Clin Exp Immunol 1997; **107**:68–75

Thiol compounds inhibit mercury-induced immunological and immunopathological alterations in susceptible mice

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(Accepted for publication 16 September 1996)

SUMMARY

In vitro mercury induces a high proliferative response in splenic lymphocytes and *in vivo* it induces a systemic autoimmune disease in susceptible mouse strains. This disease is characterized by increased serum levels of IgE and IgG1 antibodies, by the production of anti-nucleolar antibodies and by the formation of renal immune complex deposits. We have previously found that the presence of 2-mercaptoethanol (2-ME) inhibited mercury-induced cell proliferation *in vitro*. In this study, we tested the effects of four other thiol compounds, namely dithiothreitol (DTT), L-cysteine, *meso*-2,3-dimercaptosuccinic acid (*meso*-DMSA) and 2,3-dimercapto-1-propanesulfonic acid, Na salt (DMPS) on mercury-induced immunological changes both *in vitro* and *in vivo*. We found that *in vitro*, the addition of all thiol compounds abrogated mercury-induced cell aggregation and proliferation. *In vivo*, injection of *meso*-DMSA and/or DMPS (s.c. or i.p.) immediately following exposure to mercury markedly decreased IgG1 synthesis in spleen cells and serum IgE levels in mercury-susceptible SJL mice. Treatment with DMPS also prevented mercury-induced IgG1 anti-nucleolar antibody synthesis and the development of mesangial IgG1 immune complex deposits in SJL mice.

Keywords mercury thiol compounds autoimmunity anti-nucleolar antibody renal immune complex deposits

INTRODUCTION

Several studies have shown that mercuric chloride ($HgCl_2$) induces a systemic autoimmune disease in susceptible mice and rats (reviewed in [1–3]). In susceptible mice, the mercury-induced autoimmune disease is characterized by increased serum levels of IgG1 and IgE antibodies, by the production of anti-nucleolar antibodies (ANolA) and by the development of immune complex-mediated glomerulonephritis [1–3]. The cellular and molecular mechanisms by which mercury activates the immune system leading to the development of an autoimmune disorder are not fully understood. It is known that mercury is able to bind to and modify the chemical structure of cell surface proteins which contain sulfhydryl/disulfide groups [4]. This led to the postulation that mercury possibly modifies the chemical structure of the molecules of the immune system, e.g. MHC class II molecules, T cell receptors and/or self peptides, in such a way that modified structures could be recognized as foreign molecules by T cells [1].

In order to study the mechanism(s) by which mercury affects the immune system, we established an *in vitro* system and found that mercury primarily activated murine T lymphocytes, resulting in transformation and proliferation [5]. We also found that the presence of a commonly used thiol compound, 2-mercaptoethanol (2-ME), which has been shown to enhance DNA synthesis in mitogen stimulation [6–8], was highly inhibitory for mercury-induced T lymphocyte proliferation [5]. This observation and the fact that mercury has a propensity to react with the sulfhydryl group of thiol compounds led us to suggest that thiol compounds, including 2-ME, compete with cell surface proteins containing sulfhydryl groups in binding to mercury and are therefore, capable of preventing the mercury-induced immunological changes both *in vitro* and *in vivo*. The main objective of this study was to test this suggestion. We examined five thiol compounds, namely 2-ME, dithiothreitol (DTT), L-cysteine, *meso*-2,3-dimercaptosuccinic acid (*meso*-DMSA) and 2,3-dimercapto-1-propanesulfonic acid, Na salt (DMPS) for their inhibitory effects on *in vitro* mercury-induced T lymphocyte proliferation. We also tested the preventive effects of *meso*-DMSA and DMPS on *in vivo* mercury-induced antibody/autoantibody production and renal immune complex deposit formation.

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MATERIALS AND METHODS

Mice

Female BALB/c and SJL mice were bred and maintained in our animal facilities at the Department of Immunology, Stockholm

University. Both BALB/c and SJL mice were 6–8 weeks old at the beginning of the experiments.

Reagents and drugs

Lipopolysaccharide (LPS) was extracted from *Escherichia coli* 055:B5 (Department of Bacteriology, Karolinska Institute, Stockholm, Sweden). Concanavalin A (Con A) was purchased from Pharmacia (Uppsala, Sweden). $HgCl_2$ and 2-ME were purchased from Merck (Darmstadt, Germany). L-cysteine, DTT, *meso*-DMSA and DMPS were purchased from Sigma (St Louis, MO).

Cell preparation and culture conditions

Spleen cells from BALB/c mice were washed twice with Earle's balanced salt solution (BSS) and cultured at a concentration of 3×10^6 cells/ml in RPMI 1640 medium containing 10% fetal calf serum (FCS), 0·075% $NaHCO_3$, 50 U/ml penicillin, 50 μg/ml streptomycin, 10 mM HEPES and 2 mM L-glutamine (all reagents were purchased from GIBCO, Life Technologies, Gaithersburg, MD) in flat-bottomed 96-well plates in a final volume of 0·2 ml/well. Cells were cultured in the presence of different stimuli such as $HgCl_2$ (10 μM), LPS (25 μg/ml) or Con A (2·5 μg/ml). In the proliferation/aggregation inhibiting experiments, different concentrations (as indicated in the figures) of 2-ME, DTT, L-cysteine, *meso*-DMSA, DMPS were added to the medium at the start of the cultures. Cultures were incubated at 37°C in a humidified incubator containing 5% CO_2 for the indicated time periods.

^{3}H-thymidine incorporation assay

^{3}H-thymidine (Amersham International, Aylesbury, UK) at a concentration of 2 μCi/ml was added to the cultures at different time periods (as indicated in the figures). After a 6-h pulse of ^{3}H-thymidine, the cells were harvested (semi-automative cell harvester; Skatron, Lier, Norway) and the incorporated radioactivity was analysed by liquid scintillation counting in a β-counter (1218 Rackbeta Liquid Scintillation Counter; LKB Vallac, Bromma, Sweden).

