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Dose and Hg species determine the T-helper cell activation in murine autoimmunity

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Abstract

Inorganic mercury (mercuric chloride—$HgCl_2$) induces in mice an autoimmune syndrome (HgIA) with T cell-dependent polyclonal B cell activation and hypergammaglobulinemia, dose- and H-2-dependent production of autoantibodies targeting the 34 kDa nucleolar protein fibrillarin (AFA), and systemic immune-complex deposits. The organic mercury species methylmercury (MeHg) and ethylmercury (EtHg—in the form of thimerosal) induce AFA, while the other manifestations of HgIA seen after treatment with $HgCl_2$ are present to varying extent. Since these organic Hg species are converted to the autoimmunogen Hg^{2+} in the body, their primary autoimmunogen potential is uncertain and the subject of this study.

A moderate dose of $HgCl_2$ (8 mg/L drinking water – internal dose 148 μg Hg/kg body weight [bw]/day) caused the fastest AFA response, while the induction was delayed after higher (25 mg/L) and lower (1.5 and 3 mg/L) doses. The lowest dose of $HgCl_2$ inducing AFA was 1.5 mg/L drinking water which corresponded to a renal Hg^{2+} concentration of 0.53 μg/g. Using a dose of 8 mg $HgCl_2$/L this threshold concentration was reached within 24 h, and a consistent AFA response developed after 8–10 days. The time lag for the immunological part of the reaction leading to a consistent AFA response was therefore 7–9 days. A dose of thimerosal close to the threshold dose for induction of AFA (2 mg/L drinking water—internal dose 118 μg Hg/kg bw per day), caused a renal Hg^{2+} concentration of 1.8 μg/g. The autoimmunogen effect of EtHg might therefore be entirely due to Hg^{2+} formed from EtHg in the body. The effect of organic and inorganic Hg species on T-helper type 1 and type 2 cells during induction of AFA was assessed as the presence and titre of AFA of the IgG1 and IgG2a isotype, respectively. EtHg induced a persistent Th1-skewed response irrespectively of the dose and time used. A low daily dose of $HgCl_2$ (1.5–3 mg/L) caused a Th1-skewed AFA response, while a moderate dose (8 mg/L) after 2 weeks resulted in a balanced or even Th2-skewed response. Higher daily doses of $HgCl_2$ (25 mg/L) caused a balanced Th2–Th1 response already from onset. In conclusion, while metabolically formed Hg^{2+} might be the main AFA-inducing factor also after treatment with EtHg, the quality of the Hg-induced AFA response is modified by the species of Hg as well as the dose.

Keywords: Ethyl Hg; Thimerosal; $HgCl_2$; Mice; Autoimmunity; Th1/Th2

1. Background

Induction of autoimmunity by inorganic mercury (Hg^{2+}) has been described in rats, mice and rabbits (Sapin et al., 1977; Roman-Franco et al., 1978; Hultman et al., 1996). The characteristics of murine

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mercury-induced autoimmunity (HgIA) are lymphoproliferation with T cell-dependent polyclonal B cell activation and hypergammaglobulinemia (Pietsch et al., 1989; Pollard and Hultman, 1997), dose-dependent production of autoantibodies targeting the 34 kDa nucleolar protein fibrillarin (AFA) (Hultman et al., 1989; Reuter et al., 1989; Nielsen and Hultman, 2002), and systemic immune-complex (IC) deposits (Hultman and Enestrom, 1988; Robinson et al., 1997). The induction of autoantibodies against fibrillarin in Hg-treated mice is strongly linked to the mouse MHC (H-2) while other manifestations of HgIA are linked to non-H-2 loci (Kono et al., 2001). HgIA was initially considered a "Th2-type of disease" due to the phenotypic expression of the condition (Bernaudin et al., 1981; Goldman et al., 1991; van Vliet et al., 1993). However, studies in genetically HgIA-susceptible mice with inherited (Johansson et al., 1998) or induced (Bagenstose et al., 1998; Kono et al., 1998) deficiency for IL-4, or a lack of IFN-γ/IFN-γ receptor (Kono et al., 1998), showed that HgIA is dependent not on IL-4 but on IFN-γ ("Th1-type of disease").

