Immunotoxicity of Heavy Metals in Relation to Great Lakes

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Heavy metals including mercury, lead, and cadmium are present throughout the ecosystem and are detectable in small amounts in the Great Lakes water and fish. The main route of exposure of humans to these metals is via the ingestion of contaminated food, especially fish. Extensive experimental investigations indicated that heavy metals alter a number of parameters of the host’s immune system and lead to increased susceptibility to infections, autoimmune diseases, and allergic manifestations. The existing limited epidemiologic data and data derived from in vitro systems in which human peripheral blood leukocytes were used suggested that the human immune system may also be at increased risk following exposure to these metals. The magnitude of the risk that the presence of such metals in the Great Lakes may pose to the human immune system, and consequently to their health, is not known. In this review, the available data with respect to potential adverse effects of heavy metals on the immune system of humans and experimental animals are discussed, and additional data requirements are suggested. — Environ Health Perspect 103(Suppl 9):23–34 (1995)

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Introduction

Metals, including mercury (Hg), lead (Pb), and cadmium (Cd), are chemicals (elements) released into the environment following medical and industrial use and leakage from dump sites (1). Once released into the environment, metals in various forms will contaminate the air, soil, water, and food. Trace amounts of these metals are detected as contaminants in several parts of the ecosystem including the North American Great Lakes basin (2). Although their concentrations in the Great Lakes are small (Table 1) (2), they bioaccumulate in small fish and other biota present in the Great Lakes. Larger fish, feeding on these smaller fish, accumulate these metals in their body tissues and retain them for long periods of time (3,4). Humans are constantly exposed to low levels of these metals present in air, water, and food. In addition, certain populations, especially those who consume large amounts of fish, may accumulate in their tissues levels that are potentially harmful to their health and the health of their offspring (5). Studies in experimental animals and the existing scanty epidemiologic data in occupationally exposed cohorts support the view that exposure to metals may compromise the host’s immune system, leading to increased susceptibility to infections and the development of autoimmune diseases (5,6).

In view of these concerns, a review of the existing data regarding the potential immunomodulatory effects of mercury, lead, and cadmium was undertaken. The main objective of this review was to identify areas of research for which we knowl edge, with respect to parameters of the immune system affected by metals and the mechanisms involved, is insufficient for regulatory purposes.

General Comments

Heavy metals are known to elicit a number of immunomodulatory effects ultimately leading to an enhanced susceptibility to microbial agents and to the appearance of neoplastic diseases and autoimmune phenomena (5–8). The severity of these effects depends largely on the animal species and strain used, the route and duration of exposure, and the metal species employed.

Using the mouse as the animal model and the plaque-forming cell (PFC) response to sheep red blood cells (SRBC) as an indicator of immunotoxicity, Lawrence (9) was able to rank heavy metals for their immunotoxic properties in the following order: mercury > copper > manganese > cobalt > cadmium > chromium. Zinc and iron had no significant effects, while lead and nickel resulted in a significant increase of the PFC numbers. It is of interest that some of these heavy metals, notably zinc and copper as trace elements, are considered to be essential for proper body functions (10).

The mechanism(s) that mediate the immunomodulatory effects of metals are not fully elucidated. It has been suggested that metallothioneins (MTs) may play a role in the immunosuppressive properties of heavy metals. MTs are small cystine-rich proteins (6–8 kDa) that bind heavy metals (11). They are induced following

![Table 1. Concentrations of mercury, lead, and cadmium in the Great Lakes water (ppt).](image)
exposure to metals and play a role in the homeostatic regulation of essential metals and probably in the detoxification of heavy metals (11, 12). Cadmium chloride (CdCl₂), as well as mercuric chloride (HgCl₂), induced thionein production in a dose-dependent fashion in human peripheral blood lymphocytes (PBL) (12, 13). However, the concentration required for thionein induction varied among metal species (14), although no apparent differences were noted in the pattern of the induced thionein isoforms (14). In mouse splenocytes, CdCl₂ was the more potent inducer of MTs followed by HgCl₂ and lead chloride (PbCl₂) (15), while lead acetate ([C₂H₃O₂]₂Pb) did not induce any MT in human PBL (14). The induction of MT is not restricted to metals since stimulation of mouse splenocytes with the mitogen concanavalin A (ConA) or lipopolysaccharide (LPS) had similar effects (15). These results taken together suggested that induction of MT might be related to the metabolic state of the cell and that the observed quantitative differences in the induction of MT by some metals may partly explain the variability of the immunotoxic effects obtained following in vivo exposure to these metals (16). Additional mechanistic studies are required to firmly establish the potential relationship between the induction of MTs by metals and effects on the immune system.

Mercury

Epidemiologic Studies of Hg

The elemental form of Hg occurs naturally in the environment. Once released into the environment, Hg may combine with other elements to form inorganic and organic Hg compounds. Estimated levels of Hg in Great Lakes fish range from 0.14 to 0.32 μg/g for commercial fish taken from the four Great Lakes bordering Canada and have reached 0.5 μg/g as reported in a survey of valleys from northern Wisconsin Lakes (17). Higher levels of 1.21 mg/kg dry weight or 0.35 mg/kg based on a wet tissue basis were reported for Lake Ontario salmon (18). Methylmercury (MeHg) is of particular concern to human health since it is the most toxic form of Hg (19). Long-term exposure to Hg may result in irreversible damage to brain, kidneys, and the developing fetus (19). Consequently, a large database exists regarding toxicologic effects of Hg, particularly effects associated with neurobehavioral abnormalities (19, 20). Based on these data, the joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives has estimated a permissible tolerable weekly intake for MeHg of 3.3 μg/kg/week equivalent to 4.7 × 10⁻⁴ mg/kg/day (0.47 μg/kg/day) (19, 20). The U.S. Environmental Protection Agency (U.S. EPA) has derived a reference dose (RfD) for MeHg, which equals 3 × 10⁻⁴ mg/kg/day (0.3 μg/kg/day) (21). For a generic 70-kg adult, this is equivalent to a daily intake of 21 μg/day, and for a 62-kg woman, this dose is equivalent to an intake of 19 μg/day. Recently, an RfD = 0.07 μg/kg/day was suggested by Stern (22) based on developmental effects.