In vivo *treatment with $HgCl_2$ and thiol compounds*

A solution of 0·4 mg/ml $HgCl_2$ was prepared in sterile saline. Five groups of SJL mice (three to four mice per group) were injected subcutaneously with 0·1 ml of $HgCl_2$ solution (1·6 mg/kg body weight) twice a week for 3 weeks. Groups 1 and 2 of the mercury-injected mice were treated intraperitoneally with thiol compounds, *meso*-DMSA (30 mg/kg body weight) or DMPS (30 mg/kg body weight) twice a week for 3 weeks, started immediately following the administration of mercury. Similar treatment procedures were used for groups 3 and 4 of the mercury-treated mice, except that treatment with thiol compounds started 1 week after the first injection with mercury. Group 5 of mercury-injected mice was left without any drug treatment. Three control groups were included in this experiment. One group was injected subcutaneously with sterile saline. The other two groups received continual injection of thiol compounds, *meso*-DMSA or DMPS at a concentration of 30 mg/kg body weight by the i.p. route.

In a separate experiment, procedures similar to the above were used, except that only DMPS was used, groups 3 and 4 were not included in the experiment, and that the s.c. route was chosen for the administration of the thiol compound. In this experiment we had only saline-injected mice as the control group.

Spleen cell suspension and serum preparation

The mice were bled by retro-orbital puncture under light ether anaesthesia. Thereafter, they were killed by cervical dislocation and their spleens were removed. Single-cell suspensions were prepared by teasing spleens gently with forceps in BSS. All cell suspensions were washed three times and resuspended in BSS before haemolytic plaque assays. The blood of each mouse was allowed to clot at room temperature. Serum was separated after centrifugation and stored at −20°C until tested for antibody content.

Protein-A plaque assay

Splenic antibody-secreting cells of different immunoglobulin classes and subclasses were enumerated by using a protein-A plaque assay as described by Gronowics *et al*. [9]. Rabbit anti-mouse IgM, IgG1, IgG3 (Organon Teknika, Durham, NC) and IgG2b (Nordic Immunological Laboratories, Tilburg, The Netherlands) were used as developing reagents.

ELISA for mouse IgE antibody

Total mouse serum IgE antibodies were determined by a sandwich ELISA assay as described previously [10], using a rat anti-mouse IgE MoAb, R35-72 (Pharmingen, San Diego, CA) as a primary antibody and a biotinylated rat anti-mouse MoAb, R35-79 (Pharmingen) as a secondary antibody.

Detection of antinucleolar antibodies

The presence of ANolA in mice sera was determined by indirect immunofluorescence using rat liver sections as a substrate [10]. The initial serum dilution was 1:30. The highest serum dilution at which nucleolar fluorescence could be detected was defined as the titre of ANolA.

Detection of renal immune complex deposits

The presence of glomerular deposits of IgG1 antibodies was detected by direct immunofluorescence as described previously [10]. Briefly, The acetone-fixed kidney cryostat sections were incubated with serial dilutions of FITC-conjugated goat anti-mouse IgG1 antibodies (Southern Biothechnology, Birmingham, AL). The initial dilution for FITC-conjugated antibody was 1:30. The highest dilution of the antibody at which a specific fluorescence could be seen was defined as the titre of the glomerular deposits.

Statistical analysis

Results were subjected to statistical analysis by Student's *t*-test.

RESULTS

Thiol compounds inhibit mercury-induced cell proliferation in vitro

Low concentration of mercury induced a proliferative response of splenic lymphocytes from high-responder mice and the presence of 2-ME was found to abrogate cell proliferation [5]. To analyse further whether the other thiol compounds also have similar effects, we tested the four thiol compounds with a chemical structure close to 2-ME, i.e. DTT, L-cysteine [6], *meso*-DMSA and DMPS [11–13] at various concentrations from 0·01 μM to 100 μM. As shown in Fig. 1a–e, the presence of either of four thiol compounds in the medium also abrogated cell proliferation induced by mercury in a dose-dependent manner. At the standard concentration of 50 μM (a routine concentration of 2-ME in a normal cell culture), five thiol compounds were effective with

