

# Alterations of Th1/Th2 Reactivity by Heavy Metals

## Possible Consequences Include Induction of Autoimmune Diseases

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**ABSTRACT:** Heavy metal pollution still represents a primary concern regarding human health. Recently, it became evident that the contribution of heavy metals extends far beyond their accepted role in allergic diseases, and that they may play a more extensive role in a variety of other diseases. Several lines of evidence indicate that heavy metals have a key role in the induction or exacerbation of several autoimmune diseases (AD). Moreover, the association between exposure to heavy metals and the signs of autoimmunity are supported by some studies. The mechanisms by which heavy metals induce the development of AD are not yet fully understood. Our objective here is to highlight the association of exposure to some heavy metals and AD. In addition, we present recent results showing the possible alterations in Th1/Th2 reactivity by some heavy metals, which may constitute the trigger for the incidence of autoimmunity in susceptible individuals.

**KEYWORDS:** autoimmune diseases; cytokines; heavy metals; infection; mice; Th1/Th2 immune response

## INTRODUCTION

The development of autoimmune diseases (AD), as in most cases of deviation from a healthy normal immune state, is likely initiated when there is a transient loss of immune homeostasis.<sup>1</sup> AD are defined to be caused by incomplete deletion and inappropriate activation of self-reactive T and/or B cells.

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T cells, the self-reactive ones, induce autoimmunity either directly by interacting with self-target cells and organs, as seen in autoimmune diabetes, or indirectly by activating self-reactive B cells.<sup>2</sup> Then, the autoreactive B cells, in turn, produce autoantibodies (AA) that can contribute to AD, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), multiple sclerosis, or myasthenia gravis.

It is becoming increasingly accepted that the development of AD involve a combination of factors including appropriate genetic predisposition and encounter with acquired risk factors in the environment, such as infections and immunotoxic agents. In the present article, we review the recent advances in our understanding of the relation between exposure to some heavy metals and the possible induction/exacerbation of AD through alterations of the Th1–Th2 balance (Th, helper T cells).

### ASSOCIATION BETWEEN HEAVY METALS AND AUTOIMMUNE DISEASES

The association of exposure to heavy metals with AD has initiated an intense debate in the last decade. Recent immunologic findings confirm that most origins of idiopathic AD can be traced to contamination of the biosphere with xenobiotic compounds, among which some heavy metals have been strongly implicated.<sup>3</sup> Indeed, there is a growing interest in the relationship between exposure to heavy metals and the allergic and AD, on the one hand, and with the increase in the susceptibility to infectious diseases, on the other hand. Because of the ubiquity of mercury (Hg) and the development of a rodent model of Hg-induced experimental autoimmunity, the biological effects of Hg have been studied the most.<sup>1,4–6</sup>

Exposure to some heavy metals, such as Hg is known to induce AD in susceptible animals, and worsen the case of ongoing autoimmune manifestations.<sup>6–8</sup> Following exposure to subtoxic doses of inorganic mercury (iHg), susceptible mouse strains rapidly develop signs of systemic and organ-specific pathology, including induction of highly specific antibodies to nucleolar antigens (ANA) and autoreactive T cells.<sup>6,9–13</sup> In addition, these animals display a general activation of the immune system represented in polyclonal activation of T and B cells, cytokine dysregulation, and formation of immunoglobulin (Ig) deposits. For instance, in the lupus-prone BXSB mouse, Pollard *et al.*<sup>7</sup> found that iHg accelerated systemic autoimmunity, including cellular and humoral features of the disease. More recently, Tchounwou *et al.*<sup>3</sup> found that low levels of Hg exposure (<40 µg/kg body weight) may be unsafe in predisposed individuals to develop SLE. Similarly, Via *et al.*<sup>14</sup> found that short-term exposure to low-level iHg (20 or 200 µg/kg every other day for 15 days) before the induction of autoimmunity can significantly worsen the subsequent course of lupus-like disease, evidenced by earlier onset of proteinuria,

more severe histologic features of lupus-like glomerulonephritis, and premature mortality. Other heavy metals, such as silver or gold (Au) compounds, are capable of eliciting a response comparable to that induced by Hg administration,<sup>15,16</sup> but the produced antibody levels are not as much as they are in case of Hg.<sup>15,17,18</sup>

