Mercuric chloride, a chemical responsible for T helper cell (Th)2-mediated autoimmunity in brown Norway rats, directly triggers T cells to produce interleukin-4.

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Research Article

Mercurials may induce immune manifestations in susceptible individuals. Mercuric chloride (HgCl2) induced autoimmunity in the Brown Norway (BN) strain but an immuno-suppression in the Lewis strain with, however, autoreactive anti-class II T cells present in both strains. In the present study we looked at modifications of cytokine production by PCR and cytofluorometric analyses in normal BN and Lewis rat splenocytes, cultured with or without HgCl2. Unfractionated BN rat splenocytes and purified T cells exposed to HgCl2 expressed high levels of IL-4 mRNA. Increase in class II and CD23 molecule expression on B cells was partly inhibited by anti-IL-4 mAb showing that IL-4 was produced. By contrast, no overexpression of IL-4 mRNA could be seen in Lewis rats. Although an increase in class II molecule expression was observed suggesting that other T helper cell 2 cytokines were produced, there was also a concomitant decrease in CD23 molecule expression that was abrogated after addition of an anti-IFN-gamma mAb to the culture. IFN-gamma mRNA production was induced in unfractionated spleen cells and T cells from both strains after HgCl2 exposure. Altogether these findings demonstrate that HgCl2 has very early direct effects on cytokine production and that these effects differ depending on the strain. The early effect on IL-4 production observed on BN rat spleen cells and T cells may explain that the autoreactive [...]

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Mercuric Chloride, A Chemical Responsible for T Helper Cell (Th)2-mediated Autoimmunity in Brown Norway Rats, Directly Triggers T Cells to Produce Interleukin-4

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Abstract

Mercurials may induce immune manifestations in susceptible individuals. Mercuric chloride ($HgCl_2$) induces autoimmunity in the Brown Norway (BN) strain but an immunosuppression in the Lewis strain with, however, autoreactive anti–class II T cells present in both strains. In the present study we looked at modifications of cytokine production by PCR and cytofluorometric analyses in normal BN and Lewis rat splenocytes, cultured with or without $HgCl_2$. Unfractionated BN rat splenocytes and purified T cells exposed to $HgCl_2$ expressed high levels of IL-4 mRNA. Increase in class II and CD23 molecule expression on B cells was partly inhibited by anti–IL-4 mAb showing that IL-4 was produced. By contrast, no overexpression of IL-4 mRNA could be seen in Lewis rats. Although an increase in class II molecule expression was observed suggesting that other T helper cell 2 cytokines were produced, there was also a concomitant decrease in CD23 molecule expression that was abrogated after addition of an anti–IFN-γ mAb to the culture. IFN-γ mRNA production was induced in unfractionated spleen cells and T cells from both strains after $HgCl_2$ exposure. Altogether these findings demonstrate that $HgCl_2$ has very early direct effects on cytokine production and that these effects differ depending on the strain. The early effect on IL-4 production observed on BN rat spleen cells and T cells may explain that the autoreactive anti–class II T cells that are found in $HgCl_2$-injected BN rats have a Th2 phenotype. (*J. Clin. Invest.* 1995. 1484–1489.). Key words: autoimmunity • cytokines • rats • T helper cell 1/T helper cell 2 • $HgCl_2$

Introduction

Many chemicals may induce immune-mediated reactions in genetically susceptible individuals. Mercurials are good examples of such agents. Exposure to these compounds has indeed been associated with the occurrence of membranous nephropathy (1) or dermatitis (2) and has been considered as a possible etiologi-

cal agent for the mucocutaneous lymph node syndrome or Kawasaki disease (3), which is associated with hyper IgE production (4). Finally, mercuric choride ($HgCl_2$) may potentiate IgE production by human blood mononuclear cells (5). These agents have been used to develop experimental models that mimic the human situation. In Brown Norway (BN)[1] rats, $HgCl_2$ induces T-dependent B cell polyclonal activation leading to the production of antibodies against endogenous and exogenous antigens, an increase in serum IgE and IgG_1 levels, an autoimmune glomerulonephritis, and gut vasculitis (6–10). By contrast, Lewis (LEW) rats do not develop autoimmunity upon $HgCl_2$ exposure (11) but an immunosuppression (12). Similar results have also been reported in mice using susceptible and resistant strains (for review see reference 13).