The contribution of the Great Lakes fish to the overall human burden of Hg is not known. Preliminary results of an ongoing study (Kearney et al., personal communication) suggested that Great Lakes fish-eating cohorts may have higher blood MeHg levels than non-Great Lakes fish eaters. In this study, the concentrations of Hg were examined in 232 participants of which 176 were sport fish eaters (152 were Great Lakes fish eaters, 24 consumed only non-Great Lakes fish) and 56 were sport fish non-eaters. Higher blood Hg levels were reported for the 176 sport-fish eaters (geometric mean = 2.2 [2.0 ppb] with a range of 2–21 ppb) compared to the 56 sport fish non-eaters (geometric mean = 1.6 [1.8] with a range of 2–6 ppb) (Kearney et al., personal communication).

In contrast to the existing large database for neurotoxic effects of Hg, only limited data exist in humans while data on the immune effects of Hg in fish-eating populations are nonexistent.

Hg concentrations in the blood and urine, as well as total serum immunoglobulins (Ig), were measured in 89 male workers occupationally exposed to Hg vapors for 2 to 26 years (mean 13.6) (23). The blood Hg concentration did not exceed 50 μg/l (50 ppb) in any of the subjects studied. Hg concentrations in urine greater than 200 μg/l were measured in only 2 of the workers. Significantly decreased serum IgG and IgA, but not IgM, IgD, and IgE, were reported only for those workers who were exposed to metallic Hg vapors for more than 20 years compared to an unexposed reference group (23). However, there was no correlation between the immunoglobulin concentration and duration of exposure or Hg concentration in the body fluids (23). Likewise, the incidence of upper respiratory and urinary tract infections in the exposed group was not different than that in the reference group (23). In contrast, increased IgG, but not IgA and IgM, levels were observed in workers with urinary Hg levels of 29 to 545 μg/l (29–545 ppb) (24). Oral galvanism is associated with low-dose exposure of metallic Hg vapor from amalgam fillings (24) and is perhaps the second most important source of mercury, contributing approximately 0.9 to 17.5 μg to the total body burden (25). For comparison, Sweden has set a threshold limit for metallic Hg vapor in the work environment to equal 50 μg/m³ corresponding to an exposure of approximately 500 μg/dl (25). Decreased serum IgE levels and increased complement (C3d) levels were reported in a study of a small number of humans exposed to Hg in dental amalgams (26). The reduced IgE levels were observed during the period following removal of the dental amalgam, and it may have been an indication of a noncompensated consumption of IgE that might have occurred during the postexperimental period (26). Aggravation of asthma due to Hg in dental amalgams has been reported for one patient (27). On the contrary, no association was found between the number of amalgam tooth fillings and the prevalence of asthma, eczema, and allergic rhinoconjunctivitis in 348 Swedish adolescent schoolchildren (28), albeit a significant correlation could be identified between the number of amalgam surfaces and plasma Hg levels in a subgroup of these children (29). The latter included 21 schoolchildren (15 years old) with amalgams and 20 without amalgams. There were no significant differences between the two groups in several of the parameters tested including T- and B-lymphocyte levels, T-cell subsets, Ig levels (IgG, IgG1, IgG3, IgG4, IgA, IgM, and IgE), and antinuclear antibodies (29). The median for plasma mercury concentration was 1.65 and 2.10 nmol/l for males and females, respectively (28). In contrast, increased total lymphocyte, T helper/inducer (CD4⁺), and T suppressor/cytotoxic (CD8⁺) levels were reported in a group of 25 dental students (average age of 21.5 years) who were presumably occupationally exposed to Hg, compared with a reference group of medical students (30). However, the significance of these results is not clear from the data presented since the CD4⁺:CD8⁺ ratio, or the peripheral blood B-lymphocyte (CD21⁺) levels and natural killer (CD16⁺) cells did not differ between the two groups. Similarly, there was no evidence of Hg-induced cutaneous hypersensitivity reactions in the two groups tested (30).
Concentrations of Hg in tissues were not reported for these students.

Finally, the incidence of allergic contact dermatitis to a number of metals including Hg was studied in 329 Portuguese children of 14 years of age or younger. These children were patch tested by members of the Portuguese Contact Dermatitis Group between 1985 and 1989 (31). An incidence of 6.1% positive patch tests to ammoniated Hg was reported in these children, with the number of positive reactions increasing with age (31).

Overall, these data suggested that Hg, at levels found in occupational exposures, may have an adverse effect on the immune system of humans. Decreased levels of serum immunoglobulins were observed in occupationally exposed cohorts with blood Hg levels <50 µg/l, but a concurrent increase in the incidence of microbial infections was not observed in these workers. The reviewed data also suggested that Hg has the potential to elicit contact sensitization in children with a genetic predisposition to allergy; further carefully designed epidemiologic studies are needed to establish the potential role Hg may play in eliciting such reactions.

**Experimental Studies in Vivo**

Inorganic and organic forms of Hg have definite cytotoxic effects on cellular components of the immune system. HgCl₂ administered orally to B6C3F₁ mice at doses of 75 ppm for 7 days resulted in significantly reduced spleen and thymus weights compared to the control (32). A no observed adverse effect level (NOAEL) dose of 15 ppm was reported for these mice (32). Reduced thymus weight was also reported in BALB/c mice administered MeHg orally at a dose of 3.9 ppm for 12 weeks (33). A progressive systemic lymphadenopathy was observed in brown Norway (BN) rats following exposure to HgCl₂ with a 3-fold increase in the weight of the lymph nodes (34).

