sera. Immunofluorescent antinuclear antibody (ANA) patterns were determined on Hep-2 cells using a commercial kit

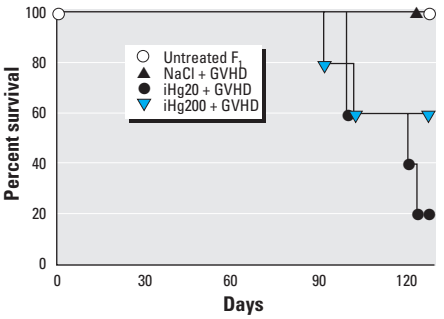


**Figure 1.** Pretreatment with iHg accelerates mortality in chronic GVHD mice ($n$ = 5 for each treatment group). See "Materials and Methods" for details.

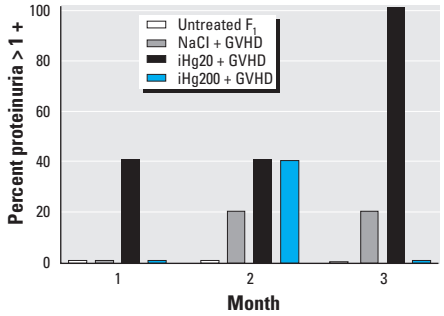


**Figure 2.** Pretreatment with iHg accelerates the appearance of proteinuria in chronic GVHD mice. Proteinuria was tested at the times indicated, as described in "Materials and Methods."

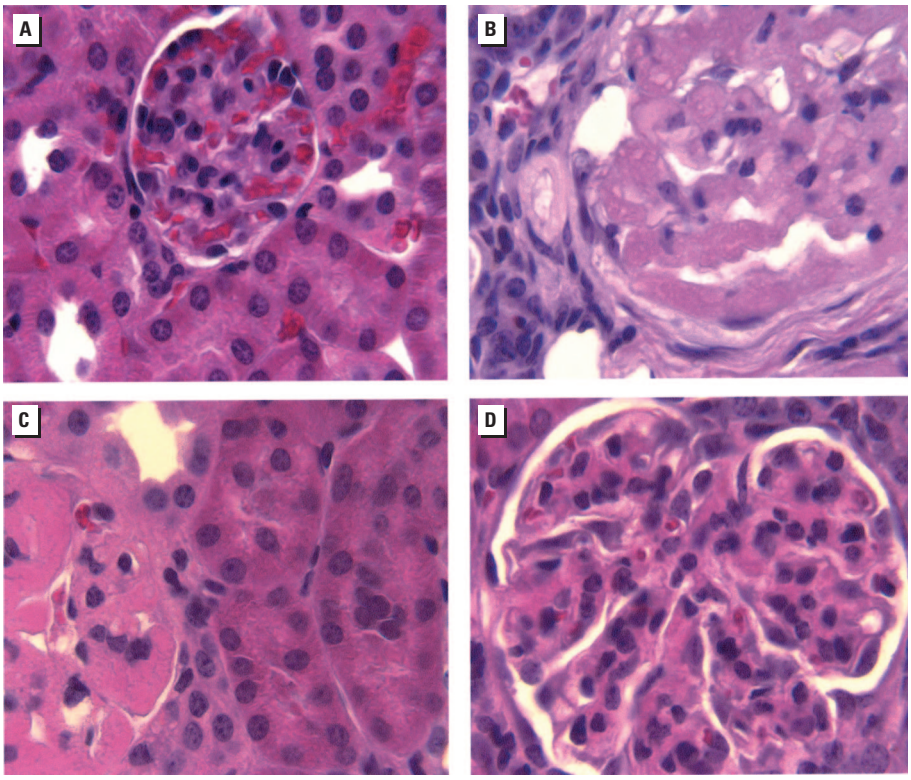


**Figure 3.** Pretreatment with iHg results in more severe glomerulonephritis in chronic GVHD mice. Mice were sacrificed 127 days after GVHD induction, and kidneys were stained as described in "Materials and Methods." Representative histopathologic sections are shown for (*A*) untreated $F_1$, (*B*) GVHD, (*C*) iHg20 + GVHD, and (*D*) iHg200 + GVHD. Magnification: 400×.

