Cytokine Regulation of a Rodent Model of Mercuric Chloride-Induced Autoimmunity

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Experimental models of chemically induced autoimmunity have contributed to our understanding of the development of autoimmune diseases in humans. Heavy metals such as mercury induce a dramatic activation of the immune system and autoantibody production in genetically susceptible rats and mice. This autoimmune syndrome is dependent on T cells, which are important for B-cell activation and cytokine secretion. Several studies have focused on the roles of T-helper (Th)1 and Th2 cells and their respective cytokines in the pathogenesis of mercury-induced disease. This article reviews recent studies that have examined the patterns of cytokine gene expression and where investigators have manipulated the Th1 and Th2 responses that occur during mercury-induced autoimmunity. Finally, we will discuss some biochemical/molecular mechanisms by which heavy metals may induce cytokine gene expression. *Key words*: autoimmunity, cytokines, mercury, rodent models, T cells. — *Environ Health Perspect* 107(suppl 5):807–810 (1999).
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In most autoimmune patients, the underlying causes of disease are unknown and the initial steps leading to loss of tolerance, activation of autoreactive T cells, and production of autoantibodies are poorly understood. Additionally, many endogenous and exogenous factors contribute to the development of autoimmune diseases, including sex hormones, genetic predisposition, infectious agents, and exposure to occupational or environmental pollutants (*1,2*). Although few animal models of chemically induced autoimmunity have been extensively studied, mercuric chloride ($HgCl_2$)-induced autoimmunity in rodents presents a well-established but poorly understood phenomenon.

The fact that mercury induces autoimmunity in rats and mice is especially relevant, since mercury is also an abundant environmental pollutant (*3*). Moreover, mercury has been used for thousands of years in mining and manufacturing and can still be found today in dental amalgam, skin creams, and fluorescent light tubes (*3*). Finally, the specificity of the autoantibody response elicited in mercury-treated mice is virtually identical to that of autoantibodies present in a subset of patients with scleroderma (or systemic sclerosis), the human autoimmune disease most frequently associated with exposure to environmental agents (*4*).

The most fascinating and compelling reason for studying this model is that it remains unknown how such a simple molecule can rapidly induce a loss of tolerance to specific self-antigens and a dramatic, self-limiting activation of the immune system. Recent investigations have examined the roles of cytokine-producing T cells in the induction and regulation of this syndrome and have provided a better understanding of mercury-induced autoimmunity.

Description of the Model

In genetically susceptible mice and rats, subtoxic doses of $HgCl_2$ (1.0 mg/kg weight) induce an autoimmune syndrome characterized by the production of specific autoantibodies, polyclonal activation of B and T cells, serum increases in IgG1 and IgE, and glomerulonephritis with renal immune complex deposits (*5*). These manifestations appear 7–10 days after the beginning of $HgCl_2$ injections and a single injection may be sufficient to induce the syndrome. Although most investigators administer $HgCl_2$ as a subcutaneous injection, the route of $HgCl_2$ exposure is not critical and $HgCl_2$ can be given intraperitoneally, orally, in drinking water, or aerosolized (*5*). Additionally, methyl mercury or pharmaceutical ointments and solutions containing organic mercury are equally effective in inducing immune-type glomerulonephritis in rats, even when these products are applied on wounds or on normal skin (*6*). In susceptible strains, the effects of $HgCl_2$ are dose dependent, with increases in serum IgE positively correlated with dosage of $HgCl_2$ in rats (*3*) and increases in serum IgG antinucleolar antibodies (ANoA) and IgG immune complexes correlated with $HgCl_2$ dosage in mice (*7*).

Rats treated with mercury develop autoantibodies of various specificities, including antiglomular basement membrane, anti–double-stranded DNA, antithyroglobulin, antiphospholipid, and antilaminin P1 (*8,9*). Whether these autoantibodies are simply a byproduct of the polyclonal activation seen in these animals or represent an antigen-specific response remains to be elucidated. In mice, however, mercury elicits a focused autoantibody response characterized by antibodies that target specific nucleolar antigens. Several studies have revealed that many of these ANoA are directed against fibrillarin, a 34-kDa basic protein that is a constituent of several nucleolar ribonucleoprotein complexes involved in preribosomal RNA processing (*10–12*).