The potential role of Hg in the development of autoimmunity was studied in genetically susceptible mice and rats (35–42). In these animals, systemic autoimmune diseases were induced by repeated injections of HgCl₂. Several Hg-induced autoimmune phenomena, including the presence of antinuclear antibodies, antinuclear and antinuclear antibodies, and the development of glomerulonephritis were observed in genetically susceptible animals (39–53). These phenomena are thought to be a result of a dysregulation of T lymphocytes, which might involve the induction of a T-dependent polyclonal activation of B cells (39–53). This would account for the development of various autoantibodies (54) and the observed increased production of total serum IgE and IgG1 in mice and rats following exposure to HgCl₂ (50,54,55).

Hg-induced autoimmune disorders are thought to be linked to the major histocompatibility complex (MHC) (35,39,41,56,57). Indeed, the development of autoreactive CD4⁺ (T-helper/inducer) cells with the ability to proliferate in the presence of normal syngeneic cells that express MHC class II antigens is well documented in HgCl₂-treated rats (58). Hg-specific T cells were detected in HgCl₂-treated mice, but their role in the development of autoimmunity is not clear (40). Fluctuations in the number of CD8⁺, MHC class-II, and interleukin-2 receptor positive (IL-2R⁺) cells were also observed in the secondary lymphoid organs of HgCl₂-exposed rats but no overall uniform effects could be identified (39,41).

MeHg may affect nonspecific host defense mechanisms since it has been shown to decrease the natural killer (NK) cell activity in rats exposed in utero and through milk to 3.9 µg/g (3.9 ppm) of the dam’s feed (59) and in mice following oral administration of the same dose over a 12-week period (33).

The effects of MeHg on humoral immune responses are well documented. MeHg at a single dose of 10 ppm administered orally for 10 weeks resulted in decreased antibody levels to pseudorabies and influenza viruses in the rabbit with a NOAEL of 1 ppm (60). Likewise, HgCl₂ at doses of 250 and 500 ppm administered to chickens orally for 35 days or at 300 µg/ml for 11 weeks diminished their ability to produce specific IgM and IgG antibodies to SRBC (61,62). A NOAEL of 125 ppm was observed in chickens. A greater sensitivity to HgCl₂ was observed in B6C3F₁ mice administered doses of 3,15, or 75 ppm orally for 7 weeks, with a lowest observed adverse effect level (LOAEL) of 75 ppm and a NOAEL of 15 ppm (32). The PFC response to LPS—i.e., a B-cell-dependent antigen—was not affected by any of these doses (32). More over, reduced PFC responses to SRBC were obtained when different strains of mice were treated with low doses (1, 5, or 10 ppm) of MeHg (63,64). A LOAEL value of 1 ppm was identified for MeHg.

Hg has the potential for eliciting contact sensitization as revealed by the classical maximization test in the guinea pig (65) and allergic contact dermatitis in mice (66). The significance of these findings is not clear since no such effects were noted in occupationally exposed cohorts (29), albeit contact sensitization was detected in children when these were patch tested with ammoniated Hg (31). The effects of MeHg on the mitogen-induced proliferative ability of leukocytes varied with the mitogen used. In B6C3F₁ mice following treatment with 15 or 75 ppm of MeHg for 7 weeks, decreases in the lymphoproliferative activity of splenocytes in response to phytohemagglutinin (PHA) and ConA were observed, whereas the response to LPS was enhanced significantly (32). A lower dose of 5 ppm of MeHg either enhanced or had no effect on the ConA-induced proliferative activity (67).

In summary, the existing experimental animal data permits the derivation of LOAELs in some species, but the obvious lack of data pertaining to tissue levels of Hg in these animals and the use of single versus multiple doses in some of the studies diminishes the merit of such data for regulatory purposes. Nevertheless, the LOAELs derived from such data clearly indicate that both forms of Hg can be immunotoxic at the levels tested and that MeHg is immunotoxic at levels lower than those observed for HgCl₂ (Table 2).

**In Vitro Mechanistic Studies of Hg**

The in vitro cytotoxicity of MeHg was shown to be 10-fold higher than that of HgCl₂, with human peripheral blood monocytes exhibiting a significantly greater sensitivity to both forms of Hg than lymphocytes (69,70). The median lethal dose (LD₅₀) values for HgCl₂ were 23 and 4 µM for human T cells and monocytes, respectively, and 6.0 and 0.4 µM for MeHg for T cells and monocytes, respectively (70). The observed cell cytotoxicity involved an apoptotic process suggesting that Hg may activate the cellular signaling pathway, which leads to the suicide of cells (70). Human peripheral blood T cells and monocytes in the presence of either form of Hg exhibited a decreased ³²P incorporation into phospholipids (69). The observed decrease was associated with a concurrent increase in intracellular calcium levels characteristic of the apoptotic process (69).
At noncytotoxic levels, HgCl₂ decreased the functional activity of either polymorphonuclear cells or monocytes isolated from human peripheral blood (70). Interestingly, a higher level of superoxides (H₂O₂) production was observed in peptone-induced murine peritoneal macrophages upon incubation with Hg, suggesting that activated macrophages may be less susceptible to the effects of this metal (70).

In vitro treatment of SJL mouse splenocytes with Hg increased the response to B- and T-cell mitogens, whereas under the same experimental conditions, DBA mouse cells showed a significant reduction in their proliferative ability (67). The latter effects were optimal at low doses of HgCl₂ (10⁻⁷⁻¹0⁻⁸ M) and were observed only when the mercury was added as a pulse 1 hr before the addition of the mitogen suggesting that lymphocyte susceptibility may be linked to the MHC (67).