(The Binding Site, San Diego, CA) according to the manufacturer's instructions.

***Assessment of glomerulonephritis.*** For histopathology studies, renal tissue was fixed in 10% phosphate-buffered formalin, embedded in paraffin, and stained routinely with hematoxylin and eosin. All slides were scored blindly by a renal pathologist (J.P.). The following glomerular features were graded: mesangial hypercellularity, neutrophilic exudate, membrane thickness, crescents, and glomerular cell apoptosis. For this purpose 15 fields were counted for each mouse and averaged, and the average score ± SEM was calculated for each experimental group. In addition, a glomerular activity score was calculated based on measurement of glomerular cellularity (composed of mesangial cells plus neutrophilic exudates as well as crescents). Tubulointerstitial features (perivascular infiltrates and tubular epithelial cell injury) were graded semiquantitatively using the following scale: 0 = normal/negative; 1+ = mild; 2+ = moderate; 3+ = severe. A cumulative glomerular and tubular/interstitial severity index was calculated for each individual mouse based on scoring of 15 microscopic fields per mouse as described (Shustov et al. 2000).

***Statistical analysis.*** Data were examined for normality and equal variance (Kolmogorov-Smirnov). If satisfactory, groups were compared by a two-tailed Student's *t*-test; if not, they were compared by the Mann-Whitney rank sum test. For studies of scalar data (histopathology scores and proteinuria colorimetry), the nonparametric data were compared by the Mann-Whitney rank sum test.

## Results

***iHg pretreatment accelerates mortality in chronic GVHD mice.*** As shown in Figure 1, pretreatment with either low-dose or high-dose iHg was associated with premature mortality after GVHD induction. Deaths were not observed within the time frame of this experiment in either normal $F_1$ mice or sham-treated (NaCl, no iHg) chronic GVHD mice. Although only the low-iHg group (iHg20 + GVHD) had a statistically significant increase in mortality compared with normal $F_1$ or NaCl + GVHD controls ($p = 0.0128$), there was no statistical difference between the high- and low-dose iHg groups. It is likely that the increased mortality in the high-dose iHg group (iHg200 + GVHD) did not reach statistical significance due to the relatively small sample size and the decision to terminate the experiment at 4 months because of the morbidity in surviving Hg + GVHD animals at that point.

***iHg pretreatment accelerates lupus-like renal disease in chronic GVHD mice.*** Lupus-like renal disease in chronic GVHD mice is mediated by glomerular deposition of immune complexes, resulting in glomerulonephritis and proteinuria (Bruijn et al. 1989; Shustov et al. 2000; van Elven et al. 1981). As shown in Figure 2, iHg20 + GVHD mice exhibited elevated proteinuria scores (> 1) at an earlier time than did GVHD mice, and by 3 months 100% of iHg20 + GVHD mice exhibited abnormal proteinuria. Only a transient proteinuria was observed in the control mice. No proteinuria has been reported in earlier studies of iHg-treated DBA/2 or C57Bl/6 mice (Goering et al. 2000; Hultman et al. 1993; Silbergeld et al. 2000). These results suggest that lupus-like renal disease in GVHD mice is accelerated and exacerbated by prior iHg treatment.

