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Genetic differences in immune reactivity to mercuric chloride ($HgCl_2$): immunosuppression of H-2^d mice is mediated by interferon-gamma (IFN-γ)

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SUMMARY

Upon treatment with $HgCl_2$, H–2^s mice, such as B10.S, develop an activation of B lymphocytes that depends, at least partially, on activation of T helper type 2 (Th2) cells and results in increased serum levels of IgG1 and IgE, appearance of IgG autoantibodies, and development of immune glomerulonephritis and vasculitis. Results of previous studies and of experiments presented here indicate that the B cell activation and systemic autoimmune disease fail to develop in MHC-congenic B10.D2 (H-2^d) and B10.BR (H–2^k) mice treated with $HgCl_2$, although B10.D2 T cells showed signs of activation by and specificity for $HgCl_2$ comparable to those seen in strain B10.S. Here, we report that following $HgCl_2$ injections the antibody response to sheep erythrocytes is normal in B10.S, but suppressed in B10.D2 mice. This suppression was prevented by MoAb to mouse IFN-γ. Conversely, treatment of B10.D2 mice with murine recombinant IFN-γ (rIFN-γ) was able to reproduce the immunosuppression seen after $HgCl_2$ treatment. In B10.S mice, it took administration of both rIFN-γ and $HgCl_2$ to suppress the anti-sheep erythrocyte response. Although rIFN-γ diminished the increase in IgE serum levels of $HgCl_2$-treated B10.S mice, it failed to prevent their autoantibody production and immune glomerulonephritis. These findings further strengthen the concept that B10.S mice react to $HgCl_2$ by preferential activation of their Th2 cells producing IL-4, whereas B10.D2 mice react to $HgCl_2$ by preferential activation of their Th1 cells, which produce IFN-γ and thus suppress antibody responses.

Keywords mercuric chloride immunosuppression Th1 cells Th2 cells interferon-gamma

INTRODUCTION

Susceptible strains of rodents treated with repeated injections of $HgCl_2$ develop a systemic, T cell-dependent autoimmune disease with increased serum levels of IgG1 and IgE, formation of autoantibodies, and development of glomerulonephritis and vasculitis (reviewed in [1,2]). In both rats and mice, MHC class II alleles determine susceptibility to $HgCl_2$-induced B cell activation [3,4]. In mice, for instance, $HgCl_2$ treatment induces antinucleolar autoantibodies (ANolA) in H–2^s strains, such as B10.S, but not in the H-2 congenic strains B10.D2 (H-2^d) and B10.BR (H–2^k) [5,6]. Nevertheless, both B10.S and B10.D2 mice were found to respond to $HgCl_2$ by a comparable degree of T cell activation, as evident from an identical increase in $CD4^+$ T cells carrying the activation marker $CD45RB^{low}$ [7] and by anamnestic responses in both strains to $HgCl_2$. This anamnestic T cell response was found to be immunostimulatory, leading to B cell activation of $HgCl_2$-treated B10.S mice, and proved to be slightly suppressive when B10.D2 T cells were studied [8]. These observations indicated that B10.D2 mice are resistant to $HgCl_2$-induced systemic autoimmunity, not because they are non-responders to $HgCl_2$, but because they mount an immune response qualitatively different from that of B10.S susceptible mice.

Based on the cytokines they produce, $CD4^+$ T cells have been divided into Th1 and Th2 subsets [9]. Th1 cells primarily produce IL-2 and IFN-γ [10,11], provide help for cell-mediated immunity, and may suppress B cell responses. In contrast, Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 [12,13] and, hence, mediate humoral responses associated with increased B cell activity [9]. In the $HgCl_2$ model in rodents, susceptibility or resistance to systemic autoimmunity seems to be the consequence of preferential activation of either Th1 or Th2 cells, with antibody-mediated autoimmune phenomena being promoted by Th2 cells and suppressed by Th1 cells [1]. Thus, autoimmunity-susceptible B10.S mice responded to $HgCl_2$ by activation of IL-4-producing $CD4^+$ T cells and an increased frequency of B cells producing IgE and IgG1 [7,14]. Moreover, in $HgCl_2$-treated H-2^s mice, blockade of

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IL-4 by MoAb abrogated the increased serum levels of IgE and simultaneously modified the isotypic composition of autoantibodies, in that the production of IgG1 autoantibodies was decreased and that of IgG2a autoantibodies increased [15]. These findings support the concept that H-2^s mice, like Brown Norway rats, respond to $HgCl_2$ by preferential activation of their Th2 cells [1,16,17].

Experimental evidence that $HgCl_2$ would preferentially activate Th1 cells in the B10.D2 strain is much weaker though, because it is mainly based on the resistance of this strain against developing the immunostimulatory and/or IL-4-dependent alterations mentioned above [6,7,18] and on its suppressive anamnestic T cell responses to $HgCl_2$ [8], rather than on direct evidence for increased production of Th1 cytokines and/or development of corresponding pathological alterations. Hence, the first aim of the present study was to characterize further the type of immunopathological response to $HgCl_2$ mounted in the B10.D2 strain and to systematically compare it with that of strains B10.S and B10.BR. As a candidate pathological alteration that might be mediated by Th1 cells, a suppressed antibody response to sheep erythrocytes was identified, as this developed in $HgCl_2$-treated B10.D2 but not B10.S mice. The second aim then was to test if, indeed, the $HgCl_2$-induced immunosuppression is mediated by Th1 cells, as represented by their key cytokine, IFN-γ.