Additional in vitro studies demonstrated that the observed Hg-related decrease in ConA-induced T-cell proliferation was associated with a decrease in phosphatidylinositol levels (71). Moreover, it was demonstrated that Hg did not change the expression of CD69, an early marker of cell activation, but significantly reduced expression of the IL-2R (70). These results suggested that following the stimuli, very early events in the G₁ phase were not affected by Hg, but later G₁ events were perturbed (67). An increase of intracellular calcium was observed in human peripheral blood leukocytes exposed for 5 min to Hg (70). The calcium flux was not mediated by calcium-specific channels since the addition of channel blockers, such as verapamil and nifedipine, failed to alter Hg-induced changes in calcium fluorescence (70). On the other hand, incubation for 1 hr with various concentrations of Hg caused an increase of tyrosine-phosphorylated proteins and p56lk kinase activity as well as an increase of phosphatase activity (72). These results taken together demonstrated that mercury could modulate cellular signaling pathways; however, the degree to which these changes influence the integrity of the immune system is not well understood. Therefore, further investigations are required to completely elucidate the mechanism by which Hg produces its immunotoxic effects.

**Lead**

**Epidemiologic Studies of Pb**

The main target of Pb toxicity is the nervous system, especially in children (73,74). Exposure to Pb has also been associated with increased blood pressure in adult populations (73) and is causally related to nephropathies in children and in adults (74). While water analysis studies suggested that Pb levels in the Great Lakes may not exceed the 10 μg/l guideline set by the Canadian Health Department for Pb levels in drinking water (17) (Table 1), different forms of Pb bioaccumulate in fish (18) and in humans, especially those who are frequent fish eaters. Preliminary results of an ongoing study suggested that Pb levels might be higher in cohorts who consumed Great Lakes fish (mean 3.7 mg/dl, range 1.1-26.8 mg/dl) compared to those who did not consume Great Lakes fish (mean 3.0 mg/dl, range <0.5-10.7 mg/dl) (Kearney et al., personal communication).

Epidemiologic data following occupational exposure to Pb suggest that the main effects of Pb might be on cellular aspects of the immune system with humoral parameters remaining relatively insensitive to Pb exposure. Thus, serum 1gs (IgG, IgM, and IgA) remained within normal levels in workers occupationally exposed to Pb for 4 to 30 years (75). The exposed workers had a mean blood lead level of 38.4 μg/dl compared to the control mean levels of 11.8 μg/dl. Similarly, no effects were noted on serum IgG and IgA levels in another cohort occupationally exposed to lead oxides with estimated blood Pb levels of 64 μg/dl. The Pb oxides concentration within the plant was 266 μg/m³ (75). The in vitro response of PBL to PHA and ConA stimulation was significantly decreased in the exposed group compared to the control (76). A decrease in serum Ig levels was reported in 14 occupationally exposed workers with blood Pb levels of 46.9 μg/dl. The duration of exposure of these workers was 36 years (77). The control (nonexposed) group had mean blood Pb levels of 10.9 μg/dl. While no significant effects were noted on serum Ig levels in 72 occupationally exposed workers compared to 53 reference subjects, the levels of secretory (salivary) IgA were significantly decreased in the exposed group (78). The exposed group had significantly higher blood Pb levels (21-90 μg/dl; mean = 51 μg/dl) compared to those of the reference group (mean = 12 μg/dl) (78). Secretory IgA plays a major role in the defense against respiratory, and gastrointestinal infections and the observed reduced levels might be responsible for the increased incidence of influenza infections reported in the exposed workers compared to that in the reference group (78). In the same study, complement (C₃) levels were not significantly different between the two groups but significant negative correlations were detected between Pb levels in the blood and C₃ levels. Similarly negative correlations were observed between blood Pb levels and total serum IgG levels, while a positive correlation was obtained between the blood Pb level and serum IgA levels (78). Positive correlations between blood Pb levels and serum IgA were also detected in a group of Pb refinery workers whose blood Pb levels were greater than 60 μg/dl (79).

Data regarding the effects of Pb on the numbers and function of lymphocytes and their subsets are available only for a small number of occupationally exposed workers. These data are generally based on one-time analysis and are therefore not easily interpretable regarding their clinical significance. In one such study 10 Pb-exposed subjects (range of blood Pb levels = 40-51 μg/dl) were compared to 10 reference subjects (blood Pb levels <19 μg/dl). This study did not report any significant differences between the two groups regarding the levels of T lymphocytes and their subsets or T-lymphocyte function in the mitogen induced proliferation assay (80). A second
study involved two groups of firearms instructors (81): group 1 (high) had mean blood Pb levels of 31 µg/dl while group 2 (low) had mean blood Pb levels of 15 µg/dl. A significant decrease in the CD3+ (total lymphocytes) attributable to a decrease in the CD4+ T-cell subset was detected for the high-exposure group compared to the low-exposure group. This may have accounted for the observed functional impairment of cell-mediated immunity that was assessed by the response of lymphocytes to T-cell mitogens and by the mixed lymphocyte culture, both of which require T-helper activity. Other cell types including the CD8+, the B-lymphocyte (CD20+), or the NK cells (CD16+) were not altered significantly (81).

The effects of Pb on immune parameters were also investigated in 12 preschool-age children with evidence of Pb toxicity (mean blood Pb levels = 45 µg/dl) compared to 7 children with mean blood Pb levels = 23 µg/dl (82). There were no significant differences between the two groups regarding total serum Ig levels, complement levels, and their ability to respond to a recall antigen such as the tetanus toxoid (82). The absence of significant differences between the two groups may be attributed to the fact that Pb levels in the low-exposure group were above the published standard of >10 µg/dl for Pb intoxication (83). In this case, correlations between blood Pb levels across all 19 children and the parameters measured might have been more revealing.

In summary, data derived from studies in epidemiologically exposed cohorts suggested that Pb may affect the immune system; however, only general parameters, including effects of Pb on serum Ig levels and peripheral blood leukocyte counts, have been examined in these studies. The absence of data on more clinically relevant parameters, such as on functional aspects of the immune system, and the lack of repeated measures of the affected parameters do not allow for a complete evaluation of the potential immunotoxic effects of Pb in humans. The observed NOAEs and LOAELs based on blood Pb levels and the associated effect on immune parameters are as follows: LOAEL = 46.9 µg/dl based on decreased serum Ig levels with a NOAEL of 38.4 µg/dl; LOAEL = 64 µg/dl based on reduced lymphoproliferative activity of PBL in response to mitogens; and a LOAEL = 31 µg/dl based on decreased T lymphocytes and T-helper/inducer cells with a NOAEL <19 µg/dl. It is apparent from these data that potentially adverse immunologic effects of Pb were noted at blood Pb levels much lower (parts per billion) than those detected in the Great Lakes fish-eating cohorts (ppm) (22). Data regarding the immunological status of the latter group are not presently available. Thus, an evaluation of the consequences of such levels on the immune system of the Great Lakes fish-eating cohorts would require the evaluation of data derived from carefully designed studies whereby quantitative and functional aspects of the immune system would be investigated.