Mercury-induced disease is self-limiting and most of the manifestations resolve spontaneously after 4–5 weeks during a regulation phase, even if $HgCl_2$ injections are continued. In rats this regulation phase is followed by a resistance phase, where rats are now resistant to further $HgCl_2$ injections (*5*). The resistance phase in the rat is mediated by $CD8^+$ T cells and IL-2–producing $CD4^+$ T cells (*5,13,14*). It is unclear whether mice undergo a resistance phase (*15*).

Major histocompatibility complex (MHC) class II genes play a major role in determining susceptibility to mercury-induced disease. Inbred Brown Norway (BN) rats of the $RT\text{-}1^n$ haplotype are susceptible to development of mercury-induced autoimmunity, whereas inbred Lewis (LEW) rats of the $RT\text{-}1^l$ haplotype are resistant to disease. In mice, expression of $I\text{-}A^s$ alleles confers a susceptible phenotype and expression of $I\text{-}A^b$ or $I\text{-}A^d$ confers resistance (*16*). Additionally, non-MHC genes may also contribute in determining the nature of glomerulopathy in rats (*17*), and the magnitude, specificity, or persistence of the ANoA response in mice (*18*). Although murine mercury-induced disease has been most often studied in $H\text{-}2^s$ mice such as A.SW, SJL, and B10.S, other strains develop ANoA, including A.CA ($H\text{-}2^f$), DBA/1 ($H\text{-}2^q$), and P/J ($H\text{-}2^p$)(*19*).

Th1/Th2 Cells

T cells participate in all phases of mercury-induced autoimmunity (*5*). Following activation, CD4+ T cells can become polarized to become either T-helper (Th)1 or Th2-type cells. This polarization is dependent on a variety of factors, including antigen dose (*20*), costimulatory molecules (*21*), type of antigen-presenting cell (*22*), and perhaps most importantly, the presence of critical cytokines (*23*). Interleukin (IL)-12, IL-18,

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and interferon (IFN)-γ promote the appearance of Th1 cells (*24–26*), which in turn secrete IL-2, IFN-γ, and mediate cellular immunity (*27*). In contrast, IL-4 biases T cells toward a Th2 phenotype (*23*). These Th2 cells can secrete IL-4, IL-5, IL-6, IL-10, and IL-13 and participate in humoral, allergic, and helminthic immunity (*28*).

Cytokine Gene Expression in the Model

Although $HgCl_2$ induces a profound activation of the immune system marked by changes in the production of various cytokines in susceptible animals, a clear and comprehensive pattern of cytokine gene expression during the syndrome has yet to be determined. Several studies have used various approaches including semiquantitative reverse transriptase polymerase chain reaction, Northern blot analysis, and intracellular staining techniques to obtain a limited picture of cytokine expression in this model. Among these, investigators have reported increases of IL-4 and IFN-γ mRNA in splenocytes of mercury-treated BN rats (*29*) as well as slight increases in IL-12 mRNA levels in spleen and lymph nodes from these animals (*30*). In these studies, $HgCl_2$ failed to induce increases of cytokines in resistant LEW rats (*29,30*). mRNA analysis revealed higher baseline levels of IFN-γ (*29*) and IL-12 (*30*) in resistant LEW rats as compared to those of BN rats, which may possibly account for this strain's resistance. Further analysis has demonstrated that treatment of BN rats with $HgCl_2$ downregulates the *ex vivo* generation of IFN-γ–producing cells in concanavalin A (ConA)-stimulated splenocytes, and that this downregulation may be mediated by nitric oxide (*31*).

Additionally, several studies in the rat have indicated that $HgCl_2$ can directly trigger naive cells to produce cytokines *in vitro*. Unfractionated splenocytes and purified T cells from BN rats express high levels of IL-4 mRNA after exposure to $HgCl_2$ *in vitro* (*32*). Similar to *in vivo* findings, mercury induces IFN-γ expression in cells of both LEW and BN rats but does not induce increases in IL-4 mRNA in cells from LEW rats (*32*). Mast cells of BN rats also respond to *in vitro* mercury exposure with induction of IL-4 mRNA and enhanced release of serotonin in response to IgE cross-linking agents (*33*). In contrast, mast cells from resistant LEW rats are less sensitive to mediator release and do not express IL-4 mRNA in response to exposure to $HgCl_2$ (*33*). Thus, cytokine production in response to $HgCl_2$ in rats differs according to genotype, and resistance may be due in part to higher baseline levels of Th1 cytokines or a failure to upregulate Th2 cytokines.