MATERIALS AND METHODS

Mice

Female B10.S (H–2^s), B10.D2 (H-2^d), and B10.BR (H–2^k) mice (6–8 weeks old) were purchased from Harlan Olac Ltd. (Bicester, UK). The mice were kept under specific pathogen-free conditions and had free access to food and water.

Chemicals, antibodies, and proteins

Stock solutions of 1 mg/ml $HgCl_2$ and 3 mg/ml $HgCl_2$ of analytical grade (Merck, Darmstadt, Germany) were prepared in sterile, pyrogen-free water (Ampuva; Fresenius AG, Bad Homburg, Germany). Before injection, $HgCl_2$ was diluted to 0·1 mg/ml or 0·3 mg/ml in isotonic, pyrogen-free saline (Fresenius), passed through sterile filters (0·2 μm filter unit) (Millipore Products Division, Bedford, MA), and frozen at −20°C. Rat MoAb XMG 1.2 (IgG1) is specific for mouse IFN-γ [19]; it was purified from hybridoma supernatants and found to exhibit an IFN-γ-neutralizing activity of 10 U murine IFN-γ/12 ng protein, as assessed on L929 cells [20]. Rat IgG1 MoAb IR 595 [21], a kind gift from Professor Hervé Bazin (University of Louvain, Brussels, Belgium), was used as an isotype control. Recombinant mouse IFN-γ (rIFN-γ) with a specific activity of 7×10^6 U/mg was produced as described elsewhere [22]. Sheep erythrocytes were