**Experimental Studies of Pb in Vivo**

The effects of Pb on the immune system of experimental animals are as divergent as those reported for Hg (67). Pb effects on rat organ weights including the thymus, spleen, and popliteal lymph nodes have been reported but these are not dose related (84,85).

Effects on total serum immunoglobulin levels and on the antibody response to specific antigens have also been reported. Rats exposed *in utero* and postnatally to 25 or 50 ppm of Pb had significantly decreased serum IgG levels while IgA and IgM were not affected (86).

Effects of Pb on humoral aspects of the immune system have been reported by several investigators, but no clear picture emerges through these data regarding the potential effects of Pb. For example, (C2H3O2)2Pb administered to mice in drinking water at concentrations ranging from 0.08 to 50 mM of Pb for 1 or 10 weeks did not affect the spleen PFC numbers to SRBC (87), whereas the *in vitro* primary response to Pb assessed in lymphocytes from Pb-treated mice gave variable results (87). In these experiments, Pb levels of 0.08 to 0.4 mM enhanced the response to SRBC while 2.0 mM did not have any effect; the 10 mM dose resulted in suppressed PFC numbers (87). Similar results were obtained in experiments in which splenocytes were exposed to Pb *in vitro* and subsequently tested for their humoral immune response to SRBC (87). Statistically significant reduced PFC numbers to SRBC were observed in CD-1 mice following oral administration of (C2H3O2)2Pb at doses of 13.75 or 137.5 ppm for 65 days or at a dose of 1375.0 ppm administered for 56 days (88), but a dose–effect relationship was not observed in other studies in which similar exposures were used. For example, no effects were noted following oral administration of 13, 130, or 1300 ppm of Pb for 70 days (68) or doses of 50, 200, or 1000 ppm administered orally to BDF1 mice for 21 days (89). On the contrary, significantly reduced PFC numbers to SRBC and to the T-independent antigens TNP-LPS or TNP-Ficoll were reported for (C2H3O2)2Pb administered at a single ip dose of 12 mg/kg body weight (bw) to B6C3F1 mice (90) or in rats treated orally with 25 ppm of (C2H3O2)2Pb for 70 days (86). Collectively, these results indicated that the effects of Pb on the PFC numbers were not consistent among studies and varied considerably across the strains of rodents used suggesting that genetic factors might be involved.

Experimental data on the effects of Pb on cellular parameters of the immune response indicated that the B lymphocyte might be affected following exposure to Pb. A significantly reduced LPS-induced splenocytic lymphoproliferative activity was observed in B6C3F1 mice following administration of a single ip dose of 12 mg/kg body weight (bw) of (C2H3O2)2Pb (90). Similarly, significantly reduced LPS-induced splenocytic proliferation was observed in CBA/J mice exposed for 28 days to 2.0 and 10 mM of (C2H3O2)2Pb added to drinking water while intermediate concentrations of 0.08 or 0.94 mM had no effect (87). There were no dose-related changes in the PHA- or ConA-induced lymphoproliferative activity or in the MLC activity (87). Similarly, the ConA-induced lymphoproliferative activity of splenocytes was not affected significantly in CBA/J mice administered (C2H3O2)2Pb orally for 28 days doses of 13, 130, or 1300 ppm (91). In contrast, a significantly reduced T-cell blasticogenic response to ConA was detected in the offspring of Sprague-Dawley rats exposed *in utero* to (C2H3O2)2Pb at doses of 25 or 50 ppm, suggesting that the developing fetus might be more susceptible to the immunotoxic effects of Pb (85).

Decreased splenic and bone marrow macrophage levels have been reported for mice treated with a single ip dose (12 mg/kg bw) of (C2H3O2)2Pb (90). The observed reduced macrophage numbers may be responsible for the impaired ability of CBA mouse peritoneal macrophages to phagocytose SRBC following administration of 1300 ppm (C2H3O2)2Pb for 10 weeks (92). The effect on macrophage function was dose dependent and possibly strain dependent since lower doses of 5, 10, or 20 µg/kg bw administered ip or single doses of 25, 50, or 100 µg/kg bw administered orally increased the clearance of colloidal carbon in NMRI mice (93).
In contrast to the observed variable effects of Pb on antibody production, the effects of Pb on host resistance were characterized by a uniformly reduced ability of the host to combat infection. A single ip dose of 24 mg/kg bw of (C₂H₃O₂)₂Pb or doses of 5 and 12.5 mg/kg bw of lead nitrate resulted in significantly reduced resistance to Klebsiella pneumoniae and Salmonella typhimurium in CD-1 mice (94, 95). Similarly, single intravenous (iv) doses of 8.5 or 20 mg/kg bw resulted in significantly reduced resistance of rats to Salmonella enteritidis and Escherichia coli endotoxin and to S. epidermidis infection (96,97). Likewise, concentrations of 0.4 to 10 mg of (C₂H₃O₂)₂Pb administered to mice in the drinking water increased the mortality rates in these animals when challenged with a facultative intracellular bacterium, Listeria monocytogenes (87).