We previously reported that, in vivo, $HgCl_2$ transiently increases the expression of MHC class II molecules on B cells in both BN and LEW rats but with different kinetics (14). This increase started after the first $HgCl_2$ injection. Similar results were recently reported in mice (15). It is well known that increase in class II molecule expression on B cells depends upon T helper cell (Th) 2 cytokines (16, 17), and it has been shown recently that IL-4 mRNA is expressed very early after the first $HgCl_2$ injection in the spleen and the gut of BN rats (18). In addition, treatment of susceptible mice with an anti–IL-4 mAb abrogated the $HgCl_2$-induced increase in serum IgE concentration (19). These findings support a crucial role for Th2 cells in these models. In both susceptible and resistant rats, we found autoreactive T cells recognizing normal class II molecules (20, 21). T cell lines have been recently derived from both strains after $HgCl_2$ or gold salt injections. Those obtained from BN rats passively transfer the disease and are Th2-like or Th0 since they produce IL-4 but no or small amounts of IFN-γ (22). Those obtained from LEW rats transfer immunosuppression (21) and are Th1-like since they produce IFN-γ but no IL-4 (Pelletier, L., manuscript in preparation). It is widely accepted that the commitment of CD4+ T cells into Th1 or Th2 subset depends upon the cytokines that are present in the environment of the precursor CD4+ T cell with the presence of IL-12 (23) and IFN-γ (24) being essential to obtain a Th1 response and IL-4 to obtain a Th2 response (25).

In the present study we have tested in vitro whether a chemical such as $HgCl_2$ could directly trigger naive spleen cells to produce cytokines that might influence the differentiation pathway of the $HgCl_2$-induced autoreactive T cells. It will be shown that $HgCl_2$ directly affects cytokine mRNA synthesis and cyto-

kine production in a different way depending upon the strain. The most striking effect observed was the induction of IL-4 mRNA synthesis and IL-4 production by spleen cells and purified T cells from BN but not from LEW rats. $HgCl_2$ exposure induced IFN-γ mRNA production by spleen cells and T cells from both strains, and indirect evidence was obtained that $HgCl_2$ induced IFN-γ production by unfractionated spleen cells from LEW rats. This divergent effect of $HgCl_2$ on cytokine production probably explains why the autoreactive anti–class II T cells found in $HgCl_2$-injected rats have a Th2 phenotype in BN but not in LEW rats.

Methods

Animals. 6–10-wk-old male and female BN and LEW rats were obtained from Charles River Laboratories (Rouen, France). They were killed after anesthesia.

Antibodies. The mouse IgG_1 OX6 and OX17 mAbs that recognize, respectively, a monomorphic determinant on rat MHC RT1B and RT1D class II molecules (mouse I-A and I-E equivalent), the mouse IgG_1 W3/13 mAb that recognizes a rat pan–T cell determinant, the mouse IgG_2a OX34, OX35, and OX12 mAbs recognizing the rat CD2 molecules, the rat CD4 molecules, and the rat κ chain, respectively, were obtained from the European Collection of Animal Cell Cultures (Salisbury, UK). These mAbs were produced in our laboratory from the corresponding hybridomas, purified by affinity chromatography on protein A–Sepharose (Sigma Chemical Co., St. Louis, MO), and fluoresceinated. The anti–rat IL-4 IgG1 OX81 mAb was kindly provided by D. Mason (Medical Research Council, Oxford, UK). Purified mouse IgG_1 anti–rat κ chain mAb, a gift from H. Bazin (Unité d'Immunologie Expérimentale, Brussels, Belgium), was used to detect cells bearing surface Ig after labeling with biotin succinamide ester (Calbiochem Behring, La Jolla, CA). Rabbit anti–human CD23 (FcϵRII) purified Igs (Rb55) that cross-react with rat CD23 (26) were also used and revealed by purified fluoresceinated sheep IgG anti–rabbit Igs (50 μg/ml) diluted in normal rat serum to prevent nonspecific binding. Macrophages were stained using the fluoresceinated IgG1 mAb ED1 (27) kindly provided by J. Aten (University of Amsterdam, Amsterdam, The Netherlands). The mouse anti–rat IFN-γ IgG1 mAb DB-1 (28) was generously given by P. H. van der Meide (Biomedical Primate Research Centre, TMO, Rijswijk, The Netherlands). The mouse IgG1 MOPC21 mAb, of unknown specificity, was given by D. Glotz (Hôpital Broussais, Paris, France).