Considerably less is known about the pattern of cytokine gene expression that occurs in mercury-treated mice. Earlier studies demonstrated that B10.S mice (H-2^s) respond to $HgCl_2$ with a massive proliferation and activation of their B cells, increased IL-4 mRNA levels, and class switching to IgE, IgG1, and IgG2a (*34*). Additionally, mercury induces dramatic IL-4–dependent increases in B-cell major histocompatability complex II expression in susceptible H-2^s mice and moderate increases in resistant H-2^d mice (34). Recently, Johansson and colleagues (*35*) examined cytokine-producing cells in lymph nodes of susceptible and resistant mouse strains receiving $HgCl_2$. Susceptible A.SW mice (H-2^s) respond to $HgCl_2$ with an early activation of T cells, indicated by increases in IL-2–producing cells and upregulation of the CD25, CD122, and CD71 activation markers. This activation was followed by increased numbers of $CD4^+$ T cells. A.SW mice also show modest increases in tumor necrosis factor-α, IFN-γ, and IL-4–producing cells at 8–10 days after $HgCl_2$ injections. In contrast, genetically resistant A.TL mice (H-2^{t1}) have minimal increases in T cells and no increases in cytokine-producing cells (*35*).

Mercury can also activate murine cells *in vitro*. In one study by Hu and colleagues, *in vitro* exposure to $HgCl_2$ induced high proliferative responses in splenocytes from susceptible SJL mice and low proliferative responses in splenocytes of resistant C57Bl/6 mice (H-2^b) (*36*). Additionally, the continuous presence of $HgCl_2$ *in vitro* induced IL-2 and IFN-γ production, but no IL-4 production, from cells of both H-2^s and H-2^b mice. In contrast, pretreatment of cells with mercury followed by washing resulted in IL-4 production in cells of susceptible and resistant mice (*36*).

Cytokine Manipulations of the Model

Several studies have employed various cytokine manipulations or interventions to further elucidate the mechanisms of disease (Table 1). These observations indicate a greater complexity to the disease than was originally ascribed and suggest independent regulation of the various manifestations.

Early studies, noting the mercury-induced increases in IgG1 and IgE (*37,38*), suggested a role for Th2-type T cells in the pathogenesis of disease, as IL-4 produced by Th2 cells promotes class switching to these isotypes (*2,5*). Ochel and colleagues (*39*) addressed the role for this cytokine in mercury-induced autoimmunity by treating susceptible A.SW mice (H-2^s) with an anti–IL-4 monoclonal antibody (mAb) prior to $HgCl_2$ injections. Although the mercury-induced increases in IgG1 ANoA and total IgE were partially or completely abrogated, respectively, levels of IgG2a, IgG2b, and IgG3 class ANoA were enhanced (*39*). These results suggested that IL-4 was required for class switching to the IgG1 and IgE isotypes in this disease but was not essential for the loss of tolerance to nucleolar antigens.

Recently, several investigators used different approaches to block the Th2 component seen in this syndrome. IFN-γ secreted by Th1 cells is important in regulating cellular immune responses and downregulating Th2 activities (*28*). Further, $HgCl_2$ induces IFN-γ–producing splenocytes in mercury-resistant LEW rats (*31*). In one study by Doth and colleagues, the effect of recombinant interferon (rIFN)-γ pretreatment prior to $HgCl_2$ injections was assessed in susceptible B10.S mice (H-2^s). In resistant B10.D2 mice (H-2^d), $HgCl_2$ induces an IFN-γ–dependent suppression of antibody formation to sheep red blood cells (*40*). To investigate whether this immunosuppression was responsible for resistance to $HgCl_2$, rIFN-γ was administered to B10.S mice prior to $HgCl_2$ treatment. This pretreatment led to reductions in serum IgE levels and anti-SRBC antibody levels but failed to prevent ANoA production and immune glomerulonephritis in B10.S mice (*40*). Conversely, mAb neutralization of IFN-γ in resistant B10.D2 mice alleviated the mercury-induced immunosuppression but could not convert the mice to a mercury-susceptible phenotype (*40*).