In summary, the derivation of NOAEL and LOAEL values based on experimental animal data is difficult since conflicting results were reported for some of the parameters measured, including the PFC response to SRBC. A LOAEL of 5 mg/kg bw based on the observed uniformly increased sensitivity to microbial infections was derived for mice and rats following either ip or iv administration of single injections or the addition of Pb to drinking water. It should be noted however, that the experimental design did not allow for the determination of a potential NOAEL value since relatively high doses of Pb were used in these studies. This limitation and the lack of data regarding levels of Pb in tissues do not permit extrapolation of these findings to humans. The increased frequency of infections observed in occupationally exposed cohorts with low blood Pb levels (51 µg/dl) and the reduced resistance to bacteria observed in Pb-exposed animals strongly indicated that the immune system can be adversely affected by low levels of Pb. Thus, additional studies using multiple doses of Pb and data relating the doses administered to levels in tissues are required to further define a safe level of exposure to Pb.

In Vitro Mechanistic Studies of Pb

The in vitro mechanistic studies also suggested that the effect of Pb might be at the B-cell level. But, unlike the suppressed LPS-induced lymphoproliferative activity observed in the exposed animals, an enhancement of this activity was observed in acute in vitro exposures.

For example, coculture of BALB/c or CBA/J mouse splenocytes with (C₂H₃O₂)₂Pb at concentrations of 10 or 100 µM for 3 days resulted in significantly increased lymphoproliferative activity in response to the LPS mitogen whereas 1 µM did not have any effect (98). The lymphoproliferative response of the mouse T-cell hybridoma DO.11.10 cell line to ConA was not affected by PbCl₂ added to cultures at concentrations of 10 or 100 µM indicating that the T cell might be resistant to Pb effects (99). Indeed, culture of human PBL with PbCl₂ at concentrations of 5.0, 10, or 100 µM for 7 days resulted in significantly increased Ig secretion in unstimulated or pokeweed mitogen (PWM)-stimulated cultures whereas lower concentrations had no effect on Ig secretion (100). The Pb-induced enhancement of B-cell proliferation correlated with the enhancing effect of Pb on the PFC numbers to SRBC (101). The potential requirement of B–T cell interaction for the enhancement of B-cell differentiation was investigated by McCabe and Lawrence (101) using membrane segregated cultures. It was shown that B→T cell interaction was not an absolute requirement for the observed immunostimulation by Pb, which suggests that Pb has the potential to increase directly B-cell responses. However, optimum levels of differentiation were observed when B and T cells were incubated simultaneously with Pb (101).

These results taken together suggested that Pb might modulate the production of helper factors leading to increased B→T helper cell interactions (9,100). Using antigen-specific T-cell clones, it was shown that Pb preferentially altered a subset of T-helper cells. Indeed, it was shown that the T-helper 1 activation was inhibited by Pb while T-helper 2 activation was enhanced (102). It is well documented, at least in the mouse model, that T-helper 1 cells produce interleukin 2 (IL-2) and interferon (INF)-gamma, whereas T-helper 2 cells produce IL-4, IL-5, and IL-6 (101). This differential influence of Pb may explain, at least in part, the observed diverse in vivo effects on immune responses.

Cadmium

Epidemiologic Studies of Cd

The commercially available Cd is largely extracted during the production of other metals including zinc, Pb, or copper. Hazardous waste disposal sites are a large source of the Cd concentrations found in soil (approximately 4 ppb) and in water (approximately 5 ppb) (103). The concentration of Cd in air is very low (range 5–40 ng/m³), with concentrations in food ranging from 2 to 40 ppb. Drinking water contains on the average less than 1 ppb (104). Cd concentrations in Great Lakes water range from 16 ppt (Lake Huron) to 98 ppt (Lake Erie) (Table 1) (2). Human exposure to Cd results from the ingestion of contaminated food especially fish and, to a much lesser extent, of drinking water (104). Adult intake levels of Cd from food in the United States were estimated to be 30 µg/day (105). Assuming a gastrointestinal absorption of 5 to 10%, the amount of Cd absorbed from the diet would be approximately 1 to 3 µg/day. The estimated dietary ingestion of Cd by the average Canadian was reported to be 14.5 µg/day (106). It should be pointed out, however, that cigarettes contain large amounts of Cd (1000–3000 ppb) and cigarette smoke may contribute up to 3 µg/day to the smoker’s daily Cd intake (103).

The acute toxic effects of Cd are generally restricted to the lung, whereas the effects following chronic Cd exposure in humans are multisystemic and include nephropathies, emphysematous alterations in the lung, and cardiovascular diseases (103). The U.S. EPA estimated oral chronic RDIs for Cd are 0.001 and 0.0005 mg/kg/day for ingestion from food and water, respectively. These are based on the observed proteinuria in humans chronically exposed to Cd (107). The World Health Organization recommends an exposure limit of 10 µg/m³ for long-term occupational exposure (108).

While a large body of data exist on the in vivo and in vitro effects of Cd on the immune system of experimental animals, little data pertaining to occupationally exposed cohorts could be located.

A significantly reduced activity (measured by chemiluminescence) was reported for zymosan-activated neutrophils in the peripheral blood of workers exposed to Cd by inhalation for 1 to 14 years compared to a reference (unexposed) group (109). Kastelan et al. (110) described a dose-dependent inhibition in PHA-induced lymphocyte proliferation while Williams et al. (111) did not detect any effects in the PHA- or the purified protein derivative-induced lymphocyte proliferation in a group of patients with chronic Cd disease. Other parameters examined, including the nitroblue tetrazolium reduction by blood polymorphonuclear cells and the antibody-dependent cellular cytotoxicity, were not affected in these patients (111).
An increase in the incidence of eczema has been reported for one group of workers with extensive exposure to Cd dust (112) while a 1% nonirritating solution of CdCl₂, used in routine patch tests in patients with clinical manifestations of dermatitis and eczema, did not result in allergic reactions in these patients (113). This was true for patients occupationally exposed to Cd or for those patients without prior exposure (113,114). Thus, the existing limited data do not present strong evidence of adverse Cd-induced effects on the immune system of humans. However, conclusions regarding the potential adverse health effects of Cd in humans should await further investigations of cohorts either occupationally exposed to Cd or following the ingestion of Great Lakes fish.