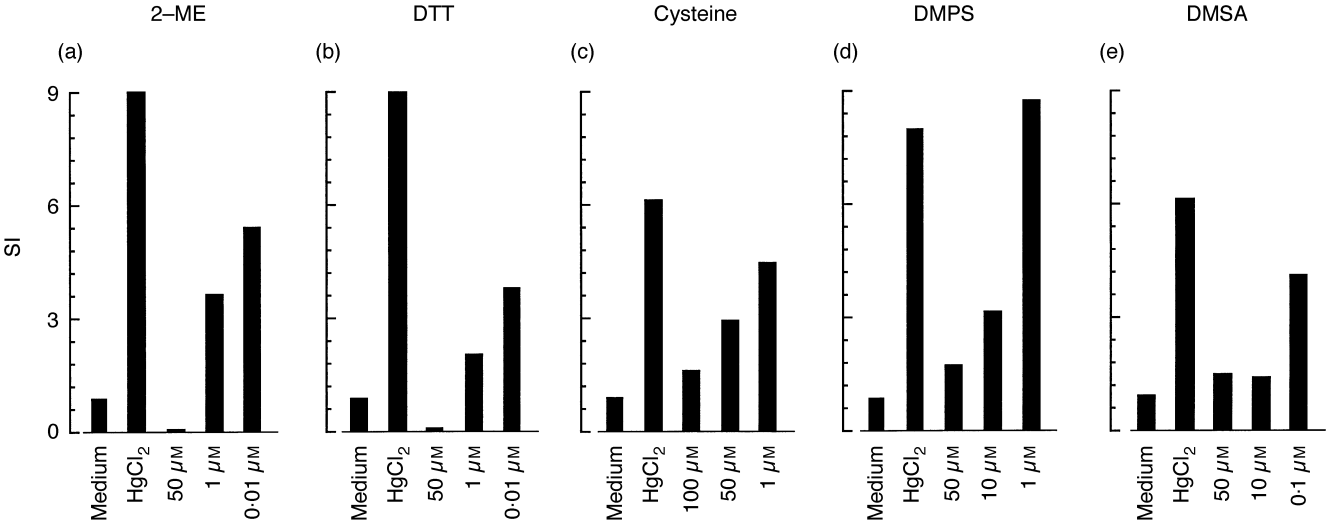

Fig. 1. Inhibitory effects of thiol compounds on mercury-induced cell proliferation. Spleen cells (3×10^6/ml) from BALB/c mice were incubated with mercuric chloride as described in Materials and Methods. Different thiol compounds were added at the beginning of the culture at indicated concentrations. ^{3}H-thymidine was added (2 μCi/ml) on day 3 or day 4 and cells were pulsed for 6 h. SI, Stimulation index, calculated by dividing ct/min from wells with mercury (or mercury plus thiol) by ct/min from medium (or medium plus thiol) wells. Results were representative of three separate experiments.

the following order of inhibitory activity: DTT ≥ 2-ME > *meso*-DMSA ≥ DMPS > L-cysteine (Fig. 1a–e).

Thiol compounds enhance mitogen stimulation

We compared the different thiol compounds with regard to their ability to enhance the proliferative response of lymphocytes to mitogens [6–8]. As shown in Fig. 2, all tested thiol compounds enhanced the proliferative responses to Con A on day 1 and to LPS on day 2. The enhancing effect varied among the thiol compounds, but 2-ME and DTT were most effective (Fig. 2).

Thiol compounds inhibit mercury-induced cell aggregation in vitro

Studies have shown that the cell–cell contact is important for cell proliferation induced by heavy metals [14]. The addition of mercury caused a rapid aggregation of lymphocytes in cell culture (Fig. 3g), and the presence of 2-ME completely blocked cell aggregation (Fig. 3b). We also tested the blocking effects of four other thiols and found they could all block mercury-induced cell aggregation in a dose-dependent manner (not shown). At indicated concentrations, as shown in Fig. 3c–f, the addition of each thiol completely abrogated cell aggregation induced by mercury.

meso-*DMSA and DMPS prevent mercury-induced IgG1 and IgE antibody production* in vivo

We [10] and others [15–17] have shown that in susceptible mouse strains such as SJL (H-2^s), continual injection with subtoxic doses of $HgCl_2$ induced a systemic autoimmune disease. Increased synthesis of IgG1 and IgE antibodies, production of high levels of ANolA and development of renal immune complex deposits were the characteristics of mercury-induced autoimmune disease. Our *in vitro* findings that thiol compounds inhibited mercury-induced cell aggregation and cell proliferation led us to test whether any of these agents could also prevent or dampen *in vivo* mercury-induced immunological alterations. Since *meso*-DMSA and DMPS have been used successfully to treat heavy metal poisoning, including mercury [11,12], and since their chemical, pharmacological and toxicological properties have been extensively studied [11,12], we selected a concentration of 30 mg/kg body weight of these two antidote drugs for our *in vivo* studies. This concentration was found to be effective and not toxic ([12], H. V. Aposhian, personal communication, and our own observation). In the first experiment, we studied the effect of *meso*-DMSA and DMPS on mercury-induced IgG1 and IgE production. SJL mice were continuously injected with either $HgCl_2$ or saline for 3

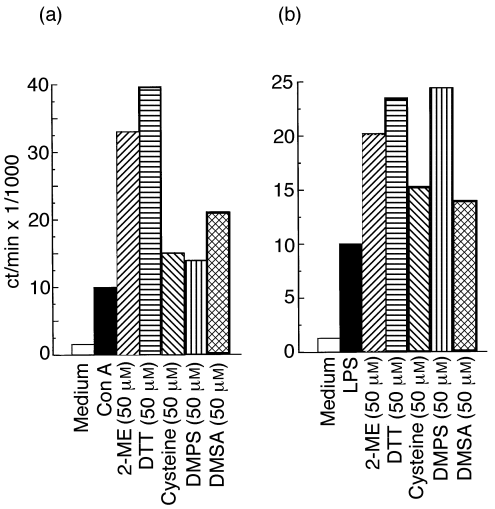

Fig. 2. Enhancement effects of thiol compounds on concanavalin A (Con A)- or lipopolysaccharide (LPS)-induced cell proliferation. Spleen cells (3×10^6/ml) from BALB/c mice were incubated with Con A (a) or LPS (b) as described in Materials and Methods. Different thiol compounds were added at the beginning of the culture at indicated concentrations. ^{3}H-thymidine was added (2 μCi/ml) on day 1 or day 2 and the cells were pulsed for 6 h. Each column represents the mean ct/min of triplicate cultures. Results were representative of three separate experiments.