To fully define the extent of renal disease, blinded glomerular scoring by light microscopic analysis of the kidneys from all surviving mice was performed by a renal pathologist at 12 weeks. Glomerulonephritis was observed in both NaCl + GVHD and iHg + GVHD mice as evidenced by glomerular enlargement, increased glomerular lobularity, mesangial hypercellularity, and membrane thickening, compared with control $F_1$ mice (Figure 3A–D). These changes are similar to previous reports of glomerulonephritis in chronic GVHD mice (Shustov et al. 2000); however, glomerular disease was significantly more severe in iHg200 + GVHD mice, compared with either untreated $F_1$ mice ($p < 0.0001$) or NaCl + GVHD mice ($p < 0.0001$) (mean glomerular scores: normal $F_1$ = 2 9.6 ± 0.7, $n = 5$; NaCl + GVHD = 33.3 ± 1.2, $n = 5$; iHg200 + GVHD = 53.62 ± 1.5, $n = 3$). The glomerular score of the single survivor from the iHg20 + GVHD group was 27.5.

Further analysis of the renal histopathologic features demonstrated that GVHD alone induces a tubular disease, which is not seen in control $F_1$ mice (mean tubular scores: normal $F_1$ = 0 vs. NaCl + GVHD = 6.2; $p = 0.008$).

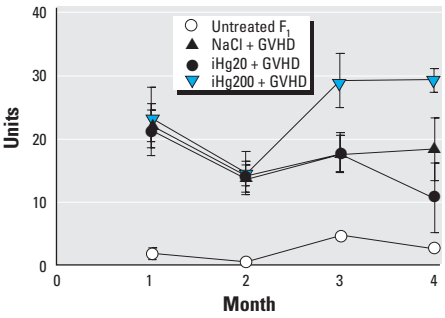


**Figure 4.** Effect of pretreatment with iHg on serum anti-ssDNA antibodies in chronic GVHD mice. Mice were bled at monthly intervals and anti-ssDNA antibodies were determined as described in "Materials and Methods." Results are shown as the mean ± SE for each group; $n = 5$ for all groups except the following: $n = 4$ for iHg20 + GVHD at 3 months and $n = 3$ for iHg20 + GVHD and iHg200 + GVHD at 4 months.