Table 1. Effect of various cytokine manipulation strategies upon the manifestations of mercury-induced autoimmunity.

Treatment	Antinucleolar antibodies	Serum IgG1	Serum IgG2a	Serum IgE	Renal IgG deposits	Reference
Anti–IL-4	IgG1 decrease IgG2a and IgG2b increase	Decreased	Unchanged	Decreased	Not tested	(*39*)
IL-4 gene deletion	IgG1 decrease IgG2a and IgG2b unchanged	Decreased	Unchanged	Decreased	Unchanged	(*47,48*)
IFN-γ	Unchanged	Not tested	Not tested	Decreased	Unchanged	(*40*)
IL-12	Decreased (all subclasses)	Decreased	Increased	Unchanged	Unchanged	(*45*)
IFN-γ gene deletion	Absent	Unchanged	Decreased	Not tested	Absent	(*47*)

Abbreviations: IFN-γ, interferon-γ; IL, interleukin.

Using an alternate approach, we have tried to modulate the response to $HgCl_2$ observed in A.SW mice to that of a Th1 phenotype by administration of IL-12 prior to treatment with mercury. IL-12 is critical for the development of the Th1 phenotype and for the initiation of inflammatory immune responses (*27*). IL-12 also contributes to certain Th1-mediated autoimmune disorders (*41,42*). Further, in mice susceptible to *Leishmania major* infection, IL-12 administration can bias toward a protective Th1 response (*43,44*). Thus, IL-12 can serve as a potentially important immunoregulator of disease outcome. In A.SW mice, IL-12 treatment dramatically reduced ANoA titers of all classes and partially inhibited the $HgCl_2$-induced increase in IgG1 but had no effect on serum levels of IgE and renal Ig deposits (*45*). This last finding was somewhat unexpected, as induction of IFN-γ by IL-12 can downregulate class switching to both IgG1 and IgE. Nevertheless, IL-12 can enhance IgE synthesis under certain circumstances (*46*); its ability to influence Th2 cytokine-dependent IgE production varies according to the immunologic setting. We also observed that IL-12 further potentiated the $HgCl_2$ induction of IL-4 (*45*). This may be related to our observation that IL-12 had no effect on serum IgE levels. Specifically, treatment of mice with both $HgCl_2$ and IL-12 may have led to the development of Th cells with a mixed Th0 phenotype. The balance of cytokines produced by these cells may thus explain differential effects of IL-12 on isotype switching to IgG1 and IgE (*45*).

Although these studies are important in their attempt to understand the complex regulatory nature of this syndrome, their findings are somewhat difficult to reconcile with a simple Th1/Th2 imbalance. Moreover, a somewhat artificial environment may be introduced in that administration of recombinant cytokines can result in kinetics and distribution that differ greatly from those of the proteins secreted *in vivo*. Similarly, the use of neutralizing antibodies can result in incomplete or short-term inactivation. Recently, we and others have utilized a targeted gene-disruption or knockout approach to more accurately assess the roles of cytokine genes in this model. These studies have examined mercury-induced disease in IL-4–deficient H-2^s mice and confirmed findings made in IL-4 mAb-treated A.SW mice (*47,48*). Although mercury-treated IL-4–deficient mice lack the characteristic increases in serum IgG1 ANoA and total IgG1 and IgE, levels of ANoA of other IgG subclasses are comparable to mercury-treated wild-type H-2^s mice (*47,48*). Using IFN-γ–deficient H-2^s mice, Kono et al. have observed that mercury treatment does not elicit ANoA or tissue lesions in these animals (*47*). If confirmed, this novel finding would suggest that IFN-γ plays a hitherto unidentified role in the loss of tolerance to nucleolar antigens that takes place in this model.

How Does Mercuric Chloride Induce Cytokine Expression?

As indicated before, it is unclear how mercury triggers an activation of T cells and an upregulation of cytokine production in genetically susceptible animals. mercury and other heavy metals can have mitogenlike effects on cells and are strong activators of the immune system (*32,49,50*). However, most of the available evidence, including the presence of self-reactive and metal-specific cells (*51–53*) as well as the specificity of the autoantibody response (*12*), suggests that mercury induces a specific antigen-driven response .