**Experimental Studies of Cd in Vivo**

The documented effects of Cd on the immune system of experimental animals are numerous and divergent (8). This reflects the fact that many different chemical forms of Cd were used in different species of experimental animal models, with various routes and duration of exposure and dose regimens. Moreover, different assays, each with a different sensitivity to detect immunotoxic effects, were used.

The weight of the thymus was reported to either decrease or remain unchanged in the rat or mouse following exposure to Cd (115,116). Similar findings were reported for the spleen weight in the mouse model (115,116). At the cellular level, a decrease in the white blood cell counts and lymphocyte counts were observed in the gold fish (Carassius auratus) following a whole body exposure to CdCl₂ at concentrations ranging from 90 to 445 μg/l of water for periods of 3 and 6 weeks (117). No such changes were reported in rainbow trout following a 12-week whole-body exposure to low CdCl₂ concentrations (0.7-6 μg/l of water) (118). Whole-body exposure of the *Rana* tadpole—a cold-blooded animal—to CdCl₂ concentrations ranging from 0.1 to 0.2 ppm for 6 weeks did not affect the number of μB lymphocytes in peripheral blood, while CdCl₂ concentrations four times higher resulted in significantly increased numbers of the μB lymphocytes (119). Similarly, increased lymphocyte numbers were reported for rats treated orally with 200 or 400 ppm of CdCl₂ for 6 months (120). On the contrary, oral administration of Cd at doses of 3, 30, or 300 ppm to mice did not affect the number of B and T lymphocytes significantly while five subcutaneous doses of CdCl₂ at levels of 0.5 or 1.0 mg/kg bw resulted in approximately 60% reduction in B-lymphocyte levels compared to the control (116). The total number of T lymphocytes was not affected by this treatment regimen (116). Thus, the route of exposure to Cd greatly influences the direction of effects on the immune system, presumably due to the different amounts of Cd absorbed by the various routes of exposure.

Cd has also been shown to affect non-specific parameters of the immune response. Uniformly decreased NK cell activity was observed following administration of various doses of CdCl₂ using different routes of exposure (121). Furthermore, the number of macrophages in the spleen and the bone marrow were reduced significantly in B6C3F₁ mice administered a single ip dose of 0.9 mg/kg bw of cadmium acetate (Cd₂H₂O₄Cd) (90). The clinical significance of the observed decreased numbers of macrophages is not clear since CdCl₂ administered orally to B6C3F₁ mice for 90 days at doses of 10, 50, and 250 ppm resulted in a significantly increased rather than decreased phagocytic activity of peritoneal macrophages when these were incubated with *K. pneumoniae* (122). However, a significant reduction in the phagocytic ability of peritoneal macrophages was noted when Cd-1 mice were administered CdCl₂ at a dose of 300 ppm orally for 70 days; this suggested that genetic factors may influence the outcome of effects on the immune system (91). No effect on the phagocytic activity of peritoneal macrophages was detected when doses of 3 or 30 ppm were used (91).

The effects of Cd on humoral immune responses varied with the route of exposure, the dose administered, and the end point measured. In general, CdCl₂ at doses of 50 ppm administered to mice orally for 3 to 4 weeks did not affect the level of total serum IgG (115). Similarly, administration by inhalation of 9.02 mg/m³ of Cd did not affect the specific antibody response to influenza virus in the mouse (123), or to *Trypanosoma lewisi* in the rat (124). However, increased production of specific antibodies to *Vibrio anguillarum* was noted in the rainbow trout as well as in the antibody levels to SRBC in the *Rana* tadpoles following whole-body exposure to low levels of CdCl₂ (118,119). In mice, doses of 5, 10, or 50 ppm of CdCl₂ administered orally for 26 days increased the number of PFC to the hapten-carrier dinitrophenol-Ficoll (DNP-Ficoll) (125); moreover, a dose of 5.9 mg/kg bw administered sc for 7 days increased the number of PFC to the hapten-carrier trinitrophenol-lipopolysaccharide (TNP-LPS) (126).

The direction of effects of Cd on the PFC response to SRBC varied with dose, the route of exposure, and the animal model used. For example, oral administration of CdCl₂ at doses of 3 or 30 ppm to CD-1 mice had no effect, whereas a dose of 300 ppm resulted in increased PFC numbers (68). Furthermore, a single dose of 0.15 mg of CdCl₂ administered orally to CD-1 mice resulted in a 25% decrease in PFC numbers to SRBC (91). However, the same dose administered ip (127) or single doses of 1.48, 2.96, 5.91, or 11.81 mg/kg bw administered im had no effect (128). Similarly, the studies by Malave and De Ruffino (129) indicated that a dose of 50 ppm of CdCl₂ administered orally to CD-1 mice for 3 to 9 weeks resulted in an increase of the PFC numbers. Doses of 10, 50, or 250 ppm administered to B6C3F₁ mice for 90 days had no detectable effect on the PFC numbers (122).

Cd has also been reported to alter the cellular immune response in rodents. Splenocytes of mice exposed to CdCl₂ for 10 weeks at doses of 3 and 30 ppm displayed no modifications in their responses to LPS while at a dose of 300 ppm they increased the blastogenic response to LPS significantly (91). The blastogenic response of lymphocytes to other mitogens varied. For example, the blastogenic response of lymphocytes to PHA, ConA, and PWM was significantly reduced when mice were treated orally with daily doses of 10 or 250 ppm of CdCl₂ for a period of 90 days; at an intermediate concentration of 50 ppm, the response to these mitogens was not affected (122). The MLC response of leukocytes was suppressed following ip administration of 2 mg/kg bw of CdCl₂ for 40 days (130). In other experiments whereby mice were treated orally with daily doses of 50 or 200 ppm of CdCl₂ for a period ranging from 3 to 9 weeks or with Cd₂H₂O₄Cd at doses of 300 or 600 ppm for 10 weeks, the response to PHA and ConA were significantly increased (129,131).

