Effects of deviating the Th2-response in murine mercury-induced autoimmunity towards a Th1-response

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Summary

T-helper cells type 1 (Th1) and type 2 (Th2) play an important role in the pathogenesis of autoimmune diseases. In many Th1-dependent autoimmune models, treatment with recombinant interleukin-12 (rIL-12) accelerates the autoimmune response. Mercury-induced autoimmunity (HgIA) in mice is an H-2 regulated condition with antinucleolar antibodies targeting fibrillarin (ANoA), systemic immune-complex (IC) deposits and transient polyclonal B-cell activation (PBA). HgIA has many characteristics of a Th2 type of reaction, including a strong increase of IgE, but disease induction is critically dependent on the Th1 cytokine IFN-γ. The aim of this study was to investigate if a strong deviation of the immune response in HgIA towards Th1 would aggravate HgIA. Injections of both rIL-12 and anti-IL-4 monoclonal antibody (α-IL-4) reduced the HgCl₂-(Hg)-induced concentration of the Th2-dependent serum IgE and IgG1, but increased the Th1-dependent serum IgG2a. The IgG-ANoA developed earlier and attained a higher titre after combined treatment, and the ANoA titre of the IgG1 isotype decreased while the ANoA titre of the Th1-associated IgG2a, IgG2b and IgG3-ANoA isotypes increased. Treatment with rIL-12 alone increased the Hg-induced IgG2a and IgG3 ANoA titres, the PBA, and the IC deposits in renal and splenic vessel walls, while treatment with α-IL-4 + Hg inhibited renal but not splenic vessel wall IC deposits. We conclude that manipulating the cytokine status, by altering the Th1/Th2 balance, will influence autoimmune disease manifestations. This might be an important way of modulating human autoimmune diseases.

Keywords autoimmunity interleukin-12 anti-interleukin-4 mercury mice

Introduction

The paradigm of T helper cells type 1 (Th1) and type 2 (Th2) [1] is well established and plays a crucial role in autoimmune diseases [2]. For example, transfer of Th1 cells induces autoimmune disease in murine allergic encephalomyelitis [3] and insulin-dependent diabetes mellitus (IDDM) [4]. Other Th1-dependent autoimmune diseases in mice are collagen-induced arthritis (CIA) [5] and autoimmune myasthenia gravis [6]. Because of the reciprocal regulation of Th1 and Th2 cells, it has been proposed that activated Th2 cells may inhibit Th1-dependent autoimmune diseases (reviewed in [7]). In agreement with this, treatment with the Th2 cytokine IL-4 confers protection from IDDM development [8]. However, Th2 cells may, under certain conditions, cause diseases otherwise induced by Th1 cells [9,10]. Therefore, linking the Th1 or Th2 type of reaction to susceptibility or resistance to particular autoimmune conditions is an oversimplification.

In murine mercury-induced autoimmunity (HgIA), mice with a specific MHC haplotype (H-2s) rapidly develop a systemic autoimmune disease characterized by antinucleolar antibodies (ANoA), systemic immune-complex (IC) deposits, transient polyclonal B-cell activation (PBA) and transient hyper-IgE condition [11,12]. Induction of ANoA seems to be an antigen-specific reaction caused by modification of fibrillarin due to binding of Hg [13,14] and/or necrosis-induced proteolytic modification [15]. In support of an antigen-specific mechanism, Hg-induced AFA are dependent on CD4+ cells [16], and T cells recognizing Hg-modified fibrillarin have been identified [14].

In 1991 Goldman et al. have suggested that HgIA was a prototypic Th2 disease [17]. Studies have been conducted in the mouse with the intention to attenuate or ameliorate HgIA by deviating the response towards Th1. Deviation has been accomplished by treatment with α-IL-4 [18] or recombinant interferon-gamma (rIFN-γ) [19]. The Th2-dependent parameters such as IgE...
are reduced efficiently by such deviation, but the ANoA response is not affected. By using mice with targeted mutations Kono et al. showed subsequently that IFN-γ but not IL-4 is required for induction of HgIA [20]. This implies that Th1 cells play a key role in the induction of HgIA.