Cell culture and cell purification. Spleen cells were teased apart in DME (Biochrom, KG, Berlin, Germany). Unfractionated or fractionated cells (10^6/ml) were cultured in regular DME containing 10% FCS, nonessential amino acids, sodium pyruvate, and L-glutamine (Biochrom), no mercaptoethanol, in the absence or in the presence of 5 μM $HgCl_2$ (Sigma). This concentration was chosen because, in separate experiments, it allowed recovery of 90% viable cells as assessed by trypan blue exclusion after up to 18 h of culture. In some experiments, rat recombinant IL-4, kindly provided by D. Mason, or 10 μg/ml cycloheximide (Sigma) was added to overnight culture of unfractionated spleen cells, in the absence or in the presence of $HgCl_2$.

To obtain adherent-depleted spleen cells, spleen cells were incubated at 37°C for 45 min in petri dishes at a concentration of 10^7/ml. Nonadherent cells were then gently recovered, counted, adjusted to 10^6/ml, and cultured as above. It was verified by FACS® analysis that the preparation was completely depleted in ED1-positive cells and that only OX34-positive (T) and mouse IgG_1 anti–rat κ chain mAb–positive (B) cells were present in a 60:40 ratio. T and B cells were negatively selected from the spleen cell population depleted in adherent cells as described (29). For T cell purification, spleen cells were incubated with a mixture of OX6 and OX12 mAbs for 30 min at 4°C and mixed with sheep red blood cells coated with rabbit anti–mouse IgG (Serotec Ltd., Oxford, UK). After 30 min at 4°C, the preparation was centrifuged for 30 s at 70 g at 4°C. The supernatant was collected and the remaining sheep red

blood cells were lysed by osmotic shock. The T cell preparation thus obtained was verified to be 95–98% pure by flow cytofluorometry on a FACScan® (Becton Dickinson & Co., Sunnyvale, CA). It was checked that this preparation did not contain class II–positive cells. B cells were purified according to a similar procedure except that spleen cells were directly incubated with W3/13 mAb-coated sheep red blood cells. The preparation was centrifuged, and the supernatant was collected and treated as above. The B cells recovered were 95–98% pure as assessed by FACS® analysis.

Analysis of cytokine mRNA. Semiquantitative reverse transcriptase–PCR (RT-PCR) was performed as follows. Total spleen cells or purified T cells were pooled from three animals and 10^8 cells were cultured for 2, 4, 6, or 18 h with or without $HgCl_2$ as described above. Spleen cells were subjected to RNA extraction by the guanidium thiocyanate phenol chloroform procedure (30), and cDNA was synthesized following the manufacturer's recommendations (Boehringer Mannheim, Mannheim, Germany). Quantification of cDNA was performed as previously described (31) using primers designed to amplify specifically the rat hypoxanthine phosphoribosyltransferase gene: sense primer, 5'-TGC TGG ATT ACA TTA AAG CGC-3' and antisense primer, 5'-CTT GGC TTT TCC ACT TTC GC-3' (32). Quantified cDNA from each sample was then amplified as previously reported (33), using primers as follows: rat IL-4 sense primer, 5'-TGA TGG GTC TCA GCC CCC ACC TTG C-3'; rat IL-4 antisense primer, 5'-CTT TCA GTG TTG TGA GCG TGG ACT C-3'; rat IFN-γ sense primer, 5'-ATG AGT GCT ACA CGC CGC GTC TTG G-3', and rat IFN-γ antisense primer, 5'-GAG TTC ATT GAC AGC TTT GTG CTG G-3' (34). These primers were designed to specifically amplify cDNA fragments, representing mature mRNA transcripts, of the following sizes: 378 bp (IL-4) and 405 bp (IFN-γ). Rat IL-4 and rat IFN-γ mRNAs were amplified by 30, 35, 38, and 40 cycles of PCR, carried out using a DNA thermal cycler (PHC-3; Techne, Cambridge, UK): 1 min at 93°C, 2 min at 60°C, and 3 min at 72°C, preceded by an initial denaturation step (2.5 min at 93°C). Under these conditions, amplification reactions were found to be in exponential phase up to 35 cycles of PCR. RNA extractions, cDNA syntheses, and PCR reactions were run in the same time for both strains of rats. Aliquots of the PCR products were analysed by electrophoresis on a 2.5% agarose gel in Tris acetate EDTA buffer plus ethidium bromide and documented on photographic film (Polaroid Ltd., St. Albans, UK). Photographs of ethidium bromide–stained gels were then numerized into 512 × 512 pixel images within 256 gray levels, using an studioscan scanner (Agfa Corp., Orangeburg, NY). The amount of nucleic acids was determined by the densitometric analysis of the bands (National Institutes of Health Image software). The amount of nucleic acids is related to the optic density by a logarithmic equation. For one band, the sum of the logarithms of the pixel grey level values allows the estimation of the amount of nucleic acids. The results are expressed in arbitrary units.