Druet and colleagues recently examined $HgCl_2$-induced cytokine expression in autoreactive T-cell lines derived from mercury-treated LEW rats. These cells can transfer resistance to naive (BN × LEW)F_1 hybrids and produce IL-2, IFN-γ, and transforming growth factor-β (*14*). In contrast, T-cell lines derived from mercury- and gold-treated BN rats passively transfer susceptibility to mercury-induced disease in CD8-depleted BN rats and produce IL-4 *in vitro* when cultured with $HgCl_2$ (*51*). This induction of IL-4 is mediated by protein kinase C-dependent Ca^{2+} influxes through L-type channels (*54*). In these experiments, $HgCl_2$ was able to activate protein kinase C directly and upregulate IL-4 mRNA expression without *de novo* protein synthesis.

Several recent lines of evidence suggest a role for intracellular thiol levels in determining Th1/Th2 cytokine expression. Sulfhydryl compounds have many important biologic functions, including maintenance of intracellular redox balances (*55*). GSH, the most abundant intracellular thiol, is required for ConA-mediated induction of IFN-γ–producing cells *in vitro* (*56*). This induction is suppressed in BN rats, but not LEW rats, after exposure to $HgCl_2$ *in vivo* (*56*). Furthermore, *N*-acetyl-L-cysteine, a GSH precursor, inhibits IL-4 and IgE production by human T cells *in vitro* (*57*), whereas *in vivo* depletion of GSH in mice results in decreased IFN-γ production and increased IL-4 production *in vitro* (*58*). Taken together, these studies indicate that high thiol levels favor development of Th1 responses, whereas low levels favor Th2 responses. A recent study has reported strain-dependent differences in thiol levels between LEW and BN rats (59). In particular, $CD4^+$ T cells from LEW rats have higher levels of total thiols than those of BN rats (*59*). Because mercury has a strong affinity for thiol groups, it is possible that mercury may bind intracellular thiols, resulting in decreased thiol levels and a shift toward a Th2 profile in susceptible animals. Supporting this hypothesis, administration of thiol compounds in mice prevents development of mercury-induced IgG1 ANoA and IgG1 immune complexes and reduces serum IgE levels in SJL mice (*60*). Strain differences in intracellular thiol levels may contribute to the differential responses elicited by $HgCl_2$ in either resistant or susceptible animals, thus explaining predominantly Th1 or Th2 patterns of cytokine expression.

In summary, $HgCl_2$-induced autoimmunity in rodents is a useful model of chemically related autoimmunity in humans. Much is still unknown concerning the T-cell–dependent induction and resolution of this disease. The conventional view is that $HgCl_2$ triggers a Th2 immune response in susceptible strains, whereas resistant strains develop a Th1 response after mercury treatment. Thus, in this scenario Th2 cells mediate induction of the syndrome, and Th1 cells are important in the resolution of disease. Early studies demonstrating increases in both Th2 cytokine mRNA and Th2 cytokine-regulated immunoglobulins in susceptible animals supported this hypothesis. However, recent examination of the roles of these cytokines has demonstrated that IL-4 production is not required for $HgCl_2$-induced disease, and the question remains whether Th1 cells participate in both the induction and regulation of this syndrome. Th1 and Th2 cytokines indeed play important roles in the B-cell activation seen in this model, although it is likely that loss of tolerance to nucleolar antigens arises by mechanisms independent of conventional Th1/Th2 regulation. Thus, interventions such as administration of rIFN-γ, rIL-12, or anti–IL-4 mAb affect isotype switching and total levels of serum immunoglobulins but cannot prevent the loss of tolerance seen in this model.

Future studies on $HgCl_2$-induced autoimmunity are most likely to focus on dissecting the biochemical and molecular pathways by which Hg interacts with self-antigen to yield an autoimmune response. Linked to these studies are questions such as: How does a metal render a self component immunogenic? and Why are nucleolar antigens (fibrillarin) specifically targeted? Recent works by several investigators have begun to address these issues (*1,61,62*). Further studies are also needed to clearly elucidate the pattern of cytokine expression in this syndrome and to determine their exact requirement for disease development. The knowledge gained from studying $HgCl_2$-induced autoimmunity in rodents will contribute to our understanding of the relationships between genetics, environment, and human autoimmunity.

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