Recently, we assessed the expression of mRNA for several cytokines during induction of HgIA. We found an early expression of Th1-derived IL-2 and IFN-γ that vanished rapidly. The latter might be due to the massive increase of IL-4 [21], which inhibits Th1 cells [22]. Our objective with the present study was to examine if a strong deviation towards Th1 would aggravate the autoimmune disease manifestations in HgIA. We used a treatment which has proved to be very efficient for ameliorating progressive cutaneous leishmaniasis in mice [23], a disease condition which shares with HgIA the dominance of Th2-associated cytokines [21]. We found that Th1 deviation accelerated several aspects of the HgIA condition.

**MATERIALS AND METHODS**

**Animals**

Female A.SW (H-2b) mice were obtained from M&B A/S (Ry, Denmark). The mice were housed in steel-wire cages under 12-h dark/12-h light cycles and given type R36 pellets (Lactamin, Vadsø, Sweden) and drinking water ad libitum. All mice were 9–10 weeks old at onset of the experiment.

**Substances used for treatment**

HgCl₂ (Hg) of analytical grade (Fluka, Seelze, Germany) was dissolved and diluted to 10 mg/l in tap water. NaCl was a sterile, 0.9% solution. Rat IgG1 (Rlg) (hybridoma IR 871) was a sterile solution, free from endogenous toxins, in 0.15 M phosphate buffered saline (PBS) (Technopharm, Paris, France). Monoclonal antibodies to IL-4 (α-IL-4 antibody) (clone 11B11 [24]) were obtained as a sterile solution in PBS from National Institute of Health (Bethesda, MD, USA). Recombinant interleukin-12 (rIL-12), Cat. 419-ML (R&D Systems Inc., Minneapolis, MN, USA) was freshly prepared as a 1-μg/ml solution in NaCl and aliquots for daily injections were kept at −70°C and thawed just prior to administration. Monoclonal antibodies to CD4 (α-CD-4 Ab) (clone GK1.5) were prepared as a 10-mg/ml solution in NaCl.

**Treatment**

Ten mg Hg/l was given ad libitum in the drinking water. Treatment with rIL-12 was started 18 h prior to Hg treatment and consisted of daily intraperitoneal (i.p.) injections of 0.2 μg rIL-12 for 10 days. Treatment with α-IL-4 consisted of 2 mg antibody given as i.p. injections 24 h prior to the start of Hg treatment and after 4 days of Hg treatment (Fig. 1). Controls were instead given 0.2 ml NaCl i.p. and 2 mg Rlg i.p. The different treatment groups and the treatment schedule are described in Table 1 and Fig. 1.

In addition, in the pilot study, groups were given 1 mg α-CD-4 antibody i.p. 7 days prior to Hg treatment, in combination with rIL-12 + α-IL-4 treatment.

**Blood and tissue sampling**

Blood samples were obtained from the mice by weekly bleeding from the retro-orbital plexus (Fig. 1). Animals were sacrificed after 4 weeks and tissues from the spleen, kidney and liver were snap-frozen in isopentan-CO₂ for direct immunofluorescence and fixed in HistoCHOICE™ (Amresco Inc., Solon, OH, USA) for light microscopy.

**Serum ANA**

Indirect immunofluorescence was performed as described previously [25]. Briefly, sera diluted 1 : 30–1 : 20 480 were applied to monolayers of fixed Hep-2 cells (Binding Site Ltd, Birmingham, UK), and bound serum antibody detected by FITC-conjugated goat antimouse IgG antibodies (Sigma Chemical Company, St Louis, MO, USA) diluted 1 : 50. The presence and pattern of fluorescence were observed using an epi-illuminescence microscope (Nikon Instech Co. Ltd, Kanagawa, Japan). The titres of ANoA of the IgM, IgG1, IgG2a, IgG2b and IgG3 isotype were assessed similarly using FITC-conjugated goat anti(α)-mouse IgM, goat α-mouse IgG1, α-mouse IgG2a, α-mouse IgG2b and α-mouse IgG3 antibodies (SouthernBiotech, Birmingham, AL, USA) diluted 1 : 80, 1 : 50, 1 : 160, 1 : 80 and 1 : 50, respectively.
For analysis of serum IgM [26] microtitre plates were coated with rat-α-mouse IgM (clone LO-MG1, Technopharm). Following blocking, the wells were incubated with diluted serum, and bound IgM was detected using diluted horseradish peroxidase (HRP) conjugated rat antianimalise IgM (clone LO-MM-3) MoAb (Technopharm). A standard curve was constructed by using mouse myeloma protein of the IgM isotype (clone MADNP-5, Technopharm). For measuring serum IgG2a, microtitre plate wells were coated with rat antianimouse IgG2a MoAb (clone R8-140, Pharmingen) overnight and then blocked with fat-free milk. Serum diluted 1 : 200 was added and ALP-conjugated antianimouse IgG2a antibodies (clone R19-15, Pharmingen) were used to detect bound IgG2a. A standard curve was constructed by using purified mouse IgG2a (Pharmingen).

