



Organic mercury compounds and autoimmunity[☆]

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Abstract

Based on *in vitro* studies and short-term *in vivo* studies, all mercurials were for a long time considered as prototypic immunosuppressive substances. Recent studies have confirmed that organic mercurials such as methyl mercury (MeHg) and ethyl mercury (EtHg) are much more potent immunosuppressors than inorganic mercury (Hg). However, Hg interacts with the immune system in the presence of a susceptible genotype to cause immunostimulation, antinuclear antibodies targeting fibrillar, and systemic immune-complex (IC) deposits, a syndrome called Hg-induced autoimmunity (HgIA). Recent studies in mice with a susceptible genotype has revealed that the immunosuppressive effect of MeHg and EtHg will within 1–3 weeks be superseded by immunostimulation causing an HgIA-like syndrome. At equimolar doses of Hg, MeHg has the weakest immunostimulating, autoimmunogen, and IC-inducing effect, while the effect of thimerosal is similar to that of inorganic mercury. The immunosuppression is caused by the organic mercurials per se. Since they undergo rapid transformation to inorganic Hg, studies are being undertaken to delineate the importance of the organic substances per se and the newly formed inorganic Hg for induction of autoimmunity.

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1. Introduction: mercury and mercuric compounds

Mercury (Hg) is a naturally occurring metal due to erosion from earth crusts and volcanoes, but anthropogenic sources have increased the exposure in recent time. The most common forms of Hg in the environment are elemental Hg (Hg^0), inorganic Hg (Hg^+ and Hg^{2+}), and organic compounds such as methyl mercury (MeHg), while ethyl mercury (EtHg) and phenyl mercury (Phe-Hg) are more uncommon [1]. Elemental Hg is oxidized to mercuric mercury (Hg^{2+}), which can be methylated by microorganisms forming MeHg, a process which takes place mainly in aquatic environments and brings MeHg into the food chain making it the most important source for Hg exposure in non-amalgam bearers. Thimerosal consists of an organic radical, ethyl mercury (EtHg), bound to the sulfur atom of the thiol group of salicylic acid, and contains 49.6% Hg by weight. Following tissue absorption, EtHg rapidly dissociates from the thiosalicylic moiety and binds to thiol ligands in tissue proteins. Thimerosal has been extensively used as a preservative in medical preparations, including vaccines [2]. A very important biochemical property of Hg is the strong affinity to sulfhydryl groups (thiols), which leads to formation of complexes called mercaptides. Sulfhydryls are especially common in cysteine-rich proteins, and binding of Hg to thiols even at a low concentration may have distinct effects on cell function [3]. Methyl mercury compounds are also able to form methyl mercury cysteines, which is probably a major compound in tissues as recently demonstrated in fish muscles [4].

2. Suppressive effects of mercurials on the immune system

2.1. In vitro studies

The effect of mercurials on the immune system was for a long time synonymous with immunosuppression [5], as expected from the cytotoxic effect of all mercurials, which varies from toxic to supertoxic (dialkylmercuric derivatives) [1]. In vitro, the LD_{50} for HgCl_2 is ca. 10^{-5} – 10^{-6} M for T- and B-cells [6–8]. Mercuric chloride affects the function of cells and organs at lower doses. For an example, mitogen-induced lymphocyte proliferation is inhibited at 10^{-7} M [6]. The LD_{50} for MeHg in lymphocytes is 10^{-6} M, but lymphocyte functions are inhibited already at a dose of 10^{-7} – 10^{-8} M. In addition, the LD_{50} is 2.5-fold higher in T- as compared with B-cells, and a 10-fold higher concentration is needed to inhibit mitogen-induced T-cell proliferation as compared with B-cell proliferation [8].