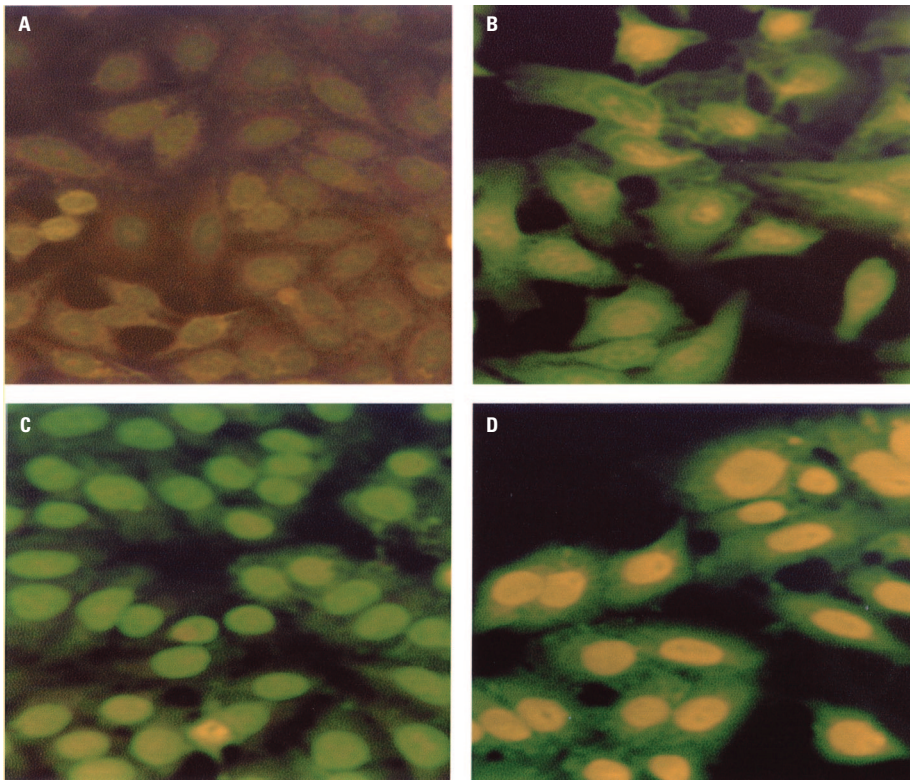


**Figure 5.** Pretreatment with iHg does not alter the pattern of ANA in chronic GVHD mice. Representative ANA patterns are shown for (*A*) untreated $F_1$, (*B*) NaCl + GVHD, (*C*) iHg20 + GVHD, and (*D*) iHg200 + GVHD. Mice were bled 4 months after GVHD induction, and the ANA pattern was determined as described in "Materials and Methods" at a serum dilution of 1:80. Magnification: 400×.

Exposure to iHg did not increase these histopathologic findings; that is, there was no significant difference in tubular damage in iHg200 + GVHD mice (mean tubular score = 5.3, not significant) compared with NaCl + GVHD mice. Taken together, these data support the conclusion that the doses of iHg used in these studies do not induce an Hg-like renal disease (tubular disease), but instead, iHg accelerates ongoing lupus-like renal disease in chronic GVHD mice

***iHg pretreatment affects anti-ssDNA antibody production in chronic GVHD.*** We also found interactions between iHg and GVHD in molecular markers of autoimmunity. Serum anti-ssDNA autoantibodies are an early and reliable marker of B-cell hyperactivity and autoimmunity in chronic GVHD and have been shown to be quantitatively related to the degree of T-cell–driven B-cell hyperactivity. That is, the serum levels of anti-ssDNA in the first 2–4 weeks after parental cell transfer are linearly related to the number of donor splenocytes injected up to a dose of $10^8$ cells (Via and Shearer 1988b).

As shown in Figure 4, levels of anti-ssDNA antibodies did not differ among the three GVHD groups (NaCl, iHg20, or iHg200) during the first 8 weeks of disease. Thus, initial B-cell activation and autoantibody production in response to equivalent numbers of donor T cells are not altered by iHg pretreatment. However, by month 3, the iHg200 + GVHD mice exhibited a significant increase in anti-ssDNA autoantibodies compared with either the NaCl + GVHD or iHg20 + GVHD mice. These results suggest that lupus-like disease undergoes an acceleration due to iHg exposure between months 2 and 3 in iHg200 + GVHD mice.

***iHg pretreatment does not induce autoantibodies characteristic of iHg-associated autoimmunity.*** The interactions between iHg and GVHD do not appear to relate to an exacerbation of HgIA. That is, iHg is well known to induce the production anti-nucleolar antibodies, including anti-fibrillarin, and a nucleolar ANA pattern (Mirtcheva et al. 1989; Monestier et al. 1994), which have been related to the presence of antibodies to fibrillarin (Pollard et al. 1997). In contrast, chronic lupus-like GVHD is associated with a homogeneous ANA pattern of fluorescence (Figure 5B). Importantly, as shown in Figure 5C and D, iHg + GVHD mice (both high and low dose) exhibit only homogeneous ANA patterns at 4 months after treatment. All mice in the cohort were tested, and a nucleolar ANA pattern was not observed. All GVHD mice (with or without iHg) exhibited homogeneous ANA patterns, consistent with the conclusion that iHg did not convert chronic GVHD to HgIA. In the representative examples shown in Figure 5A–D, at a serum dilution of 1:80, the intensity of ANA staining was greater for GVHD mice that received iHg pretreatment (~3+ for iHg200 + GVHD mice and 2+ for iHg20 + GVHD mice, vs. 1+ for control GVHD mice and negative for uninjected $F_1$ mice).