**Tissue immune complex deposits**

Cryostate sections were prepared from samples of the left kidney and examined by direct immunofluorescence as described before [16] using FITC-conjugated goat antianimouse IgG antibodies (Southern) and anti-C3c antibodies (Organon-Technica, West Chester, PA, USA). The titre of immune reactants in the tissues was determined by serial dilution of the antibodies to 1 : 5120. The actual titre was defined as the highest dilution that still resulted in a specific staining. Deposits in vessel walls were graded as 0, absent; +1, scattered deposits; +2, moderate amount of deposits; +3, abundant deposits; +4, vessel walls filled with deposits. The slides were examined without knowledge of the treatment given.

**Light microscopy**

The tissue was dehydrated, cleared and embedded in paraffin blocks. Four-μm sections were cut in a microtome and stained with periodic acid Schiff reagent and periodic acid silver-methamine [29].

**Statistical methods**

ANOVA followed by Tukey’s test was used for comparisons of results obtained by ELISA. The differences between the groups with regard to the presence and titre of ANoA and IC deposits were analysed with Fisher’s exact test and the non-parametric Kruskal-Wallis test followed by Dunn’s test. If P <0.05 was considered statistically significant.

**RESULTS**

**Animal health**

The animals showed no signs of disease during the experiment. A few mice died during or immediately after the blood samplings. Among the Hg controls two mice died at the blood samplings.

Table 1. IgM-α-ssDNA and IgM-α-DNP antibody titre in the different treatment groups

<table>
<thead>
<tr>
<th>Antibody titre</th>
<th>Treatment group</th>
<th>Weeks after onset of treatment with Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>IgM-α-ssDNA</td>
<td>Hg controls</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Hg + rIL-12 + α-IL-4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Hg + rIL-12</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Hg + α-IL-4</td>
<td>5</td>
</tr>
<tr>
<td>IgM-α-DNP</td>
<td>Hg controls</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Hg + rIL-12 + α-IL-4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Hg + α-IL-4</td>
<td>7</td>
</tr>
</tbody>
</table>

<sup>a</sup>(OD<sub>405</sub>).<sup>b</sup>Mean antibody titre ± s.d. Significantly different antibody titre when comparing groups using ANOVA followed by Tukey’s post-test: *P < 0.05, versus Hg controls; †P < 0.05, versus Hg + rIL-12; ‡P < 0.01, versus Hg controls; §P < 0.01, versus Hg + rIL-12; ¶P < 0.05, versus Hg + rIL-12 + α-IL-4.
after 1 and 2 weeks. Two mice died in the group given Hg + rIL-12 + α-IL-4 during the blood sampling after 1 week. In the group given Hg + rIL-12 one mouse died during the blood sampling after 1 week, and another mouse during the blood sampling after 2 weeks.

**Pilot study using α-CD4 + rIL-12 + α-IL-4 to deviate the immune response in HgIA**

We first used treatment with α-CD4 followed immediately by treatment with rIL-12 and α-IL-4 for modulating the Th1/Th2-balance in HgIA. The rationale for this therapy was to deplete existing CD4+ cells and subsequently deviate developing Th0 cells into Th1 cells. However, this regimen abolished the induction of ANoA (data not shown), indicating that the development of new CD4+ cells was not sufficient to support the induction of ANoA, known to be a reaction dependent on T cells and specifically CD4+ cells [16]. However, treatment with rIL-12 combined with α-IL-4, without depleting CD4+ cells, reversed the Th2 response to a Th1 response, as evidenced by suppression of IgE and IgG1 and increase of IgG2a, both with regard to the antigen-specific ANoA response and the total serum Ig isotype pattern (see below).