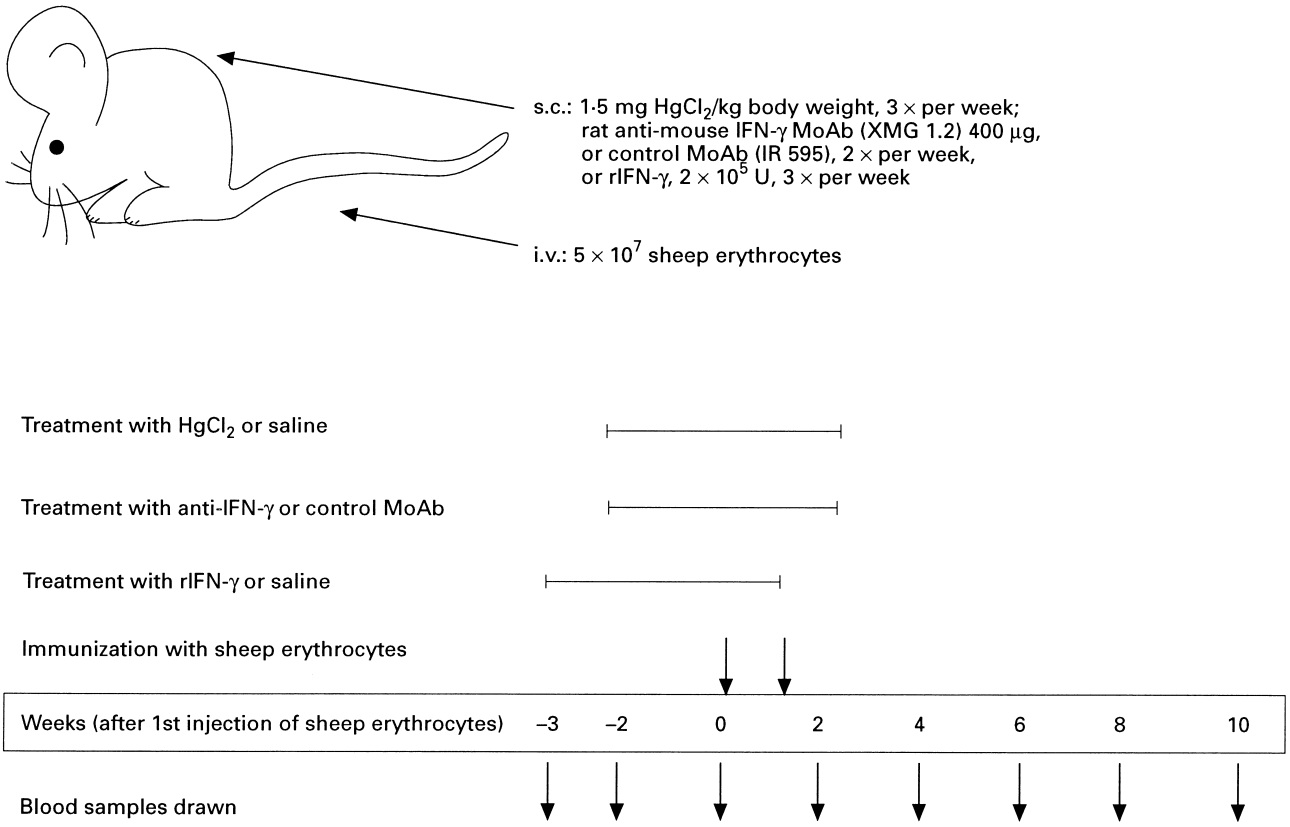

Fig. 1. Design of experiments investigating the effect of $HgCl_2$ on antibody responses to sheep erythrocytes and modulation of these responses by treatment with anti-IFN-γ MoAb and rIFN-γ, respectively. Mice were treated with 1·5 mg $HgCl_2$/kg body weight, or with saline alone, three times per week over a period of 4 weeks (from week −2 to week 2). In each, week 0 and week 1, animals received an i.v. injection of 5×10^7 sheep erythrocytes, as indicated by arrows. From week −2 till week 2, some groups received two weekly i.p. injections of anti-IFN-γ (MoAb XMG 1.2) or control MoAb (IR 595). Alternatively, from week −3 till week 1 groups of mice received three weekly i.p. injections of mouse rIFN-γ in saline, or saline alone. Starting before the onset of treatment and continuing until week 10 or 12, blood samples were taken at the times indicated by arrows.

purchased from Gesellschaft für Labordiagnostik (GLD; Mülheim, Germany).

$HgCl_2$ treatment and experimental design

For studying MHC effects upon $HgCl_2$ treatment, groups of B10.S, B10.D2, and B10.BR mice (5–11 animals per group) were injected with $HgCl_2$ (0·1 mg/ml) at a dose of 0·5 mg/kg body weight, given subcutaneously three times a week through the entire experiment (12 weeks) [6,7,14,15]. For measurement of serum IgE and formation of ANolA, mice were bled retroorbitally under ether anaesthesia at the time points indicated in Fig. 2. Immunofluorescence staining for renal IgG1 deposits was performed on kidney sections of mice treated for 12 weeks.

For studying suppression by $HgCl_2$ of anti-sheep erythrocyte antibody formation and for analysing the effects of anti-IFN-γ MoAb and rIFN-γ, respectively, the experimental design outlined in Fig. 1 was used. Mice (7–10 animals per group) were injected subcutaneously with 1·5 mg $HgCl_2$/kg body weight (0·3 mg/ml $HgCl_2$) three times a week for 4 consecutive weeks. The higher dose of $HgCl_2$ was chosen because repeated injections of 0·5 mg $HgCl_2$/kg body wt proved incapable of suppressing the anti-sheep erythrocyte antibody response. The XMG 1.2 MoAb against IFN-γ and the isotype-matched control MoAb IR 595 were administered intraperitoneally during the same period, with the first injection being placed 1 day before the first $HgCl_2$ application; each injection consisted of 400 μg of the MoAb indicated. Two other groups of B10.S and B10.D2 mice received i.p. injections of either rIFN-γ in sterile saline (2×10^5 U/injection) or sterile saline alone three times weekly for 4 weeks. In this case, treatment was started 1 week before the onset of $HgCl_2$ injections, and it also was terminated 1 week earlier. After both the second and third week of $HgCl_2$ treatment, mice were immunized by i.v. injection of 5×10^7 sheep erythrocytes; blood samples were taken at the days indicated (Fig. 1).

ELISA for serum IgE

Total serum IgE was measured by a previously described solid-phase ELISA [14], with the following modifications. Maxisorb microtitre plates (Nunc, Wiesbaden, Germany) were coated with 50 μg rat anti-mouse IgE (EM 95.3; kindly donated by Dr Z. Eshhar, Rehovot, Israel) [23]. After washing with PBS containing 0·5% Tween 20, serum samples were added. After further washing, biotinylated rat anti-mouse IgE (3 μg/ml; Dianova, Hamburg, Germany) was added as second antibody. For development of colorimetric reaction, a horseradish peroxidase–streptavidin complex (Zymed, San Francisco, CA) and the substrate were added. Dinitrophenol (DNP)-specific mouse MoAb of the IgE isotype (Sigma Chemie GmbH, Deisenhofen, Germany) [24] in the range between 1 and 10 μg/ml was used as a standard.

Antinucleolar autoantibodies

ANolA were studied by indirect immunofluorescence technique. Diluted serum samples from individual mice were incubated with cryostat kidney sections obtained from untreated mice from the same strains, as described elsewhere [6,15]. FITC-labelled rabbit anti-mouse IgG (20 μg/ml; Dakopatts, Glostrup, Denmark) was used as second antibody.

IgG1 deposits in the kidney

The incidence of IgG1 deposits at the glomerular basement membrane, in the mesangium, and in renal blood vessels was evaluated by direct immunofluorescence. Mice were killed after 12 weeks of continuous treatment with either $HgCl_2$ or saline, and their kidneys removed and snap frozen at −80°C. Cryostat sections were prepared and stained with FITC-labelled rabbit anti-mouse IgG1 (20 μg/ml; Dakopatts).

Anti-sheep erythrocyte antibodies

Serum samples were serially diluted from 1:20 to 1:5120 in 50 μl PBS in round-bottomed microtitre plates (Greiner Labortechnik, Nürtingen, Germany). A suspension of sheep erythrocytes (50 μl; 1×10^9/ml in PBS) was added to each well. After overnight incubation at room temperature, the highest serum dilution giving macroscopically visible haemagglutination was recorded [25].