Detection of modification in class II and CD23 molecule expression on splenic B cells. There is presently no available technique to directly measure IL-4, IL-10, and IL-13 in the rat. IL-4, IL-10, and IL-13 are known to increase class II molecule expression on B cells (16, 17, 35). Moreover, increase in CD23 molecule expression on mouse B cells and monocytes has been shown to be IL-4 (36) and IL-13 (35) dependent but IL-10 independent. In this study, we looked at I-A and CD23 molecule expression on splenic B cells treated or not with $HgCl_2$ in the presence or in the absence of various mAbs (anti–rat IL-4 OX81, anti–rat IFN-γ DB-1, or the isotype-matched MOPC21 control mAb). Modification of cell-surface molecule expression was studied by measuring the mean fluorescence intensity (MFI) after labeling cells with different mAbs, as previously described (14). The MFI variation was defined as the following ratio: (MFI of treated cells − MFI of untreated cells)/MFI of untreated cells.

Detection of IL-2 and of IFN-γ activity in culture supernatants. IL-2 activity in supernatants from spleen cells cultured in the absence or in the presence of $HgCl_2$ was assessed according to the method of Gillis (37), using the IL-2–dependent cytotoxic T lymphocyte line (CTLL)-2 cell line and mouse recombinant IL-2, both kindly provided by P. Truffa-Bachi (Institut Pasteur, Paris). This cell line does not respond
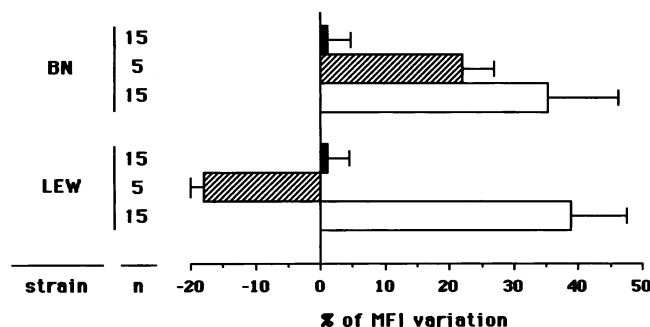
Figure 1. Effect of HgCl₂ on CD2, CD23, and I-A molecule expression on unfractionated spleen cells from BN and LEW rats. Spleen cells were cultured for 18 h in the presence of 5 μM HgCl₂. Modification in CD23 and I-A molecule expression was targeted on B cells by double staining (see Methods). Modification in CD2 (■), CD23 (■), and I-A (□) molecule expression was expressed as the variation (mean±SD) of the MFI between normal spleen cells and HgCl₂-treated spleen cells.

Figure 2. Effect of HgCl₂ on I-A (□) molecule expression on fractionated spleen cells from BN and LEW rats. Procedures were the same as those described in legend for Fig. 1.

to rat IL-4 (Saoudi, A., & D. Mason, unpublished observations). 1.2 × 10⁴ CTLL-2 cells in 150 μl conventional medium were cultured for 18 h with 50 μl of supernatant collected from untreated or HgCl₂-treated BN or LEW spleen cells, in the absence or in the presence of 2.5 U of mouse recombinant IL-2. Microcultures were then pulsed for 6 h with [³H]thymidine. Results were expressed as the mean of cpm values from three different experiments performed in triplicate. IFN-γ was assayed in culture supernatants using an ELISA kit (Gibco BRL, Cergy-Pontoise, France).

Statistical analysis. Comparisons between groups were performed by using the paired Student's *t* test.