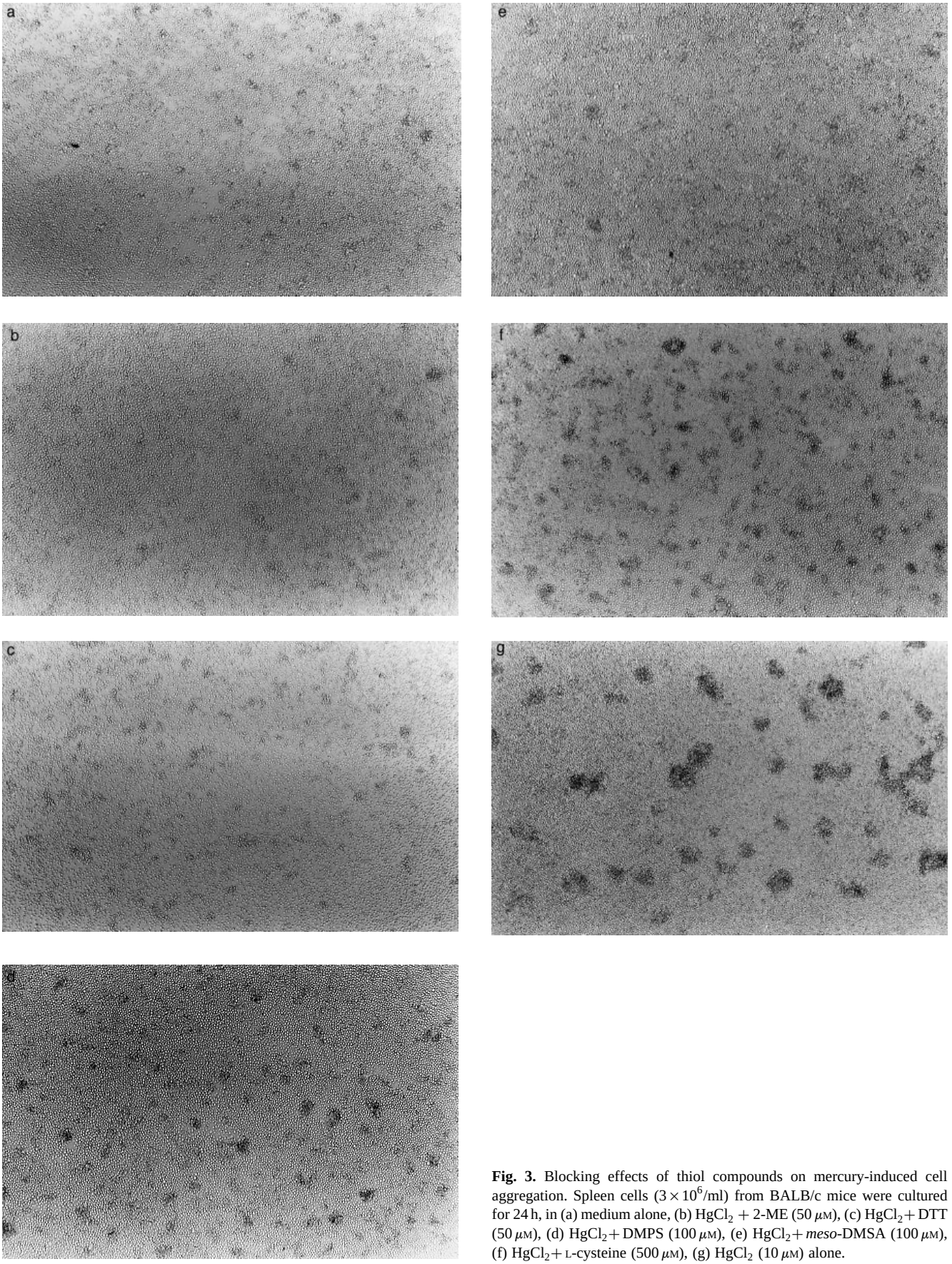

Fig. 3. Blocking effects of thiol compounds on mercury-induced cell aggregation. Spleen cells (3×10^6/ml) from BALB/c mice were cultured for 24 h, in (a) medium alone, (b) $HgCl_2$ + 2-ME (50 μM), (c) $HgCl_2$+ DTT (50 μM), (d) $HgCl_2$+ DMPS (100 μM), (e) $HgCl_2$+ *meso*-DMSA (100 μM), (f) $HgCl_2$+ L-cysteine (500 μM), (g) $HgCl_2$ (10 μM) alone.