Statistical analysis

Data of experiments are shown as arithmetic mean values ± 1 s.d. Statistical analysis was performed by ANOVA analysis of variance. For anti-sheep erythrocyte antibody concentration log 2 values of measured antibody titres were computed for statistical analysis.

RESULTS

Differential immunopathological reactions to $HgCl_2$ of MHC-congenic mouse strains

Following $HgCl_2$ treatment, 91% or 100%, respectively, of B10.S mice developed IgG1 deposits in the glomerular mesangium and

Table 1. Induction by $HgCl_2$ of renal IgG1 deposits depends on the MHC haplotype

Strains studied and their H-2 haplotype	Treatment*	Percent of animals with IgG1 deposits at the sites indicated		
		Glomerulus mesangium	Basement membrane	Blood vessels
B10.S	$HgCl_2$	91 (10/11)†	100 (11/11)	82 (9/11)
($H\text{-}2^s$)	Saline	0 (0/5)	0 (0/5)	0 (0/5)
B10.D2/n	$HgCl_2$	14 (1/7)	14 (1/7)	14 (1/7)
($H\text{-}2^d$)	Saline	0 (0/5)	0 (0/5)	0 (0/5)
B10.BR	$HgCl_2$	0 (0/7)	0 (0/7)	0 (0/7)
($H\text{-}2^k$)	Saline	20 (1/5)	20 (1/5)	0 (0/5)

* Animals were treated 3 times a week with 0·5 mg $HgCl_2$/kg body weight for 12 weeks.
† Number of mice positive/total number of animals studied per group.

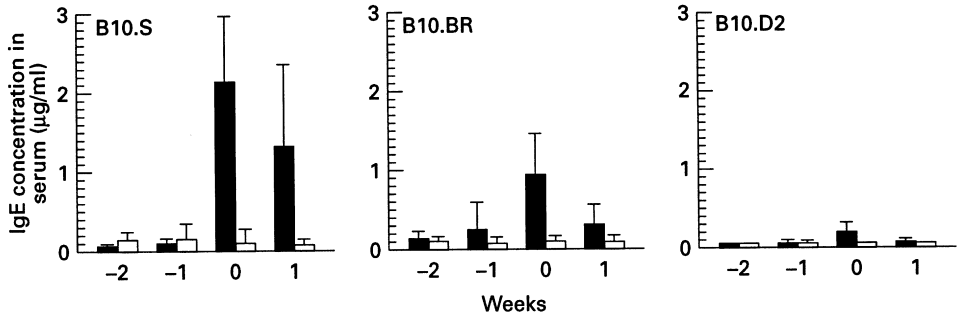

Fig. 2. Differential effect of $HgCl_2$ treatment on total serum IgE levels in three different MHC-congenic mouse strains. Groups of 7–10 animals were repeatedly injected with 0·5 mg $HgCl_2$/kg body weight (■) or saline (□) starting at week –2. Control mice received saline only. Data represent mean values ± s.d.

along the glomerular basement membrane. Almost all of them showed additional IgG1 deposits of the granular type in renal blood. In contrast, only 14% of B10.D2 mice and 0% of B10.BR mice showed IgG1 deposits in response to $HgCl_2$ treatment (Table 1). Moreover, IgE concentrations in B10.S mice increased sharply during week 0 with a significant increase persisting during week 1 (Fig. 2). In contrast, the $HgCl_2$-induced IgE increase was intermediate in strain B10.BR and modest in B10.D2.

$HgCl_2$ treatment suppresses anti-sheep erythrocyte antibody formation in B10.D2, but not in B10.S mice

Next, we compared the effect of $HgCl_2$ treatment on the antibody response to sheep erythrocytes in strains B10.S and B10.D2. Results shown in Fig. 3 indicate virtually identical anti-sheep erythrocyte responses in saline-treated animals of both strains. $HgCl_2$ treatment, by contrast, induced a significant suppression of anti-sheep erythrocyte antibodies in strain B10.D2, but completely failed to do so in B10.S mice.

Alleviation by anti-IFN-γ treatment of $HgCl_2$-induced immunosuppression of B10.D2 mice

To test whether IFN-γ is involved in the defective formation of anti-sheep erythrocyte antibodies observed in $HgCl_2$-treated B10.D2 mice, such animals were additionally treated with either anti-IFN-γ MoAb or isotype-matched control MoAb. We found that treatment of B10.D2 mice with neutralizing anti-IFN-γ MoAb abrogated the suppression of anti-sheep erythrocyte antibody formation induced by $HgCl_2$ (Fig. 4a), whereas treatment with control MoAb failed to do so (Fig. 4b).

Reproduction of immunosuppression by rIFN-γ in B10.D2 mice not treated with $HgCl_2$

The experiments shown in Fig. 4 established that endogenous IFN-γ is a mediator of the immunosuppression seen in $HgCl_2$-treated B10.D2 mice. Confirming this conclusion, treatment of B10.D2 mice with rIFN-γ resulted in the same retarded and decreased antibody response towards sheep erythrocytes as treatment with $HgCl_2$ (Fig. 5).