In humans, however, no associations have been found between Hg exposure and any clinically defined AD, even in highly exposed workers.<sup>6,19</sup> To the best of our knowledge, few studies have indicated signs of autoimmunity in humans exposed to heavy metals, indicating for example, presence of circulating antilaminin antibodies in cadmium (Cd)-<sup>20</sup> or Hg-exposed workers,<sup>21</sup> or an association between increased urinary Hg levels and serum antifibrillar AA.<sup>22</sup> In addition, the metal was also implicated in the induction of contact sensitivity occurring together with ANA,<sup>23</sup> and as reviewed by Ene-strom and Hultman,<sup>24</sup> a case of systemic AD has been described by Röger *et al.*<sup>25</sup> in an occupationally Hg-exposed worker. The authors assigned the case as an analogue to animal model of Hg-induced autoimmune reactions and recommended that exposure to Hg and other chemicals should be considered when taking the history of patients with systemic AD. These findings suggest common pathogenic mechanisms that favor development of AD, and a direct relationship between heavy metal pollution and the incidence or exacerbation of autoimmune disorders. Because of the similarities between the various animal models and the result of human exposure to heavy metals, there were many attempts to explore the mechanisms behind heavy-metal-induced autoimmunity.<sup>4,24</sup>

## WHICH MECHANISMS COULD BE INVOLVED IN THE DEVELOPMENT OF AUTOIMMUNE DISORDERS?

Countless reports have addressed the effects of heavy metal exposure on the immune system in humans as well as in animals. Heavy metals, such as lead, Cd, and Hg are known of their immunomodulatory potency especially with respect to lymphoproliferation and Th1–Th2 balance.<sup>1,3,19,26–29</sup> Based on their produced cytokine and effector functions, naïve Th0 have been shown to differentiate into at least two subsets, Th1 and Th2, both in mice<sup>30,31</sup> as well as in humans.<sup>32</sup> These cells secrete different but overlapping sets of cytokines. Whereas Th1 cells produce interleukin (IL)-2, interferon (IFN)- $\gamma$ , tumor necrosis factor (TNF)- $\beta$ , and low levels of IL-10, Th2 cells produce IL-4, IL-5, IL-9, IL-10, IL-13,<sup>30</sup> and IL-6.<sup>33</sup> Differentiation of Th0 toward Th1 or Th2 is believed to be a consequence of several cellular influences, such as the cytokine milieu. Th0 cells differentiate into Th1 cells when stimulated in the presence of IL-12 and into Th2 cells when stimulated in the presence of IL-4.<sup>34</sup> Moreover, there is cross-regulation between Th1 and Th2 in a variety of ways. Secretion of IL-4 by Th2 cells blocks differentiation of Th0 into Th1

cells. Production of IL-4, IL-13, and IL-10 by Th2 cells may suppress Th1 cells. Conversely, Th1 cells secrete IFN- $\gamma$ , which inhibits the proliferation of Th2 cells.<sup>34,35</sup> The dominance of one of these subsets results in either a predominantly cellular (Th1-mediated) or an antibody (Th2-mediated) response.<sup>3</sup> The importance of Th1/Th2 cell subsets and the association with AD focuses attention on the factors involved in determining whether an immune response will be principally of the Th1 or Th2 type.<sup>36</sup> Therefore, altering the Th1/Th2 balance may lead to the onset and/or exacerbation of AD<sup>1,36,37</sup>; however, it also may assist in the discovery of therapeutic treatments.<sup>38–40</sup> Because they are immunosuppressive or immunostimulative, heavy metals, such as Hg<sup>13,41–43</sup> and Cd,<sup>44</sup> have been suspected in the induction or exacerbation of AD. Considering these data and the possible mechanisms involved, several reviews have recently appeared.<sup>4–6,17,45</sup>

Animal studies have revealed that manifestations of autoimmune disorders are strain dependent. In mice, the genotype influences not only the disease susceptibility but also the specific AA produced.<sup>14</sup> One of the genes involved in disease susceptibility lies within the MHC region. Thus, H-2<sup>s</sup> mice (SJL, B10.S, A.SW) are highly susceptible to Hg-induced autoimmunity. This includes production of antinucleolar and antifibrillar antibodies, whereas mice from other MHC genotypes, for example, CBA (H-2<sup>k</sup>), C57Bl/10 (H-2<sup>b</sup>), and DBA/2 (H-2<sup>d</sup>), are resistant to disease induction.<sup>9–11,46,47</sup> Because of the variation in susceptibility to the induction of autoimmunity, it has been suggested that, in humans, exposure to low doses of heavy metals may trigger or aggravate disease in individuals who are prone to systemic autoimmunity.<sup>4</sup>