**Effect of rIL-12 and/or α-IL-4 treatment on IgG ANoA**

A.SW mice treated for 1 week with Hg in combination with rIL-12 + α-IL-4 showed a significantly accelerated IgG-ANoA response compared with the Hg controls, both with regard to the fraction of ANoA positive mice (100% compared with 22%) (P < 0.05; Fisher’s exact test) and the titre (P < 0.01) (Fig. 2). The IgG-ANoA titre also continued to be increased significantly after 2 weeks’ treatment. rIL-12 is important for the acceleration of ANoA development because 71% of mice given Hg + rIL-12 but none of the mice given Hg + α-IL-4 showed IgG-ANoA after 1 week’s treatment (Fig. 2).

**Effect of rIL-12 and/or α-IL-4 treatment on the isotype pattern of Hg-induced ANoA**

The only significant effect of treatment with α-IL-4 alone on Hg-induced ANoA compared with the controls was an increase of IgG2b-ANoA after 3 weeks (Fig. 2).

In contrast, after 2 and 3 weeks of treatment with Hg in combination with rIL-12 + α-IL-4 the IgG1-ANoA titre was significantly higher (P < 0.01) compared with the Hg controls (Fig. 2).

After 2 weeks of treatment with Hg and either rIL-12 alone or in combination with α-IL-4, the IgG2a-ANoA titre was significantly higher (P < 0.005) and P < 0.05, respectively), compared to the titre in the Hg controls. The same pattern was seen after 3 weeks, although the difference was significant (P < 0.05) only in the group treated with rIL-12 + α-IL-4 (Fig. 4).

The IgG2b-ANoA titre induced by Hg was significantly higher (P < 0.05) increased after 3 weeks in mice given either rIL-12 or α-IL-4, or rIL-12 + α-IL-4 compared with the Hg controls (Fig. 2).

After 2 weeks of Hg treatment, the mean IgG3-ANoA titre was higher in mice given rIL-12 or r-IL-12 + α-IL-4 compared with the Hg controls, but the difference was significant for the group given rIL-12 only (P < 0.05) (data not shown).

**Effect of rIL-12 and/or α-IL-4 treatment on the Hg-induced polyclonal B-cell activation**

During the 3 weeks the Hg controls showed a reduction of the mean values for the PBA markers (IgM-α-ssDNA and IgM-α-DNP and serum IgM) of 14–41%, but the differences between the three time-points were not significant. Mice receiving rIL-12 showed increasing mean levels of all PBA markers during the 3 weeks of Hg treatment (19–93%), and they were significantly different compared to Hg controls after 2 and 3 weeks (Tables 1 and 2). Treatment with Hg + α-IL-4 + rIL-12 increased the mean level of the PBA markers 40–72% during 3 weeks’ treatment, and the differences were significant for serum IgM and IgM-α-DNP compared with Hg controls (Tables 1 and 2). Treatment with Hg + α-IL-4 caused a maximum increase of 19–35% in the mean level of PBA markers, which was significantly higher for serum IgM compared with the Hg controls but significantly lower for both IgM-α-ssDNA and α-DNP compared with the groups given rIL-12 ± α-IL-4 (Tables 1 and 2). Therefore, rIL-12 treatment alone but also in addition with α-IL-4 clearly enhanced the polyclonal B-cell activation in HgIA, while the effect of α-IL-4 alone was weak and lower than after treatment with rIL-12 ± α-IL-4.