Failure of anti-IFN-γ MoAb to establish a Th2-like response in $HgCl_2$-treated B10.D2 mice

The experiments described in Fig. 4 demonstrated that anti-IFN-γ treatment of $HgCl_2$-treated B10.D2 mice alleviates their immunosuppression. However, when sera of these animals were searched for autoantibodies and their kidneys examined by immunofluorescence technique, the characteristic Th2-like response seen in $HgCl_2$-treated B10.S mice, i.e. increased serum levels of IgE, ANolA formation and immune glomerulonephritis, failed to be detectable (data not shown). Hence, the neutralization of IFN-γ, although capable of alleviating the immunosuppression, proved unable to shift the entire Th1-like response to $HgCl_2$ of B10.D2 mice to the Th2-like response of strain B10.S.

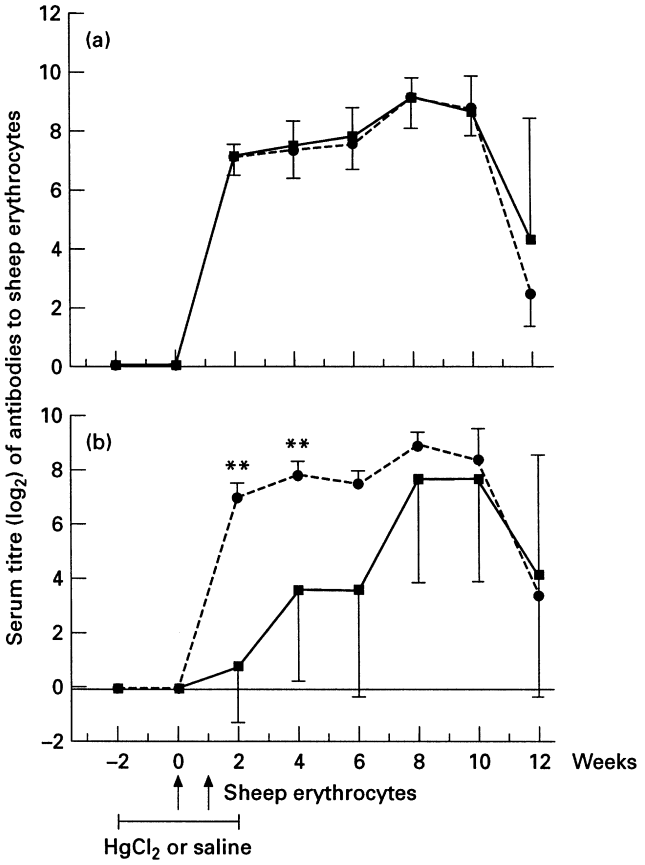

Fig. 3. Differential effect of $HgCl_2$ treatment on anti-sheep erythrocyte antibody titres in B10.S (a) and B10.D2 mice (b). From week –2 till week 2 (horizontal bar), groups of 7–10 mice were treated with 1·5 mg $HgCl_2$/kg body weight (■), or with saline alone (●), and in weeks 0 and 1 they were immunized against sheep erythrocytes (arrows). The mice were bled at the times indicated and their sera tested for antibodies to sheep erythrocytes. $^{**}P < 0{\cdot}01$.

Effects of rIFN-γ in B10.S mice treated or not with $HgCl_2$

In the experiments described in Fig. 4, the Th1-like reaction to $HgCl_2$ of B10.D2 mice was partially altered by injections of neutralizing MoAb to IFN-γ. In a complementary experimental approach using B10.S mice therefore, we tried to shift their Th2-like reaction to $HgCl_2$ by pretreating them with rIFN-γ. This treatment led to a reduced antibody formation to sheep erythrocytes (Fig. 6) and to a less intense increase in serum IgE levels (Fig. 7). Here, too, however, the shift in the response pattern to $HgCl_2$ was not complete, since neither the serum titre of ANolA nor the incidence and severity of glomerulonephritis were modified by rIFN-γ (data not shown).