In Brown–Norway (BN) rats, administration of iHg or Au compounds causes Th2-associated autoimmune disorders characterized by induction of IL-4 at gene and protein levels, production of AA, elevation of serum IgE, and tissue injury in the form of vasculitis and arthritis.<sup>42,48</sup> The generation of these Th2-driven autoimmune responses has been attributed to oxidant stress through generation of free radicals and the following activation of natural killer (NK)- $\kappa$ B, a transcription factor for the IL-4 gene.<sup>49</sup> These results are consistent with other studies where chronic administration of iHg developed AD and elevated levels of serum IgE, whereas Lewis (LEW) rats do not develop the characteristic autoimmune glomerulonephritis and produced very low levels of IgE.<sup>50</sup> Similar results have been found by Fournie *et al.*<sup>26</sup> who found that Th2-dependent autoimmunity induced by Hg and Au only in BN rats that are genetically committed to develop Th2 responses, and that analyzing the effects of HgCl<sub>2</sub> on T cells led the authors to suggest a new signaling pathway implicated in IL-4 production. In mice, studies on Hg-induced autoimmunity<sup>51</sup> revealed similar effects as those observed in BN rats, in that susceptible strains display a general activation of the immune system, especially pronounced for the Th2 subset and a transient glomerulonephritis with Ig deposits. However, the *in vivo* treatment with anti-IL-4 antibodies caused only a partial

reduction in autoantigen-specific IgG1 indicating a minimal role of IL-4.<sup>52</sup> These and other reports,<sup>4</sup> suggest that Hg administration in susceptible mice results in AD with a mixed Th1–Th2 phenotype, and that other cells may be probably involved. However, the dependence on T cells, especially Th cells,<sup>53–55</sup> IFN- $\gamma$ /IFN- $\gamma$  receptor,<sup>56</sup> cluster of differentiation (CD)28, and CD40L<sup>55,57</sup> for autoimmunity induction has been confirmed.

Our results on the production of serum cytokines in BALB/c mice (FIG. 1) which are low rodent responders in terms of AA and lymphoproliferation,<sup>9</sup> in response to heavy metal exposure, showed a significant increase in IL-2, IL-4, IL-5 measured after 5 weeks of exposure to Hg accompanied by decrease in IFN- $\gamma$  release, indicating possible proliferation of Th2 rather than Th1 subsets. However, the increase in Th2 cytokines was not significant in the 3-week exposure group, and was accompanied by increase in IFN- $\gamma$  release. This indicates changing of cytokine profiles along different exposure periods, and thereby distinct roles of cytokines in different phases of AD. IL-10 was not inhibited following short-term exposure but was rather inhibited following 5 weeks of exposure. The proinflammatory cytokines TNF- $\alpha$  and IL-6 show insignificant increase in comparison to control mice. In case of Cd exposure, on the other hand, 5-week exposure led to stimulation of IFN- $\gamma$  release, which was prominent following long exposure accompanied by increase in the Th2 cytokine IL-5. These results also indicate differences in response to heavy metals depending on the metal ion itself as well as duration of exposure, and highlights the dependence on T cells, or at least specific cytokines, for example, IFN- $\gamma$ , in different phases of the disease. The increase in IFN- $\gamma$  release in Cd-exposed BALB/c mice following longer exposure may be consistent with IgG2a increase and the subsequent ANA production in NZBW mice,<sup>44</sup> and recall the involvement of IFN- $\gamma$  in the development of AD in this model.

Previous *in vitro* findings of our group indicated that, in contrast to lead,<sup>27</sup> Cd and Hg<sup>28,29</sup> exposure induced different immunomodulatory effects on cultured human peripheral mononuclear cells depending on the immune stimulation pathways. Therefore, taking into consideration the constraints of extrapolating *in vivo* into *in vitro* mechanisms, bacterial infections or parasites<sup>6</sup> may affect the prevalence of the disease induced by heavy metals and add a new co-factor in AD, increasing the risks and severity in presence of other triggering agents. This warrants further experimental and epidemiological studies to elucidate the relation between infectious diseases and incidence of AD, which may be related to alterations in patterns of T cell differentiation.