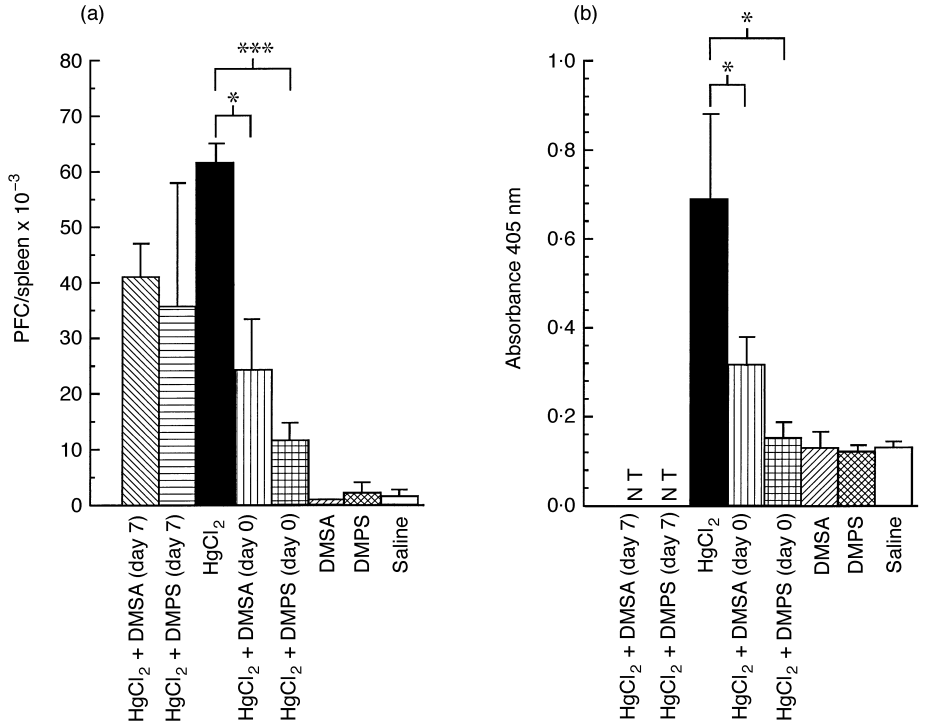

Fig. 4. Prevention of mercury-induced IgG1 and IgE antibody production by thiol compounds, *meso*-DMSA and DMPS. Groups of SJL mice were repeatedly injected subcutaneously with $HgCl_2$ for 3 weeks. Different groups of mercury-injected mice received *meso*-DMSA and/or DMPS immediately after (day 0) or 1 week after the first injection with mercury by the i.p. route. One group was left without treatment. The control groups received continual injection of either sterile saline (s.c.) or thiol compounds, *meso*-DMSA and DMPS (i.p.). At the end of the experiment, the spleens were tested for IgG1 antibody-secreting cells (a) and sera were tested for IgE antibodies (b). Data are shown as means $\pm$ 1 s.d. Significant differences between mercury- and mercury + thiol compounds (*meso*-DMSA and DMPS)- injected mice were calculated by Student's two-tailed *t*-test. $*P < 0{\cdot}05$; $***P < 0{\cdot}001$. NT, Not tested.

weeks. Groups of mercury-treated mice received continual i.p. injections of *meso*-DMSA and/or DMPS started immediately after or 1 week after the first injection with mercury, as described in Materials and Methods. After 3 weeks of treatment, spleens and sera were tested for the presence of different immunoglobulin antibody-secreting cells and IgE antibodies, respectively. As shown in Fig. 4, mice injected with mercury alone exhibited a high increase in the numbers of splenic IgG1 antibody-secreting cells and in serum IgE levels compared with saline-injected control mice. Mercury-injected mice treated with either *meso*-DMSA or DMPS, which started immediately after the first injection of mercury, showed a marked reduction in the numbers of IgG1 antibody-secreting cells as well as in serum IgE levels compared with mice injected with mercury alone (Fig. 4), and DMPS was more effective than *meso*-DMSA. Treatment with both *meso*-DMSA and DMPS 1 week after the first injection with mercury also decreased the numbers of IgG1-secreting cells, but it was not statistically significant compared with mercury-injected mice. Treatment with *meso*-DMSA or DMPS alone did not affect antibody production in SJL mice (Fig. 4). In addition, no significant differences were observed between saline-, mercury-, mercury plus *meso*-DMSA- and mercury plus DMSP-injected mice with regard to IgM, IgG2b and IgG3 antibody-secreting cells (not shown).

Evaluation of the routes for administration of thiol compounds in mercury-induced IgG1 and IgE antibody production

The results from the experiment in Fig. 4 showed that *meso*-DMSA and DMPS were most efficient in prevention of mercury-induced IgG1 and IgE antibody production if administered immediately and systematically (i.p.) following exposure to mercury. Since in most of the studies on mercury-induced autoimmunity, mercury was injected locally (s.c.) [15–17], we thought that thiol compounds would have more preventive effects if they are given by the same route as mercury. To test this, groups of SJL mice were continuously injected either with $HgCl_2$ or with saline. One group of mercury-injected mice was treated with continuous s.c. injections (close to the site for injection of mercury) of DMPS immediately after the first injection with mercury. After 3 weeks of treatment, spleens and sera were tested for the presence of IgG1 antibody-secreting cells and IgE antibodies, respectively. As shown in Fig. 5, mice receiving both mercury and DMPS showed high reduction in both IgG1 and IgE antibody production compared with mercury-injected mice.

DMPS prevents mercury-induced IgG1 ANolA formation in vivo

One of the major characteristics of mercury-induced autoimmune disease in mice is the formation of high levels of autoantibodies against nucleolar antigens [10,15–17]. Most of these autoantibodies are found to be of IgG1 isotype [18] and to be directed against fibrillarin, a 34-kD protein, which is a component of the U3 and U8 small nuclear ribonucleoprotein [19,20]. To test whether treatment with DMPS also prevented the production of mercury-induced ANolA in the same experimental groups, we determined the serum titres of IgG1 ANolA. Injection with mercury alone