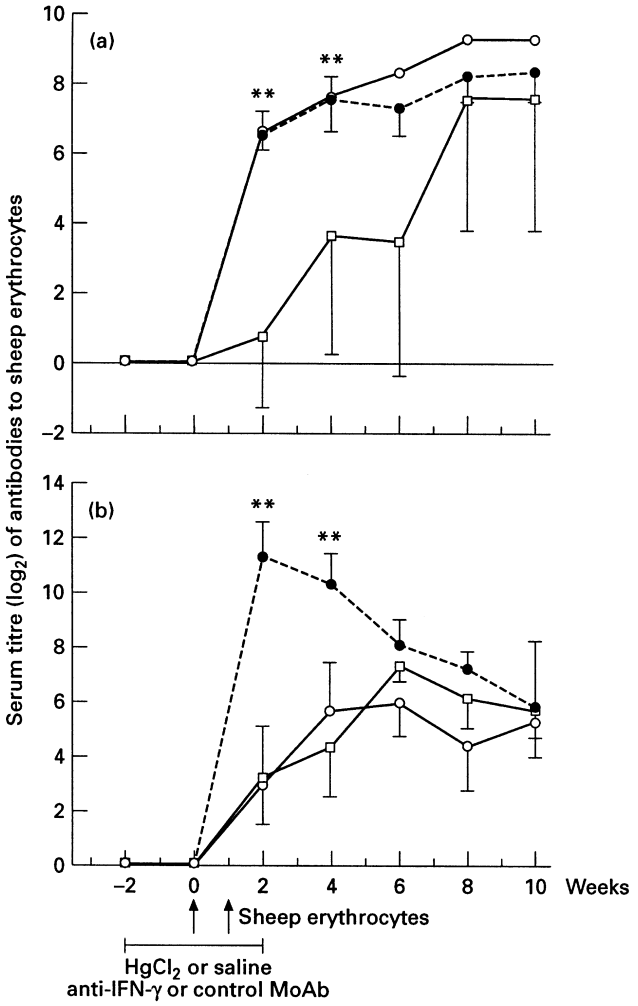

Fig. 4. Treatment with anti-IFN-γ MoAb alleviates the $HgCl_2$-induced suppression of anti-sheep erythrocyte antibody response in B10.D2 mice. From week −2 till week 2 (horizontal bar), B10.D2 mice (7–10 animals per group) were treated with 1·5 mg $HgCl_2$/kg body weight, or with saline, and they were immunized against sheep erythrocytes in week 0 and week 1. During the same period of time but starting 1 day before the onset of treatment with $HgCl_2$ and saline, respectively, some groups were treated with XMG 1.2 anti-IFN-γ MoAb (a) or IR 595 control MoAb (b), as indicated. Mice were bled at the times indicated and their sera tested for antibodies to sheep erythrocytes. Statistical differences between the saline-treated group and each of the other two groups were: $^{**}P < 0{\cdot}01$. (a) ○, $HgCl_2$ + anti-IFN-γ MoAb; ●, saline + anti-IFN-γ MoAb; □, $HgCl_2$ + saline. (b) ○, $HgCl_2$; ●, saline; □, $HgCl_2$ + control MoAb.

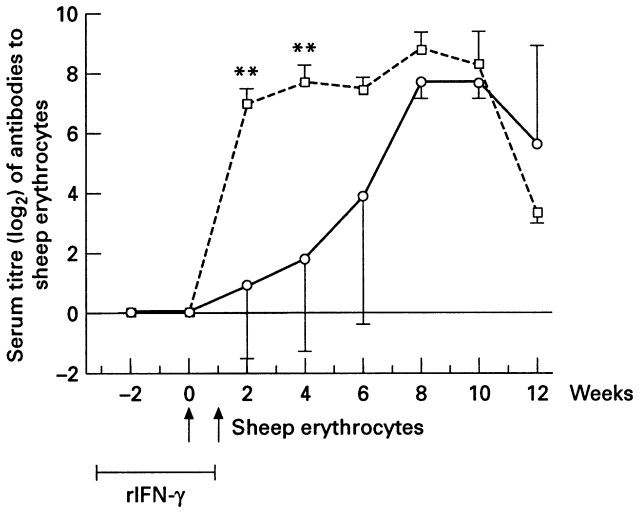

Fig. 5. Treatment with rIFN-γ suppresses the anti-sheep erythrocyte antibody response of B10.D2 mice. From week −3 till week 1, two groups of B10.D2 mice (seven animals each) received three weekly i.p. injections of rIFN-γ in sterile saline (○; 2×10^5 U/injection) or sterile saline alone (□), as indicated; in weeks 0 and 1, all animals were immunized against sheep erythrocytes. Mice were bled at the times indicated and their sera tested for antibodies to sheep erythrocytes. $^{**}P < 0{\cdot}01$.

Unexpectedly, some IgG deposits were detected along the glomerular basement membrane of B10.S mice treated with rIFN-γ (without $HgCl_2$) by the usual regimen, i.e. a course of 4 weeks. Since two out of six of these animals also had ANolA in their serum (titre 1:2560), this points to adverse immunological

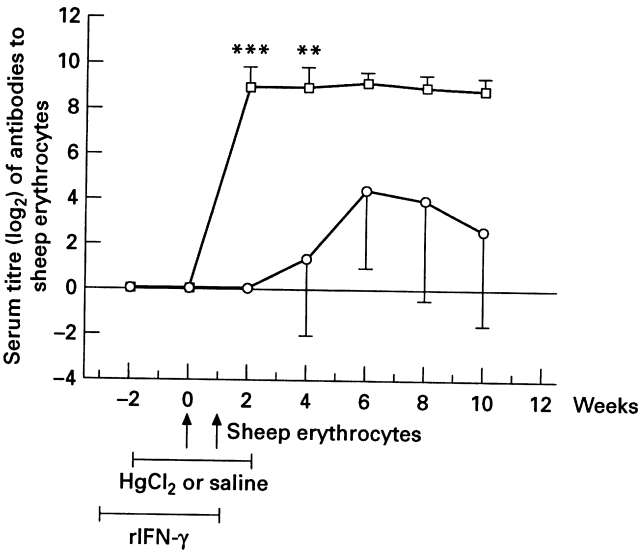

Fig. 6. In B10.S mice, administration of rIFN-γ was immunosuppressive only when combined with $HgCl_2$ treatment. From week −3 till week 1, groups of 7–10 B10.S mice were treated with rIFN-γ, and from week −2 till week 2 they received three weekly injections of 1·5 mg $HgCl_2$/kg body weight, or of saline, as indicated; in weeks 0 and 1, all animals were immunized against sheep erythrocytes. Mice were bled at the times indicated and their sera tested for antibodies to sheep erythrocytes. $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}005$. ○, $HgCl_2$ + rIFN-γ; □, saline + rIFN-γ.