2.2. In vivo studies

100 mg HgCl_2 /L drinking water (ca. 1800 μg Hg/kg body weight [bw]/day) for 28 days did not affect the T- and B-cell populations in the bone marrow and spleen in outbred CD-1 mice [9]. A dose of ca. 900 μg Hg/kg bw/day as HgCl_2 for 7 weeks to B6C3F1 hybrid mice did not affect the mitogen-induced lymphocyte response [10], and ca. 300 μg Hg/kg bw/day did not reduce the number of T- and B-cells in inbred A.SW mice [11]. In contrast, MeHg doses of 280–600 μg Hg/kg bw/day reduced the immune response in outbred mice [12,13]. Subcutaneous injections of 540 μg Hg/kg

bw/day as MeHg caused after 5 days 35% reduction of splenocytes, 47% reduction of B-cells, and 9% reduction of T-cells in A.SW mice [14]. The higher susceptibility of B-cells is in accordance with in vitro observations [8]. A similar dose of Hg (590 µg Hg/kg bw/day), given as thimerosal, caused 65% reduction in the number of T- and B-cells in the spleen during the first weeks after onset of treatment [15]. These observations clearly demonstrate that both methyl mercury and thimerosal (ethyl mercury) are much more potent immunosuppressive substances than inorganic Hg.

3. Stimulating effect of mercurials

3.1. In vitro studies

Inorganic mercury induces a cell- and concentration-dependent proliferation of adult T-cells but not B-cells or immature T-cells (thymocytes) [16,17]. The in vitro proliferation is dependent on MHC class II [18] as well as costimulatory molecules, especially IL-1 [17], and there are evidence of an oligoclonal T-cell proliferation [19]. Mercury may cause cell proliferation by perturbing lymphocyte signalling [20,21], and by attenuating apoptosis of lymphocytes due to interference with the *Fas–Fas* ligand interaction [22], or by acting on the nuclear level increasing the Bcl-2 content [23]. Proliferation and defective apoptosis might lead not only to expansion of peripheral lymphocytes, but also allowing autoreactive T-cells to escape IFN- γ -dependent activation-induced cell death.

MeHg may increase mitogenic T- and B-cell responses [10,13,24], and a low concentration of MeHg induces tyrosin phosphorylation of Jurkat T-cells [25]. MeHg may promote apoptosis, but, unlike HgCl₂, is unable to induce Bcl-2 expression [23]. In vivo, Stiller-Winkler et al. reported that injection of MeHg in several mouse strains caused a more profound and systemic lymphoproliferative effect than inorganic Hg, also in the DBA/2 strain, which is markedly resistant to the effect of HgCl₂ [26].

3.2. Systemic autoimmune reactions after exposure to inorganic mercury

Mercury-induced autoimmunity (HgIA) has been described in rats [27] and mice [28], but almost

exclusively after exposure to inorganic Hg. The characteristics of HgIA include lymphoproliferation with T-cell-dependent polyclonal B-cell activation, hypergammaglobulinemia [11,29], dose-dependent production of autoantibodies targeting the 34-kDa nucleolar protein fibrillarin [28], and development of systemic immune-complex deposits [30,31]. Development of autoantibodies against fibrillarin (AFA) in mice treated with Hg is strongly linked to the mouse MHC (H-2) haplotypes *s* and *q*, whereas most other haplotypes are resistant to induction of AFA [32]. The other immunoproliferative and autoimmune manifestations are not linked to H-2.

3.3. Autoimmune effects of MeHg and thimerosal

A Hg²⁺ dose of approximately 300–600 µg/kg bw/day in the form of HgCl₂ [11], MeHg [14,33], or thimerosal [15] caused autoantibodies targeting the 34-kDa nucleolar protein fibrillarin (ANoA/AFA) in mice with a susceptible H-2 haplotype (H-2^s) (Fig. 1). Although the H-2A locus regulates susceptibility to ANoA/AFA development, background (non-H-2) genes also play a role, affecting the titre in the following way: A.SW>SJL>B10.S. This relationship was observed for all three Hg species [14,15,33]. A dose–response relationship exists for HgCl₂ [34] and thimerosal [35] with regard to ANoA/AFA. This was not seen for MeHg, but ANoA/AFA developed more rapidly with higher doses of MeHg [14]. Basic requirements for induction of ANoA/AFA by HgCl₂, such as T-cells [36], IFN- γ /IFN- γ receptor [37], CD28 and CD40L [38], have been confirmed also for thimerosal [15], but not yet studied for MeHg (Table 1).