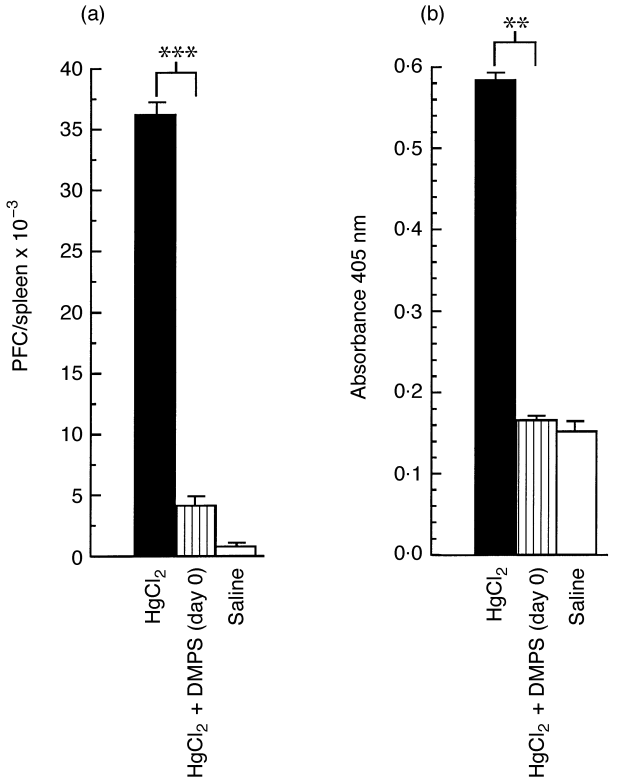

Fig. 5. Evaluation of the routes for administration of thiol compounds in mercury-induced IgG1 and IgE antibody production. Groups of SJL mice were repeatedly injected subcutaneously with $HgCl_2$ for 3 weeks. One group of mercury-injected mice received DMPS immediately (day 0) after the first injection with mercury by the s.c. route. One group was left without treatment. The control group received continual injection of sterile saline (s.c.). At the end of the experiment, spleens were tested for IgG1 antibody-secreting cells (a) and sera were tested for IgE antibodies (b). Data are shown as means ±1 s.d. Significant differences between mercury- and mercury + thiol compounds (*meso*-DMSA and DMPS)-injected mice were calculated by Student's two-tailed *t*-test. $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

induced high levels of IgG1 ANolA synthesis in SJL mice, whereas injection with saline was ineffective (Fig. 6). Treatment with DMPS almost completely prevented mercury-induced IgG1 ANolA production (Fig. 6).

DMPS prevents mercury-induced renal immune complex deposits in vivo

The hallmark of mercury-induced autoimmune disease in susceptible mice is the development of renal immune complex deposits [10,15,21]. It has been shown that in mercury-treated SJL mice, the dominant autoantibody deposited in the kidney is ANolA [22]. The finding that mercury-induced IgG1 ANloA production was highly reduced by treatment with DMPS led us to test whether it also prevents the formation of ANolA immune complexes in the kidneys of mercury-treated SJL mice. Kidneys from mercury-, mercury plus DMPS- and saline-injected mice were tested for the presence of deposited IgG1 antibodies by using a direct immunofluorescence method. As shown in Fig. 7, SJL mice injected with mercury alone showed a high increase in the titres of mesangial and vessel wall IgG1 deposits compared with saline-injected mice. Treatment with DMPS nearly completely inhibited the deposition of IgG1 antibodies in the kidneys of

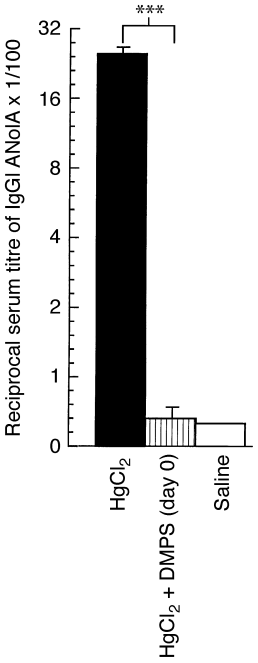

Fig. 6. DMPS prevents mercury-induced IgG1 anti-nucleolar autoantibody (ANolA) formation *in vivo*. Titres of IgG1 ANolAs in sera from the same SJL mice treated with mercury alone, mercury + DMPS and/or saline (as described in Fig. 5) were measured by indirect immunofluorescence. Significant differences between serum titres of IgG1 ANolAs in mercury- and mercury + DMPS-injected mice were calculated by Student's two-tailed *t*-test. *** $P < 0{\cdot}001$.

mercury-injected SJL mice, comparable to the levels of saline-injected mice (Fig. 7).

DISCUSSION

Several thiol compounds, such as 2-ME, L-cysteine and DTT, have been reported to promote the growth of lymphoma cells [6], to enhance the proliferative response of lymphocytes to mitogens [6–8], to augment the primary immune response *in vitro* [8,23,24], to substitute for macrophages [25], and to be an essential ingredient in the *in vitro* cell culture [26,27]. Previous studies [6–8] and also this study showed that the addition of thiol compounds, including *meso*-DMSA and DMPS, enhanced the proliferative response to Con A and LPS. It is believed that effects of thiol compounds on the activities of lymphocytes are due to the low redox potential of thiol compounds to either maintain monothiols completely in a reduced state on cell surface proteins or to interrupt disulfide bonds between cell membrane proteins [6,28,29]. Such modulation on lymphocyte activities has been found to be related to chemical structure of thiol compounds [6], in which the substitution of non-thiol group on thiol compounds with other functional groups may alter the activity of the molecule [6,29]. The differences in the enhancing effects on mitogen-stimulation among various thiol compounds (2-ME and DTT being most effective), especially in Con A stimulation, might be due to the differences in their chemical structures. This would also explain why 2-ME and DTT at routine concentrations completely abrogated mercury-induced cell aggregation, whereas L-cysteine, *meso*-DMSA and DMPS were less efficient.

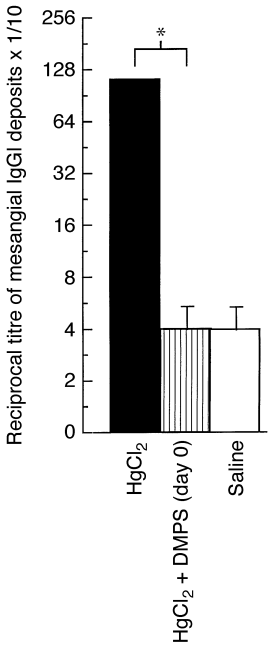

Fig. 7. DMPS prevents mercury-induced immune complex deposits *in vivo*. Kidneys from the same SJL mice treated with mercury alone, mercury + DMPS and/or saline were analysed for the presence of glomerular deposits of IgG1 antibody by direct immunofluorescence. Significant differences between the titres of granular mesangial IgG1 deposits in mercury- and mercury + DMPS-injected mice were calculated by Student's two-tailed *t*-test. * $P < 0{\cdot}05$.