Results

Effect of HgCl₂ on I-A and CD23 molecule expression on spleen cells from BN and LEW rats. When unfractionated spleen cells from BN or LEW rats were exposed to 5 μM HgCl₂ for 2 or 4 h, 95% of the cells were viable, and no modification in I-A, I-E, class I, CD23, CD2, or CD4 molecule expression was observed (not shown). By contrast, after 18 h of culture in the presence of 5 μM HgCl₂, a significant increase ($P < 0.001$) in I-A molecule expression on spleen cells from both BN and LEW rats was evident (not shown). Double staining FACS® analysis showed a significant increase ($P < 0.001$) in class II molecule expression on B cells that did not differ between both strains (Fig. 1); it was inhibited by cycloheximide, a protein synthesis inhibitor (not shown). No significant increase in I-A molecule expression was observed on surface Ig negative cells. CD23 molecule expression increased significantly ($P < 0.01$) on splenic B cells from BN rats but, by contrast, decreased significantly ($P < 0.01$) on B cells from LEW rats (Fig. 1). No modification in CD2 (Fig. 1), I-E, CD4, or class I (not shown) molecule expression on spleen cells was seen. In other experiments, it was shown that the increase in I-A molecule expression on splenic B cells, as assessed by double staining, was still observed to the same degree in both strains after removal of adherent cells (Fig. 2); it was verified that this preparation was devoid of ED1-positive cells and contained only T (OX34⁺) and B (mouse IgG₁ anti–rat κ chain mAb⁺) cells. When purified T or B cells (95–98% pure) were incubated for 18 h with 5 μM HgCl₂, no increase in I-A molecule expression was observed. Again, I-E, CD2, CD4, and class I molecule expression was not affected (not shown). These experiments
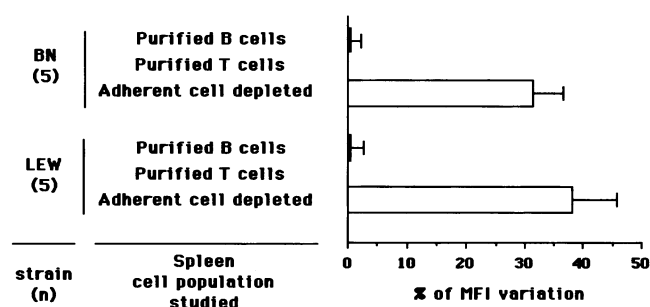
strongly suggested that exposure of normal spleen cells from BN or LEW rats to nontoxic amounts of HgCl₂ induced the production of various cytokines.

Culture supernatants from the different experiments mentioned above were tested for their ability to increase I-A molecule expression on normal splenic B cells, an effect dependent on Th2 cytokines. No significant effect could be obtained even when undiluted supernatants were tested. Neither IL-2 nor IFN-γ could be detected in the supernatants. Indeed, the CTLL-2 cell line did not proliferate when cultured in the presence of the different supernatants. Addition of 2.5 U/ml of mouse recombinant IL-2 in supernatants induced CTLL-2 cells to proliferate showing that residual HgCl₂ had no toxic effect on this cell line (not shown). Supernatants were also tested for the presence of IFN-γ using an ELISA. This assay allows the detection of as few as 1,250 pg/ml of IFN-γ. Five supernatants were tested in duplicate for each experiment. No significant IFN-γ production could be detected in any supernatant.