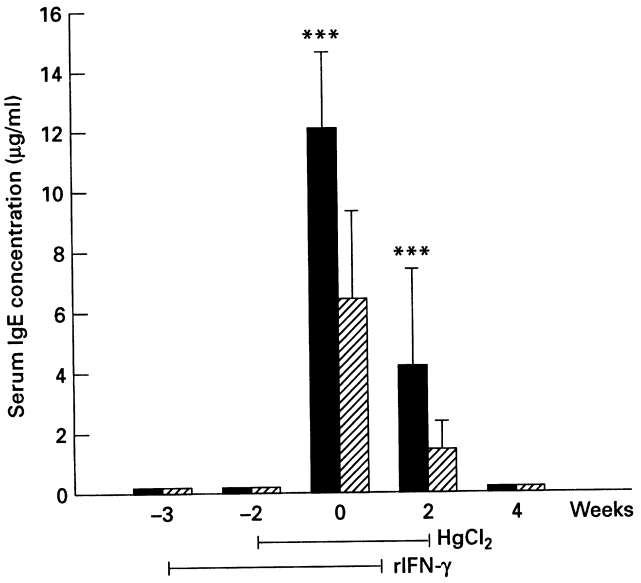

Fig. 7. Administration of rIFN-γ suppresses the $HgCl_2$-induced increase of serum IgE in B10.S mice. From week –3 till week 1, mice were treated with rIFN-γ (▨), or received injections of saline (■). From week –2 till week 2, mice of both groups received three weekly injections of 1·5 mg $HgCl_2$/kg body weight. The animals were bled at the times indicated and their serum IgE levels determined. ***$P < 0{\cdot}005$.

side-effects of rIFN-γ *per se*. Conceivably, the known proinflammatory and autoimmunity-promoting properties of IFN-γ [26–28] were involved here.

DISCUSSION

We demonstrated that, upon repeated injections of $HgCl_2$, B10.S mice promptly developed signs of systemic autoimmunity with ANolA, increased serum IgE levels, and IgG1 deposits in the renal glomeruli and blood vessels; in contrast, these immunostimulatory reactions to $HgCl_2$ were greatly reduced in B10.BR and hardly or not at all detectable in B10.D2 mice, demonstrating their MHC dependence. In B10.S mice, the immune deposits in the renal mesangium and at the glomerular basement membrane and vessel walls described in the present study were identical to those reported by Hultman *et al.* [4]. In B10.D2 mice, the results of Hultman *et al.* [18] were slightly different from ours. Whereas they detected renal immune complex deposits in the glomerular mesangium and the vessels in all examined animals [18], we found only 14% (one out of seven) of $HgCl_2$-treated B10.D2 mice to be positive for these renal immune complexes. Possibly, this difference is due to different $HgCl_2$ doses used in the respective experiments, namely 1·6 mg/kg body wt [18] *versus* 0·5 mg/kg $HgCl_2$ in the present study.

Conforming to previous results obtained in rats [25], here we demonstrate an MHC-dependent suppression of antibody formation to sheep erythrocytes in MHC-congenic mouse strains. A novel observation made in the present study is that this immunosuppression was mediated by endogenous IFN-γ. The experimental evidence for this is two-fold. First, the suppression of anti-sheep erythrocyte antibody formation seen in $HgCl_2$-treated B10.D2 mice was prevented by neutralizing MoAb against endogenous IFN-γ, and second, administration of murine rIFN-γ was able to reproduce this immunosuppression in B10.D2 mice not treated with $HgCl_2$. As expected in view of the IL-4-antagonizing effects of IFN-γ [29], a suppressive effect of rIFN-γ was indeed detectable in strain B10.S when the elevated IgE formation characteristic of $HgCl_2$-treated B10.S mice was studied. Taken together, the results reported here and in previous publications (see Table 2) strengthen the view [1] that strain B10.S reacts to $HgCl_2$ by preferential activation of Th2 cells, whereas B10.D2 reacts by preferential activation of Th1 cells.

A preferential activation of Th1 or Th2 cells has clearly been demonstrated in murine models of infectious disease, involving *Leishmania major* [30], *Nippostrongylus brasiliensis* [31], or *Plasmodium falciparum* [32]. Furthermore, a Th1/Th2 dichotomy

Table 2. Survey of differences in $HgCl_2$-induced* immunopathologic reactions of the two MHC-congenic strains B10.D2 and B10.S. Reactions are grouped together according to a known or assumed predominant involvement of Th1 or Th2 cells, respectively

Reaction studied	Strength and incidence of reaction† in the mouse strain indicated		References
	B10.D2 (H-2^d)	B10.S (H-2^s)	
IL-4 mRNA in $CD4^+$ T cells	+/–	+++	[7]
MHC II expression on B cells	–	+	[7]
Total IgE level in serum	+	+++	Present paper
IgG1 deposits in the kidney	–	+	Present paper
Cytoplasmic IgG1 and IgE in splenic B cells	–	+	[7]
Anamnestic T cell responses to $HgCl_2$ that increases PLN cellularity	–	+	[8]
Anamnestic T cell response to $HgCl_2$ that suppresses PLN cellularity	+	–	[8]
Cytoplasmic IgG2a in splenic B cells	+	–	[7]
IFN-γ-dependent suppression of antibody formation	+	–	Present paper

* Three weekly s.c. injections of $HgCl_2$ (0·5 mg/mg) given over the entire observation period; only for induction of immunosuppression was the $HgCl_2$ dose different (1·5 mg/kg), as described in the present paper.