3.4. Immune stimulation and lymphoproliferation of MeHg and thimerosal

HgCl₂ does not cause any initial immunosuppression (see above), but already a dose of 300 µg Hg/kg bw/day results from day 3 in an increase in splenic T- and B-cells in genetically Hg-susceptible mice (A.SW-H-2^s) [11]. In MeHg-treated A.SW mice, the initial decline in splenic T- and B-cells changed to an increase 9 days after onset of treatment [14], while this was seen after 30 days treatment with a similar dose of Hg in the form of thimerosal [15]. Although

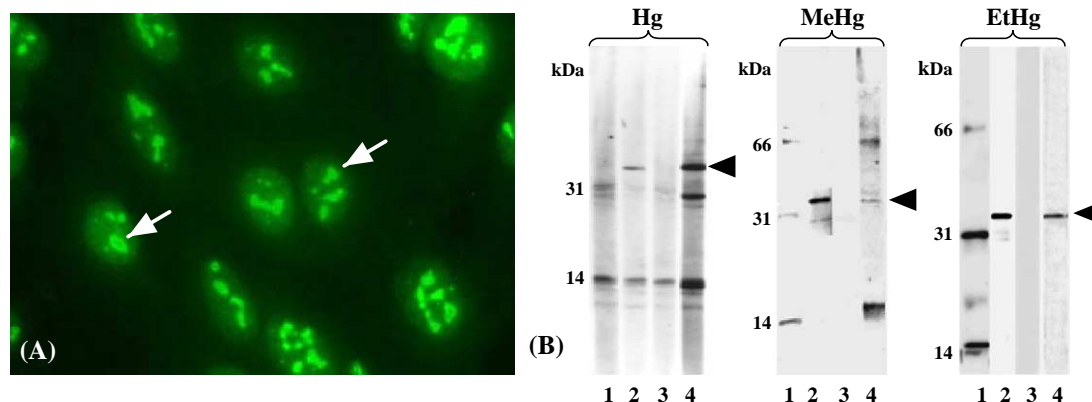


Fig. 1. (A) Serum antinucleolar antibodies from thimerosal-treated A.SW (H-2^s) mice assessed by indirect immunofluorescence using HEP-2 cells. Strong “clumpy” staining outlining the nucleoli (arrows) and a weak nucleoplasmic staining. (B) Immunoblotting of mouse sera from H-2^s mice (A.SW and SJL) treated with either inorganic mercury (Hg), methyl mercury (MeHg), or thimerosal (EtHg), and controls. Lane 1: molecular weight markers. Lane 2: human reference serum targeting an apparent 34-kDa protein (fibrillarin). Lane 3: serum from control mice. Lane 4: serum from mice treated with Hg, MeHg, or thimerosal showing a “clumpy” nucleolar staining on HEP-2 cells. Sera target a protein with an apparent molecular weight of a 34-kDa fibrillarin (arrowhead).

delayed, the lymphoproliferative response was much more vigorous in the thimerosal-treated mice, especially regarding T-cells and polyclonal B-cell activation [14,35], which makes thimerosal similar to HgCl₂ [11]. Increase of IgE is a hallmark of HgIA [34]. Because of the close link between IL-4 and IgE [39], the initial increase of IL-4 mRNA expression was

immediately followed by an increase of serum IgE [40], and the strength of the IL-4 mRNA/serum IgE increase was in the order: HgCl₂>thimerosal>MeHg [14,15,28,40].

The cytokine mRNA pattern for HgCl₂ and thimerosal was similar with an initial modest increase of IL-2 and IFN- γ , followed by a distinct increase of IL-4, which was more prominent after HgCl₂ treatment [14,40]. Equimolar dose of Hg in the form of MeHg caused an increase in only IL-4 mRNA [14].

Table 1

Immune parameters in A.SW (H-2^s) mice treated with mercury in the form of mercuric chloride, methyl mercury or ethyl mercury (thimerosal)

Immune parameter	HgCl ₂	MeHg	Thimerosal
Immunosuppression	$\pm 0^a$	+ ^b	++ ^c
Serum antinucleolar/antifibrillarin antibodies	+++ ^a	+ ^b	++ ^c
Polyclonal B-cell activation	+++ ^a	$\pm 0^b$	++ ^c
Splenic B-lymphocytes	++ ^a	+ ^b	+ ^c
Splenic T-lymphocytes	+++ ^a	+ ^b	++ ^c
Total serum IgG1 concentration	+++ ^a	+ ^b	+ ^c
Total serum IgG2a concentration	+++ ^a	$\pm 0^b$	+ ^c
Total serum IgE concentration	+++ ^a	+ ^b	++ ^c
Immune-complex deposits			
Glomerular deposits	++ ^a	$\pm 0^b$	+ ^c
Systemic vessel walls deposits	++ ^a	$\pm 0^b$	+ ^c

± 0 , no change; +, slight increase; ++, moderate increase; +++, strong increase.