Effect of anti–IL-4 and anti–IFN-γ mAbs on HgCl₂-induced increase in I-A and CD23 molecule expression. As shown in Fig. 3, 18 h incubation of unfractionated spleen cells from BN rats in the presence of 5 μM HgCl₂ together with the anti–IL-4 mAb significantly reduced but did not abrogate the increase in I-A and CD23 molecule expression on splenic B cells when compared with spleen cells incubated in the presence of HgCl₂ and of an isotype-matched control mAb ($P < 0.05$ and $P < 0.01$, respectively). We verified that the amount of anti–
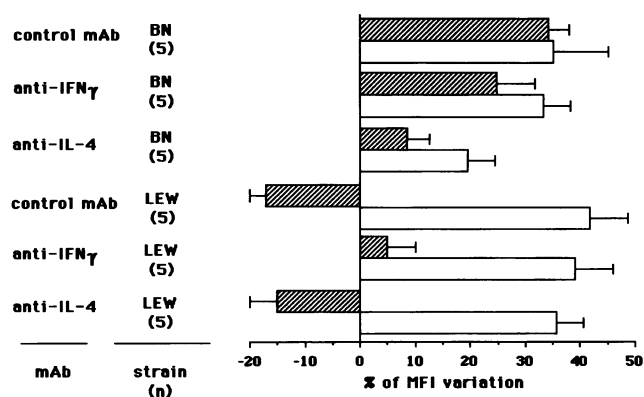
Figure 3. Effect of addition of mAb on the HgCl₂-induced variation in CD23 (■) and I-A (□) molecule expression on unfractionated spleen cells from BN and LEW rats. Cultures were performed as indicated in Fig. 1 except that the various mAbs were added.
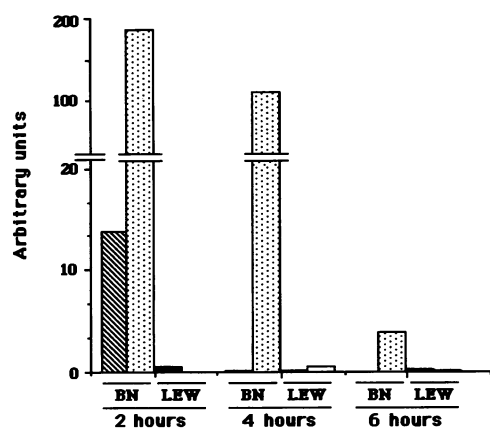
Figure 4. Semiquantitative RT-PCR analysis of IL-4 mRNA expression in total spleen cells from BN and LEW rats. A pool of spleen cells from three rats of each strain was used. The amplification process was run in the same time for untreated and treated BN rat or LEW rat cells and the amplified cDNA products were analyzed by gel electrophoresis. Relative band intensities for untreated BN spleen cells (■), HgCl$_2$-treated BN spleen cells (▨), untreated LEW spleen cells (■), and HgCl$_2$-treated LEW spleen cells (□) were expressed in arbitrary units.

IL-4 mAb used in these experiments totally inhibited a 120% increase in I-A molecule expression induced on naive rat B cells by rat recombinant IL-4 (not shown). Incubation with the DB-1 anti–IFN-γ mAb had no significant effect on either I-A or CD23 molecule expression. Completely different results were obtained when the same experiments were performed using unfractionated spleen cells from LEW rats. Incubation with anti–rat IL-4 mAb had no effect on either I-A or CD23 molecule expression, but incubation with DB-1 mAb abrogated the negative effect of HgCl$_2$ on CD23 molecule expression on B cells. However, the increase in CD23 molecule expression on B cells now observed was weak and not significant (Fig. 3).

Effect of HgCl$_2$ on cytokine mRNA expression. In the previous experiments, we could not detect cytokines in culture supernatant, although, the increase in I-A and CD23 molecule expression and the influence of anticytokines mAbs strongly suggested that cytokines were indeed produced. We therefore looked at cytokine mRNA expression using semiquantitative RT-PCR. It is clear from Fig. 4 that unfractionated spleen cells from BN rats produced high amounts of IL-4 mRNA when cultured for 2 or 4 h in the presence of 5 μM HgCl$_2$. IL-4 mRNA was barely detectable after 6 h (Fig. 4) and was undetectable after 18 h (not shown). Unfractionated spleen cells from BN rats cultured in the absence of HgCl$_2$ expressed low levels of IL-4 mRNA after 2 h of culture and no detectable IL-4 mRNA after 4, 6, or 18 h. In striking contrast, no IL-4 mRNA could be detected when spleen cells from LEW rats were cultured for 2–18 h in the presence or in the absence of HgCl$_2$. The semiquantitative RT-PCR was repeated on 98% purified T cells that were, as assessed by FACS® analysis, devoid of surface Ig$^+$ and class II$^+$ cells. T cells from BN rats cultured for 2, 4, or 6 h in the absence of HgCl$_2$ did not produce IL-4 mRNA while a strong message was observed after 4 h of culture in the presence of HgCl$_2$. This message was clearly detectable as soon as the second hour and was still detectable after 6 h of culture (Fig. 5). No IL-4 mRNA was detected either in normal or HgCl$_2$-exposed T cells from LEW rats (Fig. 5) even when anti–IFN-γ mAb was added to the culture (not shown).
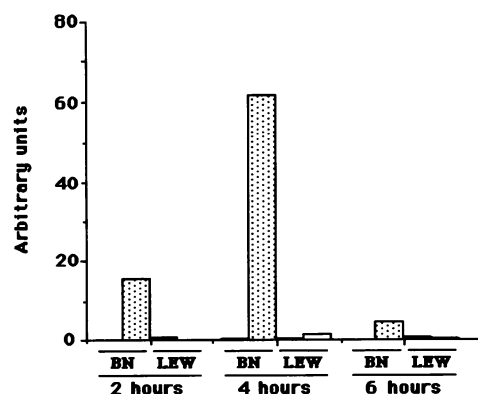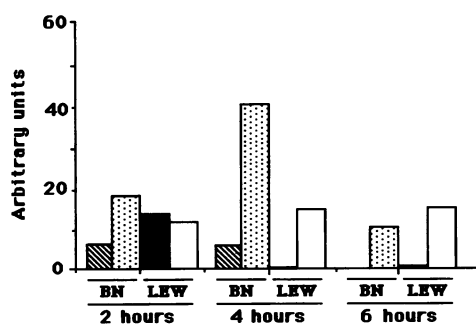