**Effects of rIL-12 + α-IL-4 treatment on Th1 and Th2 associated Ig isotypes in HgIA**

As evidence of Th2 suppression, after 1 week the Hg-treated mice receiving combined treatment with rIL-12 + α-IL-4 showed a significantly reduced serum IgE concentration compared to Hg controls (Table 2). The mean IgE concentration in this group was lower than in the group given Hg + α-IL-4. Treatment with rIL-12 alone suppressed the IgG1 concentration, similar to α-IL-4 ± rIL-12, after 1 week, but lowered the mean IgG1 concentration more than the two other treatment modes after 2 and 3 weeks, indicating that rIL-12 had a distinct Th2-suppressing effect. However, in the group given rIL-12 alone the IgE concentration did not decrease significantly after 1 week, and showed a significant increase after 2 weeks compared with α-IL-4 ± rIL-12 (Table 2), which indicates a specific effect of rIL-12 on serum IgE. Combined treatment with rIL-12 + α-IL-4 gave rise to the highest mean concentration of IgG2a after 2 and 3 weeks, and it was significantly different compared with the other treatment modes after 2 weeks. We interpret this as a Th1 switching effect of rIL-12.

**Tissue IC deposits**

The mean titre of IgM and C3 in the mesangial deposits was higher in all treatment groups compared with the Hg controls, but the difference did not reach statistical significance (Table 3).

Treatment with rIL-12 enhanced the deposits of IgG and C3 induced by Hg in the renal and splenic vessel walls (Table 3). Treatment with α-IL-4 abolished the induction of IC deposits in the kidney vessel walls, but caused no significant change in the splenic deposits. Treatment with rIL-12 + α-IL-4 attenuated Hg-induced IC deposits in the renal vessels but had no effect on IC deposits in the splenic vessels compared with Hg controls.

**DISCUSSION**

The major objective of this study was to investigate if an autoimmune disease condition (HgIA) with characteristics of a Th2 type of reaction, but dependent on the Th1 cytokine IFN-γ [20], is aggravated by deviating the immune response pattern strongly from Th2 to Th1. We found that the Th1 deviation, accomplished by combined treatment with rIL-12 + α-IL-4, aggravated the HgIA by causing an earlier development of IgG-ANoA and by inducing a higher titre of IgG-ANoA. The group receiving Hg + rIL-12 also showed accelerated ANoA development,
Fig. 2. The reciprocal titre of IgG, IgG1, IgG2a, IgG2b ANoA in the different groups 1, 2 and 3 weeks after onset of treatment. *, ** and *** denote significant differences (P < 0.05, P < 0.01 and P < 0.001, respectively) compared with Hg controls as determined by Kruskal-Wallis test followed by Dunn's post-test.
Interleukin-12 in mercury-induced autoimmunity

Table 2. Serum Ig concentrations in the different treatment groups

<table>
<thead>
<tr>
<th>Serum Ig conc.</th>
<th>Treatment group</th>
<th>n</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE (μg/ml)</td>
<td>Hg controls</td>
<td>9</td>
<td>29.55±23.92</td>
<td>27.21±25.53</td>
<td>14.64±11.76</td>
</tr>
<tr>
<td></td>
<td>Hg + rIL-12 + α-IL-4</td>
<td>7</td>
<td>18.92±8.957</td>
<td>17.33±11.71</td>
<td>6.34±7.859</td>
</tr>
<tr>
<td></td>
<td>IgG1</td>
<td>5</td>
<td>2.08±2.02</td>
<td>0.65±0.078</td>
<td>0.77±0.178</td>
</tr>
<tr>
<td></td>
<td>IgG2a</td>
<td>7</td>
<td>0.57±0.027</td>
<td>0.56±0.064</td>
<td>0.58±0.069</td>
</tr>
<tr>
<td></td>
<td>IgG2b</td>
<td>5</td>
<td>0.52±0.004</td>
<td>0.67±0.043</td>
<td>0.712±0.116</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>7</td>
<td>0.53±0.084</td>
<td>0.14±0.025</td>
<td>0.113±0.008</td>
</tr>
<tr>
<td></td>
<td>IgM1</td>
<td>5</td>
<td>0.12±0.014</td>
<td>0.17±0.025</td>
<td>0.169±0.023</td>
</tr>
<tr>
<td></td>
<td>IgM2</td>
<td>7</td>
<td>0.158±0.017</td>
<td>0.189±0.023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgM3</td>
<td>5</td>
<td>0.129±0.016</td>
<td>0.137±0.019</td>
<td></td>
</tr>
</tbody>
</table>

Significant differences in serum Ig concentration when comparing groups using ANOVA followed by Tukey’s post-test. Values denote mean serum Ig concentration ± s.d. *P < 0.05 versus Hg controls; **P < 0.01 versus Hg controls; ***P < 0.001 versus Hg controls; ^P < 0.05 versus Hg + rIL-12; ^*P < 0.01 versus Hg + rIL-12 + α-IL-4.