The major substance used for induction of murine HgIA is inorganic Hg ($HgCl_2$), probably because the association of organic mercury compounds with immunosuppression (Descotes, 1986). However, in 1981 Bernaudin et al. reported systemic IC deposits in genetically susceptible Brown Norway rats after exposure to methyl mercury (MeHg), similar to the reaction after $HgCl_2$ exposure (Bernaudin et al., 1981). Stiller-Winkler et al. later showed that MeHg initiates a strong systemic lymphoproliferative effect also in mouse strains which are low responders (C57BL/6) or totally resistant (DBA/2) to induction of HgIA by $HgCl_2$ (Stiller-Winkler et al., 1988). More recently MeHg (Hultman and Hansson-Georgiadis, 1999; Haggqvist et al., 2005) and EtHg (Havarinasab et al., 2004) were reported to induce AFA in H-2^s mice. Treatment with $HgCl_2$ (al-Balaghi et al., 1996) or thimerosal (Havarinasab and Hultman, 2006) in (NZB × NZW)F1 mice with a genetic predisposition for spontaneous development of systemic autoimmunity in certain respects aggravated the autoimmune syndrome. Since both MeHg (WHO, 1991; Haggqvist et al., 2005) and EtHg (Magos et al., 1985; Havarinasab et al., 2005) are converted to Hg^{2+} in the body, the autoimmunogen effect of these organic Hg species could theoretically be due solely or partly to the Hg^{2+} produced in the body from the organic compounds. If this conversion could be suppressed, the primary effect of organic Hg species might easily have been determined. However, although modification of the diet and suppression of the gut flora by antibiotics increase the ratio of organic/inorganic Hg in the tissues after MeHg exposure (Rowland et al., 1984), a substantial amount of tissue Hg will still be present as inorganic Hg following conversion from the organic mercury species.

In order to establish differences between organic and inorganic Hg species regarding induction of HgIA, it was necessary to first study the effect of different Hg concentrations on the induction phase. Following early studies in rats and mice (Druet et al., 1977; Enestrom and Hultman, 1984), the internal dose used for induction of HgIA using $HgCl_2$ has usually been 250–400 μg Hg/kg bw per day. Apart from dose–response studies focusing on the threshold for the different manifestations of HgIA (Hultman and Nielsen, 2001), few data exist on the dose-dependency of other aspects, such as the balance between T-helper type 1 and type 2 cells in the autoimmune response. We have therefore determined the treatment time and the renal Hg^{2+} concentration required for induction of AFA by $HgCl_2$, and compared these data with the renal Hg^{2+} concentration caused by treatment with EtHg close to the threshold dose for induction of AFA. In order to determine the effect of the dose and Hg species on T-helper cell subtypes, we assessed the IgG isotype of the developing AFA in analogy with studies on the T-helper cell response after immunization (Hovden et al., 2005).

2. Material and methods

2.1. Mice

Previous studies on the influence of gender on development of HgIA in genetically susceptible strains (Nielsen, 1992; Nielsen and Hultman, 2002) prompted us to use female mice because of their higher sensitivity for induction of autoimmunity. Female A.SW (H-2^s) mice were obtained from Taconic M and B (Ry, Denmark) and maintained in the animal facilities of the Faculty of Health Sciences, Linköping. All mice were 9–13 weeks old at onset of the experiments. Mice were housed under 12 h dark–12 h light cycles, kept in steel-wire cages, and given R70 pellets (Lactamin, Vadstena, Sweden) and tap water ad libitum. The pellets contained 23 ng Hg^{2+}/g and 4 ng MeHg as Hg/g, whereas the ethylmercury (EtHg) concentration was below the detection limit (<0.1 ng/g) (Qvarnstrom et al., 2003). The studies were approved by the local animal ethics committee.

2.2. Treatment

Groups of mice were treated with different doses of mercuric chloride ($HgCl_2$) (Fluka, Seelze, Germany) or thimerosal $C_9H_9HgNaO_2S$ (Fluka, Seelze, Germany) in the drinking water.