Figure 5. Semiquantitative RT-PCR analysis of IL-4 mRNA expression in purified T cells from BN and LEW rats. Procedures were the same as those described in legend for Fig. 4.

As shown in Fig. 6 and 7, unfractionated spleen cells and T cells from BN and LEW rats produced higher amounts of IFN-γ mRNA when cultured in the presence of HgCl$_2$ than when cultured in the absence of HgCl$_2$. This was true at every time point tested except for LEW rat spleen cells after 2 h of culture, at which time IFN-γ mRNA expression was similar whether HgCl$_2$ was present or not.

Discussion

This study mainly shows that HgCl$_2$, a chemical responsible for autoimmune manifestations in BN rats, induced IL-4 mRNA expression as early as 2 h after it was added to syngeneic normal spleen cells or to 98% purified T cells. IL-4 was produced later and synthetized de novo since cycloheximide had a blocking effect (38). By contrast neither IL-4 mRNA expression nor IL-4 production was affected in the resistant LEW strain.

The increase in I-A and CD23 molecule expression was specifically and significantly reduced after addition of anti–IL-4 mAb to the culture. One may therefore conclude that, although Th2 cytokines could not be detected in the culture supernatants, HgCl$_2$ induced IL-4 production by BN rat spleen cells. Incidentally, our study showed for the first time, using cross-reacting

Figure 6. Semiquantitative RT-PCR analysis of IFN-γ mRNA expression in total spleen cells from BN and LEW rats. A pool of spleen cells from three rats of each strain was used. The amplification process was run in the same time for untreated and treated BN rat or LEW rat cells and the amplified cDNA products were analyzed by gel electrophoresis. Relative band intensities for untreated BN spleen cells (■), HgCl$_2$-treated BN spleen cells (▨), untreated LEW spleen cells (■), and HgCl$_2$-treated LEW spleen cells (□) were expressed in arbitrary units.
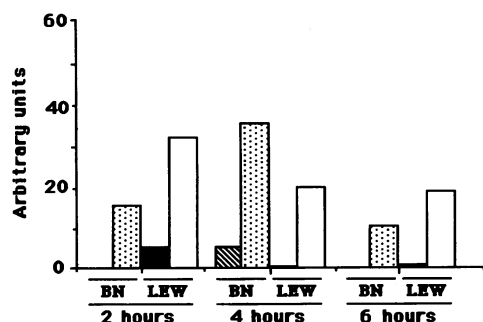
Figure 7. Semiquantitative RT-PCR analysis of IFN-γ mRNA expression in purified T cells from BN and LEW rats. Procedures were the same as those described in legend for Fig. 6.

anti–human CD23 antibodies, that increase in CD23 molecule expression on rat B cells was IL-4 dependent as in mice (for review see reference 36) and humans (35). It must also be stressed that anti–IL-4 mAb only partially abrogated the increase in I-A and CD23 molecule expression on B cells, although the amount of mAb used completely suppressed a much higher increase in I-A molecule expression induced by rat recombinant IL-4. This suggested that other Th2 cytokines (IL-10 and/or IL-13) were also produced or that cellular interactions play a role in this system. These results are in agreement with several in vivo experiments indicating a role for Th2-like cells and for IL-4 in HgCl₂-induced disease in BN rats (13, 18, 39) and in susceptible mice (15, 19).