† Strength and incidence of reactions were compared with that in syngeneic controls not treated with $HgCl_2$ and arbitrarily quantified as follows: +++, strong increase; +, clearly visible reaction or increase; +/–, hardly distinguishable from that of controls; –, no increase in reaction.

in the activation of murine $CD4^+$ T cells has been described by Pfeiffer *et al.* [33], who used human collagen IV as model antigen. They observed that the differential activation of Th subsets was dependent on the mouse strain examined, but in their system Th1 cells were activated in H-2^s mice and Th2 cells in H-2^b and H-2^d mice. Studies performed with cloned T cell lines [34–37] showed that high ligand density promotes priming of proliferative T cell responses characteristic of Th1 cells, whereas low ligand density fails to do so but retains the ability to prime Th2 cells.

In the rodent models of $HgCl_2$-induced immunopathological alterations, however, the precise antigens, let alone the epitopes, that activate $CD4^+$ T cells have not yet been identified [2,38]. Because of this, *in vitro* demonstration of the $CD4^+$ T cells involved in $HgCl_2$-induced immunopathological alterations is difficult and this, in turn, hampered analysis of the cytokines produced by them. Recently, some progress has been made [39,40], in that bulk T cells of $HgCl_2$-treated B10.S mice were found to react against a variety of different self proteins altered by Hg^{2+}, in particular fibrillarin, a small ribonucleoprotein that is mainly located in the nucleolus and is the principal target of ANolA in these mice [41,42]. Moreover, in $HgCl_2$-treated Brown Norway rats an activation of autoreactive $CD4^+$ T cells has been described [43,44], albeit that the MHC II-associated self proteins recognized by them have not yet been identified; recent experimental evidence indicates that these cells are of the Th2 type [17] (P. Druet, personal communication). These and other findings [7,15,16,45,46] indicate that in animals susceptible to $HgCl_2$-induced systemic autoimmune disease an activation of the immune system takes place that favours a Th2-like reaction. This pattern of reactivity to $HgCl_2$ is reduced or abolished by the presence of Th1 cytokines, such as IFN-γ, abrogating the systemic autoimmune disease [17,47,48]. Moreover, an increased induction of IFN-γ-producing splenocytes was demonstrated in $HgCl_2$-treated Lewis rats [49], pointing to a preferential involvement of Th1 cells in this strain and accounting for its resistance towards $HgCl_2$-induced systemic autoimmune disease. The results of the present study, performed in mice, conform to these findings obtained in rats. In B10.D2 mice, $HgCl_2$-induced IFN-γ accounts for the suppressed antibody formation detected in this strain, and it also may account for its resistance to the Th2-dependent systemic autoimmune disease inducible by $HgCl_2$.

In $HgCl_2$-treated B10.S mice, exogenously administered rIFN-γ suppressed the increase in serum IgE seen after $HgCl_2$ treatment. These findings, along with the pivotal role played by IL-4 in this experimental system [7,15] (Table 2), suggest that the antagonism between IFN-γ and IL-4 [29,50] also operates in $HgCl_2$-treated B10.S mice, with IL-4 inducing the isotype switch to IgE, and IFN-γ inhibiting the effects of IL-4. In spite of prolonged treatment with rIFN-γ, however, B10.S mice were not completely protected from $HgCl_2$-induced systemic autoimmunity, as they produced ANolA and developed glomerular immune deposits. These latter immunopathological alterations may develop via pathogenic pathways that are, at least partially, independent of IL-4 and cannot be antagonized by IFN-γ. Accordingly, anti-IL-4 MoAb therapy of $HgCl_2$-treated H-2^s mice, which did prevent the rise in total IgE and partially suppressed the rise in IgG1 and IgG1 ANolA, failed to reduce total ANolA titres [15].

In conclusion, our results indicate that IFN-γ plays a central role in the suppressed antibody formation to sheep erythrocytes seen in $HgCl_2$-treated B10.D2 mice. However, although neutralization of endogenous IFN-γ alleviated the immunosuppression, it did not suffice to convert the Th1-dominated response of $HgCl_2$-treated B10.D2 mice into a preferential Th2 response. Conceivably, in $HgCl_2$-treated B10.D2 mice the initial steps required for production of IL-4, and possibly additional cytokines needed for development of the systemic autoimmune disease, are not taken even if IFN-γ is blocked. IL-12 could play a role here, since it has been shown that IL-12 can up-regulate Th1-type autoimmune responses [10] and inhibit humoral autoimmunity via an IFN-γ-independent way [51]. Alternatively, the IFN-γ blockade in $HgCl_2$-treated B10.D2 mice may not have been complete. In that case, the remaining IFN-γ would still have been sufficient to prevent development of Th2 cells, but insufficient to suppress the anti-sheep erythrocyte antibody response.

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