In summary, according to a survey of literature and our own results, some heavy metals have the ability to induce AD through alterations of Th cell activities and the subsequent release of cytokines, especially IFN- $\gamma$  and IL-4, which are involved in disease induction or exacerbation. However, evidence exists that other cell types may be involved. It is most likely a multiple-factor event that can collectively initiate and/or maintain the production of autoreactive T

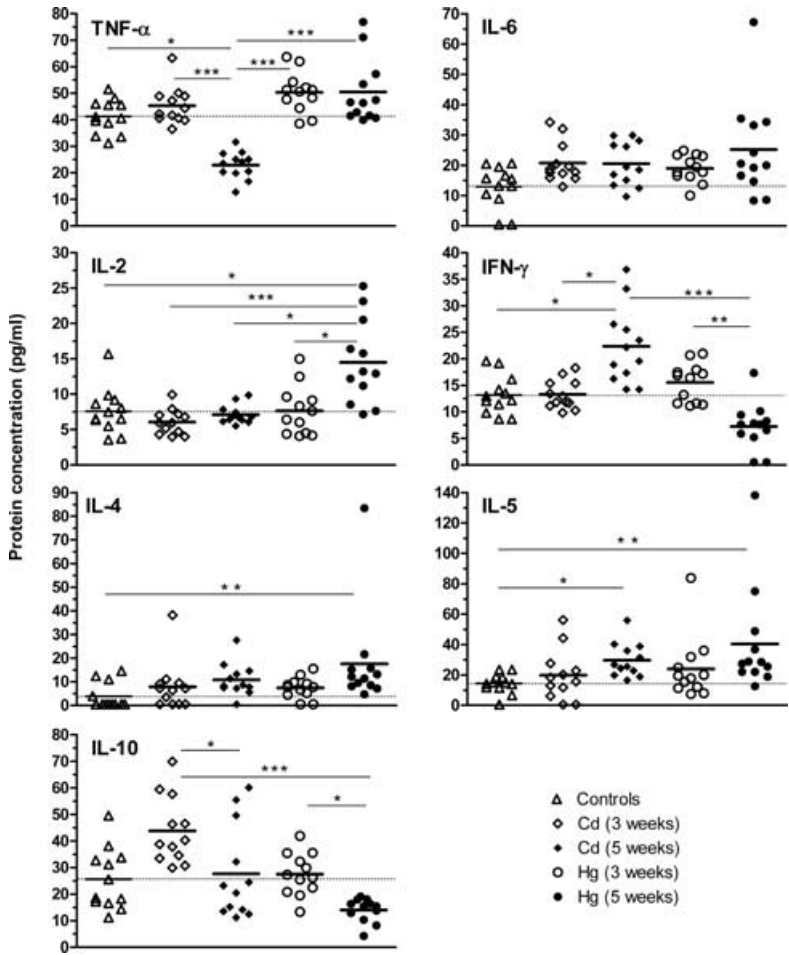


**FIGURE 1.** Serum concentrations of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, IL-2, interferon (IFN)- $\gamma$ , IL-4, IL-5, and IL-10 in wild-type Balb/c mice treated with 1.25 mg/kg B. wt. cadmium (Cd) as Cd acetate or mercury (Hg) as mercuric chloride. Controls were given sterilized isotonic NaCl solution. Horizontal bars denote mean serum concentration. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  compared with the controls (nonparametric ANOVA: Kruskal-Wallis test, followed by Dunns post-test). Cytokines were measured in serum using Cytometric Bead Array (CBA, BD Biosciences, San Jose, CA). Mice were injected intraperitoneally with subtoxic doses (1.25 mg/kg B. wt.) of cadmium or mercuric salts every third day for 3–5 weeks.

and B cells, which ultimately affects the overall reaction and the severity of the disease. The genetic background of the exposed species, duration of contact, and attending current or past infections are all events that allow a heavy metal to have multiple levels of mechanisms of immune modulation.

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