Specific cell-mediated immune responses were also shown to be affected by Cd. In mice, the delayed-type hypersensitivity (DTH) response to SRBC was decreased following oral administration of 65.2 mg/kg bw for 14 days (132); however, in another series of experiments, neither the DTH response to SRBC (132) or to KLH (118) in the mouse model or to dinitrofluorobenzene (DNFB) in the rat model...
various and Escherichia contrary, translation effects increased the systems, reducing their resistance. The mechanism of Cd-induced immunomodulation includes quantitative and functional effects on the phagocytic lineage of cells and NK cells and the antibody response to various antigens. However, the direction of the observed effects was not uniform across strains and varied with the route of exposure. In general, exposure to Cd reduced the ability of the animal to combat infection at a LOAEL = 10 ppm. Clearly, the derivation of a NOAEL based on data available from the in vivo animal studies is not possible, and further carefully designed multiple dose studies are needed.

In Vitro Mechanistic Studies of Cd

In vitro systems in which cells are exposed to heavy metals can be useful in elucidating the mechanisms whereby heavy metals produce their immunotoxic effects. In such systems, knowledge of the levels of Cd that are cytotoxic to cells is of importance. In this respect, CdCl₂ concentrations of 0.1, 1.0, or 10.0 ppm were not cytotoxic to mouse splenocytes whereas concentrations of 1000 ppm resulted in cytotoxicity (133).

Using an in vitro system, it was shown that the NK cell activity and antibody-dependent cellular cytotoxicity of human PBL were suppressed in a dose- and time-dependent manner when incubated with CdCl₂ (134,135). The cytotoxic effects were observed only when Cd was added to the cell culture very early in the assay. Moreover, preincubation of the human PBL with IL-2 for 4 days did not abrogate the Cd-mediated effects, suggesting that the effects of cadmium could be on an early signaling pathway in these cells (134).

Data on the in vitro effects of Cd on humoral immune responses indicated that Cd had an enhancing effect. For example, CdCl₂ added to spleen mononuclear cell cultures of BALB/c mice at quantities of 4 to 40 μM for 5 days, resulted in a significantly increased PFC response to SRBC (136). Similarly enhanced PFC numbers to SRBC were observed in cultures of CBA/J mice or when spleen mononuclear cells from either CBA/J or BALB/c mice were incubated with CdCl₂ at quantities of 0.1, 1.0, 10.0, or 100 μM (9,137). A significant enhancement was also observed for the PFC numbers to the T-independent antigen DNP-Ficoll when BALB/c mouse mononuclear cells were cultured with 0.04 mM of CdCl₂, whereas significantly decreased PFC numbers were detected at doses of 0.1 or 0.2 mM (138).

In contrast to the observed enhancing effect of Cd on PFC numbers, a uniformly decreased mitogen-induced lymphoproliferative activity was obtained when Fischer 344 rat splenocytes were cocultured with 0.05 to 500 μM of CdCl₂ (139). A significantly decreased proliferative activity in response to PWM and purified protein derivative was also observed when human PBL were incubated with 25 or 50 μM for 3 days (140). Likewise, the lymphoproliferative response of ConA-stimulated human PBL was significantly decreased in cocultures with 1 to 100 μM of Cd (141). The observed inhibition was dose-dependent and detected only when cadmium was added to cultures concurrently. A marginal inhibitory effect was observed if the metal was added after the first hour of cell culture, suggesting that cadmium could affect an early step in the mitogen-induced stimulation (141). When CdCl₂ in quantities of 0.05 to 1.0 μM were cultured with pooled human PBL for 7 days, a dose-independent increased proliferation of the unstimulated (no mitogen) cells and increased Ig levels in culture supernatants was observed, whereas PWM-induced Ig secretion in culture supernatants was not affected by Cd (100). The significance of these in vitro findings is not clear since a considerable interindividual variability in PWM-activation and Ig secretion was observed when each subject’s lymphocytes were tested separately; this suggests that genetic factors may play a role in the observed variable effects of cadmium (99).

Thus, data derived from the in vitro studies demonstrated that Cd is a potential immunomodulator at noncytotoxic levels of exposure. From the mechanistic point of view, it has been proposed that Cd may disrupt cell to cell contact between subpopulations of lymphocytes (142) and thus interfere with cell signaling mechanisms (143). The activation of lymphocytes for signal transduction includes hydrolysis of phosphatidylinositol-4,5-diphosphate to diacylglycerol and inositol 1,4,5-triphosphate (IP₃), which in turn activate protein kinase C (PKC) and stimulate the release of calcium from intracellular compartments (141). Two independent studies have demonstrated that hydrolysis of IP₃ was decreased up to 50% in the presence of cadmium (134,141). Another mechanism by which Cd may induce its immunotoxic effects is probably related to interference with the role of calcium. It has been demonstrated that Cd added to cultures inhibited the proliferation of lymphocytes when stimulated with phorbol myristate acetate an activator of PKC (141). These results are suggestive of a potential action of Cd downstream to PKC or of a direct Cd-induced inhibition of PKC (144). Moreover, cadmium has been demonstrated to be an inhibitor of calcium channels (142) and a competitor of Ca²⁺ ions resulting in interference with Ca²⁺-dependent enzymatic functions such as calmodulin (145). Overall, the mechanism of Cd-induced immunomodulation remains elusive, and further mechanistic studies are required to clearly define the potential adverse immunotoxic effects of Cd on human health.

Future Considerations

There is a need to design protocols using multiple doses, including low-dose exposures, and to perform experiments with built-in quality assurance and quality control methods. Further research is required to define the potential interactive effects of the heavy metals present in the Great Lakes basin, which have been shown to accumulate in fish tissues. Parallel to these requirements there is an urgent need to establish a set of immunologic assays for use in humans and to include these assays in future designs of epidemiologic studies in cohorts who are exposed to these metals either inadvertently, occupationally, or through ingestion of contaminated fish from the Great Lakes.
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