Table 3. Immune-complex deposits in A.SW mice treated mercuric chloride and rIL-12 and/or anti-IL-4 and controls

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Kidney glomerular mesangium</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgGl</td>
<td>IgMl</td>
<td>C3l</td>
</tr>
<tr>
<td>Hg controls</td>
<td>320±196</td>
<td>12±0.45</td>
<td>832±429</td>
</tr>
<tr>
<td>Hg + rIL-12 + α-IL-4</td>
<td>160±98</td>
<td>1.6±0.89</td>
<td>2560±1568</td>
</tr>
<tr>
<td>Hg + rIL-12</td>
<td>256±88</td>
<td>2.0±0.0</td>
<td>2816±1402</td>
</tr>
<tr>
<td>Hg + α-IL-4</td>
<td>144±36</td>
<td>2.0±0.0</td>
<td>1792±701</td>
</tr>
</tbody>
</table>

*See Fig. 1; †mean reciprocal titre ± s.d.; ‡mean titre ± s.d. (grading 0–4); §fraction of mice with immune complex deposits; e, significantly different compared with Hg + rIL-12 + α-IL-4 (P < 0.05, Fisher’s exact test); ‖significantly different compared with Hg + rIL-12 (P < 0.05, Fisher’s exact test); ①significantly different compared with Hg + rIL-12 (P < 0.05; Kruskal–Wallis and Dunn’s post-test); ②significantly different compared with Hg + α-IL-4 (P < 0.01, Fisher’s exact test); ③significantly different compared with Hg + rIL-12 + α-IL-4 (P < 0.01, Kruskal–Wallis and Dunn’s post-test); ④significantly different compared with Hg controls (P < 0.05, Kruskal–Wallis and Dunn’s post-test); ⑤significantly different compared with Hg controls (P < 0.01, Fisher’s exact test).

although the response was less homogenous than after combined treatment. In contrast, mice given only α-IL-4 + Hg showed no acceleration of IgG-AnoA development and the titre was not increased, which is in accordance with previous observations [18], and concurs with findings in mice with a targeted mutation of the IL-4 gene [20]. Therefore, active deviation by the Th1-promoting IL-12 cytokine is crucial for aggravating the autoimmune response in HgIA.

With regard to the isotype pattern of the Hg-induced AnoA, our results after treatment with rIL-12 + α-IL-4 are in agreement with those observed after administration of α-IL-4 to Hg-treated mice, namely an increase in IgG2a, IgG2b and IgG3 AnoA titres, but a reduced titre of IgG1 AnoA [18]. The increase in IgG2a, IgG2b and IgG3 AnoA titres was also seen after treatment with rIL-12 + Hg (present study), which is in agreement with the effect of rIL-12 treatment in another antigen-specific response [30].

Our results are at variance with those of Bagenstose et al., who reported that treatment with rIL-12 in combination with Hg suppressed the AnoA response of all IgG isotypes [31]. This discrepancy may be due to differences in the rIL-12 regimen, because we treated the mice with rIL-12 for 10 days, while Bagenstose et al. treated the mice for 4 days [31]. In addition, in
the group to which we gave rIL-12 + α-IL-4, suppression of IL-4, a cytokine abundantly expressed in HgIA [21], might have allowed for a brisker development of Th1 cells, as IL-4 has an inhibiting effect on Th1 cells [22,32].