***The two iHg pretreatment doses may have different effects on autoimmunity.*** Flow cytometric analysis of surviving mice performed at 12 weeks indicates that typical features of chronic GVHD (increased total spleen cell numbers, host B-cell expansion, and donor $CD4^+$ T-cell engraftment in the absence of donor $CD8^+$ T-cell engraftment) were present both in iHg200 + GVHD mice and in NaCl + GVHD mice (Table 1). Total spleen cell counts and host B-cell numbers were greater in Hg200 + GVHD mice compared with NaCl + GVHD mice; however, the differences were statistically significant only for host B-cell numbers. These results are consistent with the data, discussed above, indicating that iHg at 200 μg/g exacerbates autoimmune manifestations and B-cell hyperactivity in GVHD mice. Of note, the one surviving iHg20 + GVHD mouse exhibited a marked increase in engraftment of donor $CD8^+$ T cells, which is not a characteristic of chronic GVHD but is highly characteristic of acute GVHD, suggesting that the two different iHg doses may affect GVHD differently. Further studies will be required to determine whether these findings are representative of iHg20 + GVHD mice.

## Discussion

It is well recognized that iHg exposure can induce an autoimmune renal disease in genetically susceptible murine strains (Hultman et al. 1992, 1993, 1996; Hultman and Enestrom 1987, 1992; Pietsch et al. 1992; Warfvinge et al. 1995) and that iHg exposure can exacerbate disease in animal models of spontaneously developing autoimmunity (Abedi-Valugerdi et al. 1997; al-Balaghi et al. 1996; Pollard et al. 1999, 2001). Our study is the first to use normal, genetically nonsusceptible mice (DBA/2 and $B6D2F_1$) to examine the role of low-dose iHg preexposure on the subsequent course of acquired autoimmune disease. In the parent-into-$F_1$ model of lupus-like chronic GVHD, the transfer of parental (donor) $CD4^+$ T cells into a nonirradiated $F_1$ mouse (host) results in donor T-cell recognition of host allogeneic MHC II molecules, polyclonal host B-cell activation, autoantibody production (e.g., anti-ssDNA antibodies), and immune complex formation and deposition in renal glomeruli. Mortality in this model is due to immune complex glomerulonephritis (Bruijn et al. 1989; Shustov et al. 2000; van Elven et al. 1981). Of note, the severity of disease is directly related to the number of donor cells transferred (Van Rappard-Van Der Veen et al. 1983; Via and Shearer 1988b). In the present study, we have used a relatively low donor cell inoculum ($8 \times 10^7$), which is just above the threshold of disease induction. As a result, autoimmune features such as serum anti-ssDNA levels and histologic evidence of glomerulonephritis were mild in GVHD mice in the absence of iHg pretreatment, and there was no mortality in this group at 4 months after GVHD induction. This treatment allowed us to observe inhibition or acceleration of disease by iHg. We found that iHg pretreatment significantly worsened lupus-like disease, as evidenced by earlier onset of proteinuria, more severe histologic features of lupus-like glomerulonephritis, and premature mortality. It is important to note that these results were induced using doses of iHg and a duration of treatment that are substantially lower than those used by most other studies of iHg immunotoxicity. Pollard et al. (2001) reported that low-dose iHg exposure could accelerate disease in autoimmune prone BXSB mice. In addition, in our experiments there was a 5-day period between the last dose of iHg and donor cell transfer, implying that iHg does not need to be present during the induction of autoimmune disease to enhance disease expression. These results, taken together, indicate that mercury has very potent interactive effects with autoimmunity at doses considerably lower than those required to induce autoimmunity in susceptible mouse strains that do not develop disease in the absence of iHg.