Previous *in vitro* studies have shown that mercury induces a primary proliferative response in splenic lymphocytes [5]. In this study, we clearly showed that the addition of thiol compounds abolished both the cell aggregation and proliferative response induced by mercury. There are three possibilities to explain the inhibitory effects of thiol compounds on mercury-induced responses *in vitro*. First, thiol compounds interfere with the binding of mercury to free sulfhydryl groups of proteins on cells of the immune system. Therefore, it is likely that free thiol groups present on thiol compounds bind to mercury directly, thus preventing the binding of mercury to lymphocyte surface proteins containing sulfhydryl groups [11–13]. Second, as reducing reagents, thiol compounds have the potential to protect sulfhydryl groups on cell surface proteins from oxidation [28,29], and thus it is possible that they are able to release bound mercury from cell surface proteins. Third, as we proposed in our previous studies [5], mercury ions and 2-ME might form certain compounds which might be toxic to the cells. However, this seems unlikely, since by using trypan blue exclusion test we found that in mercury stimulation there were no more dead cells in the presence of 2-ME than in the absence of 2-ME (data not shown). This result indicated that in an *in vitro* system, the possible formation of mercury-thiol compound complexes may provide environmental conditions in which cells can not undergo cell division but are still alive. At present, it is not clear which of the first two proposed mechanisms, or both, are responsible for the inhibitory effects of thiol compounds. Preliminary experiments have shown that when 2-ME was added to the cultures after 24 h, the mercury-induced proliferative response, but not cell aggregation, was abolished (not shown), indicating that both mechanisms may be involved.

The novel findings that treatment with two thiol compounds, *meso*-DMSA and DMPS, prevented mercury-induced antibody/autoantibody production and renal immune complex deposit formation indicate that thiol compounds also have inhibitory effects on mercury-induced immunopathological changes *in vivo*. The observation that both *meso*-DMSA and DMPS were less effective when administered 1 week after exposure to mercury suggests that their inhibitory effects may be due primarily to prevention of mercury binding to cells and/or molecules of the immune system.

The efficacy of *meso*-DMSA and DMPS in the treatment of mercury intoxication or in mobilizing mercury compound has been extensively studied in humans as well as in experimental animals [11–13]. In rats the influence of *meso*-DMSA and DMPS on the distribution and excretion of mercuric chloride as well as methylmercury has been compared [30,31]. *Meso*-DMSA was found to remove more organic mercury (methylmercury) from the body [30], whereas DMPS removed more inorganic mercury (mercuric mercury) from most organs [31]. Therefore, it is conceivable that DMPS is more potent than *meso*-DMSA in inhibition of adverse immunological changes caused by inorganic mercury. This was supported by the finding that DMPS prevented mercury-induced IgG1 and IgE antibody production more effectively than *meso*-DMSA.

Several studies on the treatment of mercury intoxication have shown that the route of administration of *meso*-DMSA and/or DMPS has little or no influence [11,12]. In concordance, we found that identical doses of DMPS, when given by either the i.p or s.c. route, had similar preventive effects on mercury-induced autoimmunity.

Several attempts have been made to prevent/treat mercury-induced autoimmune disease [18,32]. The autoimmune disease caused by mercury has been attributed partly to IL-4 production by T helper cells [1,33]. Therefore, in most of the studies, prevention/treatment of autoimmune disease was achieved either by elimination of T helper cells, using anti-CD4 MoAb [32] or by neutralization of their product such as IL-4, using anti-IL-4 MoAb [18]. However, treatment with anti-IL-4 antibody only partially inhibited the increase of IgG1 antibodies/autoantibodies caused by mercury [18], and treatment with anti-CD4 antibodies resulted in severe suppression of T cell function and almost complete abrogation of $CD4^+$ T cells [32]. Compared with treatment with anti-IL-4 and anti-CD4 antibodies, treatment with DMPS prevented all characteristics of mercury-induced autoimmune disease without having direct interfering effects on the immune system. In addition, treatment with DMPS not only prevented mercury-induced autoimmune disease (this study), but also eliminated mercury from the body and prevented the accumulation of mercury in different organs [11–13]. More importantly, as safe antidote drugs, *meso*-DMSA and/or DMPS have already been used for treatment of humans who were exposed to toxic or subtoxic doses of mercury [11–13,34]. Therefore, they could be used for the prevention/treatment of possible immunological disorders caused by mercury in humans.

In conclusion, our *in vitro* and *in vivo* studies indicate that thiol compounds in general and *meso*-DMSA and DMPS in particular exert their inhibitory effects by interacting with mercury, and therefore prevent mercury-induced immunological and immunopathological alterations in susceptible mice.

ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Medical Research Council, the Swedish Work Environmental Fund, the Swedish Foundation

for Care Sciences and Allergy Research and a concerted action grant from the European Commission (Biomedical and Health research).

REFERENCES

1 Goldman M, Druet P, Gleichmann E. TH2 cells in systemic autoimmunity: insights from allogeneic disease and chemically-induced autoimmunity. Immunol Today 1991; **12**:223–7.

2 Eneström S, Hultman P. Does amalgam affect the immune system? A controversial issue. Int Arch Allergy Immunol 1995; **106**:180–203.

3 Griem P, Gleichmann E. Metal ion induced autoimmunity. Curr Opin Immunol 1995; **7**:831–8.

4 Utschig LM, Wright JG, O'Hallorn TV. Biochemical and spectroscopic probes of mercury (II) coordination enviroments in proteins. Methods Enzymol 1993; **226**:71–77.

5 Jiang Y, Möller G. *In vitro* effects of $HgCl_2$ on murine lymphocytes. I. Preferable activation of $CD4^+$ T cells in responder strain. J Immunol 1995; **154**:3138–46.

6 Broome JD, Jeng MW. Promotion of replication in lymphoid cells by specific thiols and disulfides *in vitro*: effects on mouse lymphoma cells in comparison with splenic lymphocytes. J Exp Med 1973; **138**:574–92.