Cells responsible for IL-4 production at the initiation of an immune response are not yet well characterized. Mast cells (40), (HSA^{lo} CD4$^+$8$^-$) thymocytes, also called recent thymic migrants (41), and NK1.1$^+$ CD4$^+$ splenic T cells (42) are good candidates. Our results strongly indicate that HgCl₂ is able to directly induce T cells from BN rats to produce IL-4. This does not rule out the possibility that interactions between T cells play a role or that interactions with other cells could amplify IL-4 production. The T cell subset responding to HgCl₂ remains to be defined. Mechanisms leading to the production of IL-4 by T cells exposed to HgCl₂ have to be clarified. HgCl₂ could control *IL-4* gene promoter by inducing the phosphorylation of nuclear proteins (43) or by interfering with *IL-4* gene regulatory elements described by Todd et al. (44). HgCl₂ could also act on cell-surface determinant in a way similar to anti-CD3 mAb (42) or, as already suggested (13), affect membrane determinants such as adhesion molecules (45), resulting in increased cell interactions.

Completely different results were obtained in LEW rats. Although I-A molecule expression was also increased on LEW rat B cells after culture of unfractionated spleen cells in the presence of HgCl₂, there was no IL-4 mRNA production. Furthermore, the increase in I-A molecule expression was not modified by addition of anti–IL-4 mAb in the culture, suggesting that Th2 cytokines other than IL-4 (IL-10 or -13) were produced. It has been recently shown that the BN *IL-4* gene has a unique CT repeat allele in the second intron which is absent in LEW rats (Kermarrec, N., manuscript in preparation). This polymorphism may explain the differential effect of HgCl₂ on IL-4 mRNA expression in these strains.

Our results also showed that IFN-γ mRNA expression was induced by HgCl₂ in spleen cells and purified T cells from both BN and LEW rats. However, the presence of IFN-γ in the

supernatants could not be directly demonstrated using either a classical ELISA or, in preliminary experiments (not shown), a much more sensitive in situ ELISA recently described for mouse IL-4 (46). We obtained however indirect evidence for IFN-γ production in LEW rats since the HgCl₂-induced decrease in CD23 molecule expression on spleen cells was reversed by the addition of anti–rat IFN-γ mAb to the culture. Others have shown that, in mice, IFN-γ down-modulates CD23 molecule expression (36). This suggests that, at least in LEW rats, IFN-γ is produced. This production may be transient due, for example, to unstable mRNA. The absence of IL-4 mRNA expression and of IL-4 production in this strain probably does not depend on IFN-γ production since the addition of the anti–IFN-γ mAb did not affect these parameters. By contrast, no evidence for IFN-γ production could be obtained either directly or indirectly in BN rats. This could be due to regulation at the posttranscriptional level (47), the enhanced production of NO by spleen cells from HgCl₂-injected BN rats which suppresses IFN-γ production (48), or down-regulation by IL-4 (49).

The potential relevance of these in vitro findings to the in vivo situation may be important. IFN-γ production is reduced (50) due to NO accumulation (48) in HgCl₂-injected BN rats while IL-4 production is induced in HgCl₂-exposed T cells from BN rats; this may explain that the autoreactive anti–class II T cells generated in this strain have a Th2 phenotype, although it cannot be ruled out that Th0 cells are also present. In agreement with this, four out of the six T cell lines obtained from gold-injected BN rats had a clear Th2 phenotype, while the two others had a Th0 phenotype (22); all the lines were able to passively transfer autoimmunity (22). In contrast, the autoreactive anti–class II T cell lines derived from HgCl₂-injected LEW rats that transfer immunosuppression (21) produce TGF-β, IFN-γ, but no IL-4 (Pelletier, L., manuscript in preparation). Reasons for the emergence of T cells with this phenotype are not yet clear, but, interestingly, spleen cells from these animals do not produce IL-4 when cultured in the presence of HgCl₂.

Although much remains to be done to understand the mechanisms of action of HgCl₂, it is probably important that, depending upon the strain, this chemical may or may not trigger the production of IL-4 and perhaps other cytokines that will drive the immune response in opposite directions. These findings may greatly contribute to the understanding of the mechanisms of drug-induced autoimmunity.

Acknowledgements

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