^a From Johansson et al. [11].

^b From Häggqvist et al. [14].

^c From Havarinasab et al. [15].

3.5. Systemic immune-complex deposits

Thimerosal had the same ability to induce renal mesangial and systemic vessel wall immune-complex (IC) deposits as HgCl₂ [34,35], while MeHg did not induce such deposits, even if the dose of MeHg was substantially increased [14].

4. Summary

The organic mercurials are much more potent immunosuppressors than inorganic mercury. The suppression is long-lived in most strains, but is superseded after 1–3 weeks in genetically susceptible strains by lymphoproliferation, hyperimmunoglobulinemia, and autoantibodies targeting the nucleolar

protein fibrillar (AFA), otherwise seen in some 10% of patients with systemic scleroderma. While the organic mercurials are directly responsible for the immunosuppressive effect, it is unknown to what extent they contribute to the autoimmune reaction. This uncertainty is due to the rapid transformation in the body of ethyl Hg and methyl Hg to inorganic Hg (mercuric mercury), which reached a maximum after 14 days thimerosal treatment, but was still rising after 30 days MeHg treatment. Increased concentrations of inorganic mercury is therefore available to the immune system almost from onset of exposure to thimerosal or MeHg, and may theoretically be either solely responsible for the autoimmune reaction, or contributing to varying extent. Studies are being pursued to try to establish the autoimmunogen potency of MeHg and thimerosal per se.

Take-home messages

- The organic mercurials methyl mercury and thimerosal (ethyl mercury) cause immunosuppression in mice, which is not seen after treatment with inorganic mercurials.
- In genetically susceptible mice, the immunosuppression effect of organic mercury compounds is superseded by immunostimulation and autoimmunity after 1–3 weeks.
- Similar to treatment with inorganic mercury treatment (HgCl_2), organic mercurials induce in genetically susceptible mouse strains autoantibodies targeting the 34-kDa nucleolar protein fibrillar (AFA).
- The genetic susceptibility for induction of AFA with mercurials is localized in the H-2A locus, but background (non-H-2) genes affect the titre and the threshold dose.
- AFA induction requires T-cells, costimulatory molecules (CD28 and CD40L), and IFN- γ .
- The cytokines IL-2, IFN- γ , and IL-4 mRNA are increased in lymph nodes after treatment with HgCl_2 and ethyl mercury, but MeHg causes an increase in mainly IL-4 mRNA.
- A general activation of the immune system and systemic immune-complex deposits are induced by HgCl_2 and ethyl mercury, but not by methyl mercury.

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Intrathecal synthesis of autoantibodies against tissue transglutaminase

Anti-tissue transglutaminase (tTG) antibodies (AtTGA) are typically found in sera of patients with untreated celiac disease (CD). tTG occurs in cerebrospinal fluid (CSF) and its assay in CSF was suggested to be diagnostically useful in neurological disorders. However, nothing is known about AtTGA in CSF. Schrodler D. et al. (*J Autoimmunity* 2004; 22: 335–340) analyzed IgA- and IgG-AtTGA (assayed by ELISA) in 129 unselected CSF-serum pairs. For comparison, IgA- and IgG- anti-gliadin antibodies (AGA), typically coexisting with AtTGA were measured. AtTGA were detected in 27 (IgA) and in 63 (IgG) CSF samples. Antibody indices (AI) could be calculated for AtTGA from 21 (IgA) and from 61 (IgG) sample pairs. AI for AtTGA was > 2 in 11 (IgA) and in 22 (IgG) sample pairs, hinting to intrathecal antibody synthesis. AI for AGA was > 2 only for 1 (IgA) and 2 (IgG) sample pairs. This is the first demonstration of AtTGA in CSF and their intrathecal synthesis.