7 Fanger MW, Hart DA, Wells JV, Nisonoff A. Enhancement by reducing agents of the transformation of human and rabbit peripheral lymphocytes. J Immunol 1970; **105**:1043–5.

8 Goodman MG, Weigle WO. Nonspecific activation of murine lymphocytes: I. Proliferation and polyclonal activation induced by 2-mercaptoethanol and α-Thioglycerol. J Exp Med 1977; **145**:473–89.

9 Gronowicz E, Coutinho A, Melchers F. A plaque assay for all cells secreting Ig of a given type or class. Eur J Immunol 1976; **6**:588–90.

10 Al-Balaghi S, Möller E, Möller G, Abedi-Valugerdi M. Mercury induces polyclonal B-cell activation, autoantibody production and renal immune complex deposits in young $(NZB \times NZW)F_1$ hybrids. Eur J Immunol 1996; **26**:1519–26.

11 Aposhian HV. DMSA and DMPS-water soluble antidotes for heavy metal poisoning. Ann Rev Pharmacol Toxicol 1983; **23**:193–215.

12 Aposhian HV, Aposhian MM. Meso-2,3-dimercaptosuccinic acid: chemical, pharmacological and toxicological properties of an orally effective metal chelating agent. Ann Rev Pharmacol Toxicol 1990; **30**:279–306.

13 Aposhian HV, Maiorino RM, Rivera M *et al*. Human studies with the chelating agents, DMPS and DMSA. Clin Toxicol 1992; **30**:505–28.

14 Warner GL, Lawrence DA. Stimulation of murine lymphocyte responses by cations. Cell Immunol 1986; **101**:425–39.

15 Hultman P, Eneström S. The induction of immune complex deposits in mice by peroral and parenteral administration of mercuric chloride: strain dependent susceptibility. Clin Exp Immunol 1987; **67**:283–92.

16 Mirtcheva J, Pfeiffer C, De Bruijn JA *et al*. Immunological alterations inducible by mercury compounds. III. H-2A acts as an immune response and H-2E an immune 'suppression' locus for $HgCl_2$-induced antinucleolar autoantibodies. Eur J Immunol 1989; **19**:2257–61.

17 Hultman P, Bell LJ, Eneström S, Pollard KM. Murine susceptibility to mercury I. Autoantibody profiles and systemic immune deposits in inbred, congenic, and intra-H-2 recombinant strains. Clin Immunol Immunopathol 1992; **65**:98–109.

18 Ochel M, Vohr H, Pfeiffer C, Gleichmann E. IL-4 is required for the IgE and IgG1 increase and IgG1 autoantibody formation in mice treated with mercuric chloride. J Immunol 1991; **146**:3006–11.

19 Hultman P, Eneström S, Pollard KM, Tan EM. Anti-fibrillarin autoantibodies in mercury-treated mice. Clin Exp Immunol 1989; **78**:470–2.

20 Reuter R, Tessars G, Vohr H *et al*. Mercuric chloride induces autoantibodies against U3 small nuclear ribonucleoprotein in susceptible mice. Proc Natl Acad Sci USA 1989; **86**:237–41.

21 Hultman P, Bell LJ, Eneström S, Pollard KM. Murine susceptibility to mercury II. Autoantibody profiles and renal immune deposits in hybrid, backcross, and H-2d congenic mice. Clin Immunol Immunopathol 1993; **68**:9–20.

22 Hultman P, Eneström S. Mercury induced antinuclear antibodies in mice: characterization and correlation with renal immune complex deposits. Clin Exp Immunol 1988; **71**:269–74.

23 Click RE, Benck L, Alter BJ. Enhancement of antibody synthesis *in vitro* by mercaptoethanol. Cell Immunol 1972; **3**:155–60.

24 Ohmori H, Yamamoto I. Mechanism of augmentation of the antibody response *in vitro* by 2-mercaptoethanol in murine lymphocytes. Cell Immunol 1983; **79**:173–85

25 Chen C, Hirsch JG. The effects of mercaptoethanol and of peritoneal macrophages on the antibody forming capacity of nonadherent mouse spleen cells *in vitro*. J Exp Med 1972; **136**:604–17.

26 Metcalf D, Nossal GJV, Warner NL *et al*. Growth of B-lymphocyte colonies *in vitro*. J Exp Med 1975; **142**:1534–49.

27 Metcalf D. Role of mercaptoethanol and endotoxin in stimulating B-lymphocyte colony formation *in vitro*. J Immunol 1976; **116**:635–8.

28 Cleland WW. Dithiothreitol, a new protective reagent for SH groups. Biochemistry 1964; **3**:480–2.

29 Noelle RJ, Lawrence DA. Modulation of T-cell function: II. Chemical basis for the involvement of cell surface thiol-reactive sites in control of T-cell proliferation. Cell Immunol 1981; **60**:453–69.

30 Planas-Bohne F. The influence of chelating agents on the distribution and biotransformation of methylmercuric chloride in rats. J Pharmacol Exp Ther 1981; **217**:500–4.

31 Gabard B. The excretion and distribution of inorganic mercury in the rat as influenced by several chelating agents. Arch Toxicol 1976; **35**:15–24.

32 Hultman P, Johansson U, Dagnaes-Hansen F. Murine mercury-induced autoimmunity: the role of T-helper cells. J Autoimmun 1995; **8**:809–23.

33 Mathieson PW. Mercury: god of Th_2 cells? Clin Exp Immunol 1995; **102**:229–30.

34 Aposhian HV, Bruce DC, Alter W *et al*. Urinary mercury after administration of 2,3-dimercaptopropane-1-sulfonic acid: correlation with dental amalgam score. FASEB J 1992; **6**:2472–6.