With regard to the effect of the serum immunoglobulin isotypes as markers of Th1/Th2 balance, injections of rIL-12 + α-IL-4 reduced the Th2-dependent serum IgE and IgG1 levels, indicating a Th2 suppression, and increased the Th1-dependent serum IgG2a, indicating a Th1 switching. Treatment with α-IL-4 alone had an expected suppressing effect on serum IgG1 and IgE concentrations [18]. Treatment with rIL-12 alone suppressed serum IgG1, as observed already in HgIA [31]. However, rIL-12 alone increased the serum IgE level, which is unexpected given the general assumption that rIL-12 down-regulates switching to both the IgG1 and IgE isotype [33]. Germann et al. showed that rIL-12 may fail to suppress or even enhance the effect of IL-4 on serum IgE, depending on both the strain and the dose of rIL-12 used [33]. Furthermore, Bagenstose et al. reported that rIL-12 enhanced IL-4 production in A.SW mice and caused an increased mean serum IgE level, although the difference was not significant compared with mice given Hg alone [31]. The stronger increase of IgE production in the present study might be related to the longer treatment time with rIL-12 in our study (see above). This might be related to the stimulating or even dominating effect on Th2 development which has been reported when IL-12 is present in vitro [34,35] or in vivo [33,36] together with IL-4.

With regard to other HgIA parameters, treatment with rIL-12 increased the renal and splenic vessel wall IC deposits. The mechanism behind the development of vessel wall deposits in HgIA is not known, but the splenic deposits are more stable and less affected by manipulating HgIA compared with the renal deposits [37,38]. This was also observed in the present study, because treatment with α-IL-4 inhibited the Hg-induced vessel wall IC deposits in the kidney but did not affect significantly the deposits in the spleen. Treatment with rIL-12 + α-IL-4 showed that the effect of IL-4 dominated, as the Hg-induced renal IC deposits were severely reduced, but the deposits in the spleen remained unchanged compared with Hg controls.

Interestingly, there was a co-variation between vessel wall IC deposits and PBA, another feature of HgIA [39]. Treatment with rIL-12 + Hg enhanced PBA compared with Hg controls, while α-IL-4 alone or in combination with rIL-12 did not affect consistently the degree of PBA induced by Hg. This co-variation between PBA and development of IC deposits has also been observed when the HgIA model is modified by other means, such as mercury species [40], dose or gender [37]. Because PBA induces antibodies of the IgM isotype, and the deposits in addition consist of IgG and C3, PBA must act as a co-factor.

Our main question, whether a strong deviation of the immune response towards Th1 in HgIA would aggravate disease manifestations, has been dealt with previously only indirectly in conjunction with studies aiming at down-regulating the Th2 response by inhibiting Th2 or stimulating Th1 [18,19,31]. Acceleration of the disease has not been found previously, although α-IL-4 treatment [18] augmented the IgG titre of ANoA induced by Hg. The observations in other Th1-dependent autoimmune disease models, such as experimental autoimmune myasthenia gravis [6], experimental arthritis [5] and autoimmune diabetes in non-obese diabetic (NOD) mice [41], indicate that treatment with IL-12 aggravates and/or accelerates disease manifestations. In these models the proposed mechanism includes a preferential expansion of IFN-γ secreting cells. While we did not measure IFN-γ secreting cells, a correlation exists between IFN-γ gene expression and the severity of HgIA [20].

Similarly, in vivo treatment of SJL (H-2') mice with rIL-12 aggravates experimental allergic encephalomyelitis (EAE) after adoptive transfer of antigen-stimulated lymph node cells [42]. However, in the EAE model the effect of rIL-12 treatment is IFN-γ independent [43]. These authors suggested that IL-12 plays an important role by suppressing IL-10 expression which would otherwise inhibit the development of autoimmune effector cells [43]. The concept of an immunoregulatory circuit comprising IL-12 and IL-10 which may modulate autoimmune diseases is interesting, as we have found previously that IL-10 expression is up-regulated in Hg-treated resistant A.TL mice [21]. This implies that IL-10 may play a role in maintaining the self-tolerance in resistant strains during Hg treatment.

We conclude that strong deviation of the immune response from Th2 towards Th1 using directly Th1-inducing cytokines does not provide protection from the development of HgIA, but instead accelerates and aggravates several of the disease parameters, including antinuclear antibodies, vessel wall IC deposits and PBA.

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