Several lines of evidence indicate that iHg pretreatment in these experiments did not directly result in Hg-induced autoimmune disease but rather exacerbated the lupus-like chronic manifestations of GVHD. First, the mouse strains used ($B6D2F_1$, DBA/2) are not known to be susceptible to iHg-induced immunotoxicity, and second, the doses of iHg

**Table 1.** Pretreatment with high-dose iHg increases host B-cell numbers but does not alter splenic donor T-cell chimerism in chronic GVHD mice.

| Group | Spleen cells | Donor T cells | | Host B cells |
|---|---|---|---|---|
| | | $CD4^+$ | $CD8^+$ | |
| Untreated $F_1$ ($n = 5$) | 86 ± 1.6 | ND | ND | 53.0 ± 1.3 |
| NaCl + GVHD ($n = 5$) | 118 ± 8.8 | 1.47 ± 0.3 | 0.25 ± 0.5 | 75.5 ± 6.7 |
| iHg20 + GVHD ($n = 1$) | 51.0 | 0.55 | 2.4 | 22.5 |
| iHg200 + GVHD ($n = 3$) | 140 ± 6.7 | 1.48 ± 0.4 | 0.47 ± 0.2 | 97.5 ± 4.8* |

ND, not detectable over background (< $0.2 \times 10^6$ cells). Splenocytes were analyzed by flow cytometry at 127 days post-parental cell transfer as described in "Materials and Methods." Values are shown as group mean ± SE × $10^{-6}$.
*$p < 0.05$, iHg200 + GVHD vs. NaCl + GVHD.

used are well below exposures associated with overt signs of autoimmunity, such as nephropathy, renal histopathology, or death (Hultman et al. 1993; Hultman and Hansson-Georgiadis 1999; Hultman and Nielsen 2001). Neither the renal histology nor the immunologic measurements were consistent with evidence of iHg-related pathology. Moreover, HgIA is associated with a nucleolar ANA pattern (Monestier et al. 1994; Pollard et al. 1997). No iHg-treated GVHD mice exhibited a nucleolar ANA pattern. Instead, all mice showed a homogeneous ANA pattern characteristic of GVHD, and iHg treatment increased the intensity of ANA fluorescence compared with GVHD mice, implying disease exacerbation.

These findings are consistent with the findings by Pollard et al. (2001) in spontaneous models of lupus, in which Hg exposure exacerbated underlying autoimmune disease, rather than the genotype exacerbating Hg toxicity. Our results extend these observations by demonstrating that iHg can also accelerate and exacerbate acquired autoimmune disease even when exposure precedes disease. Studies are in progress to determine the maximal time that iHg exposure can precede disease induction and still exert a synergistic effect on disease expression.

Of note, the effects of iHg pretreatment on GVHD appeared to vary with the doses used, although these observations are limited by the reduction in survivors available for analysis. Both doses of iHg (20 or 200 μg/kg × 8 doses) accelerated mortality; however, in iHg200 + GVHD mice, other measurements indicated an acceleration or exacerbation of disease, for example, increased anti-ssDNA titers at 3 months and increased intensity of ANA staining. In contrast, in the one surviving iHg20 + GVHD mouse, the marked increase in engraftment of donor $CD8^+$ T cells suggests that this dose of iHg may have converted chronic GVHD to acute GVHD. We have previously observed that in the GVHD model used here, chronic GVHD can be converted to acute GVHD by the administration of agents that promote $T_H1$ cytokine responses, for example, administration of recombinant interleukin 12 (Via et al. 1994), or by highly selective costimulatory blockade in which the down-regulatory signal delivered by CTLA4 through its preferential ligand CD80 is inhibited (Lang et al. 2002). Because both CD80 and CD86 appear to be required for HgIA (Bagenstose et al. 2002), we are currently investigating whether low-dose iHg preexposure promotes $T_H1$ cytokine production and/or interferes with CTLA4-CD80 expression or ligand binding.

In conclusion, it is becoming increasingly accepted that the development of autoimmune disease in humans involves a combination of factors, which include the appropriate genetic predisposition and encounter(s) with acquired risk factors in the environment, including infections and immunotoxic agents. Our results support the hypothesis that low-level environmental exposure to Hg is one potential factor in the development of autoimmune disease. Specifically, low-level iHg exposure likely does not induce disease by itself; however, it may lower the threshold for disease development in susceptible individuals who later encounter the appropriate infectious or toxic triggers of disease.

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