Mercury-induced Autoreactive Anti-class II T Cell Line Protects from Experimental Autoimmune Encephalomyelitis by the Bias of CD8+ Antiergotypic Cells in Lewis Rats

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Summary

Brown-Norway (BN) rats injected with HgCl2 develop a systemic autoimmune disease associated with a polyclonal B cell activation, due to autoreactive T cells specific for self-class II molecules, while Lewis (LEW) rats injected with HgCl2 do not exhibit autoimmunity and develop a non-antigen-specific, CD8-mediated immunosuppression assessed by a depression of T cell functions, and a protection against experimental autoimmune encephalomyelitis (EAE). Resistance to HgCl2-induced autoimmunity is not due to these suppressor cells since treatment with an anti-CD8 monoclonal antibody (mAb) did not allow autoimmunity to appear. The absence of autoimmunity in this strain could result from the absence of autoreactive T cells, or from quantitative or qualitative differences of these cells between susceptible and resistant strains. In the present study, we show that CD4+ anti-class II T cells are present in HgCl2-injected LEW rats and are as frequent as in BN rats when assessed by limiting dilution analysis. LEW CD4+ autoreactive T cell lines were derived. They proliferated in the presence of normal class II-bearing cells, secreted interleukin 2, and did not induce B cells to produce immunoglobulins. Transfer of one of these lines, LEW Hg A, into normal LEW rats led to the appearance of CD8+ cells responsible for a non-antigen-specific immunosuppression that induced complete protection from EAE. Immunosuppression was abrogated after treatment with an anti-CD8 mAb. In vitro, CD8+ cells from rats injected with the LEW Hg A T cell line proliferated in the presence of activated T cells whatever their origin. We conclude that HgCl2 induces CD4+ autoreactive T cells that proliferate in the presence of class II+ cells in susceptible BN as well as in resistant LEW rats. But while these cells collaborate with B cells to produce autoantibodies in BN rats, they initiate in LEW rats a suppressor circuit involving antiergotypic CD8+ suppressor cells.

Brown-Norway (BN) rats injected with HgCl2 develop a lupus-like systemic autoimmune disease in the context of a polyclonal B cell activation (1, 2), due to autoreactive T cells specific for self-class II molecules (3). In contrast, Lewis (LEW) rats injected with HgCl2 do not develop autoimmunity but exhibit a non-antigen specific CD8-mediated immunosuppression (4). This immunosuppression is mediated by non-antigen-specific CD8+ cells responsible for depression of T cell functions (4). HgCl2-injected LEW rats are protected against Heymann’s nephritis (5) and experimental autoimmune encephalomyelitis (EAE) (6, 7). Protection is not absolute, probably due to the emergence of contrasuppressor cells (7). Until now, the way these suppressor cells were induced and their specificity were unknown. Since anti-class II T cells were present in BN rats injected with HgCl2 (3) and since in another situation anti-class II T cells have been shown to induce suppressor cells (8), it was tempting to look for the presence of anti-class II T cells in HgCl2-injected LEW rats and to assess their possible connection with the mercury-induced suppressor cells observed in that strain.

We show here that anti-class II T cells are detectable in LEW rats injected with HgCl2, with a frequency similar to that found in susceptible BN rats. Autoreactive anti-class II CD4+ T cell lines have been derived from LEW rats injected with HgCl2. One of these lines has been transferred in naive LEW rats and it acted as a suppressor inducer line. It triggered the appearance of CD8+ antiergotypic and per-
hps antidiotypic suppressor cells. These autoreactive anti-class II T cells differed from those found in HgCl$_2$-injected BN rats by their inability to help in vitro or in vivo B cells to produce antibodies.

Materials and Methods

Rats. LEW and BN rats were initially obtained from the Centre de Selection et d'Elevage des Animaux de Laboratoire (Orléans-La Source, France), and spontaneously hypertensive rats from Ifla Credo (Lyon, France). These strains were then maintained by brother-sister mating in our facilities. 8-12-wk-old males were used.

Antigens. Myelin and myelin basic protein (BP) were prepared as described elsewhere (6). Purified protein derivative (PPD) from Mycobacterium tuberculosis was obtained from Statens Serum Institut, Copenhagen, Denmark. OVA was obtained from Sigma Chemical Co. (St. Louis, MO).

Injections of HgCl$_2$, Immunization with OVA, and EAE Induction. HgCl$_2$ was subcutaneously injected at a dose of 100 μg/100 g body weight, three times a week, as described elsewhere (1). LEW rats were immunized with 100 μg of OVA (Sigma Chemical Co.) emulsified in CFA in the rear footpads and killed after 10 d. EAE was induced as described (5). Briefly, myelin was suspended in PBS and an equal volume of CFA containing 0.2 mg/ml M. tuberculosis (H-37RA; Difco Laboratories, Detroit, MI); 0.25 ml of this emulsion was injected subcutaneously in the two rear footpads.

mAbs and FACScan® Analysis. W3/13, W3/25,OX8,OX39,OX6,OX17, and OX22 mAbs were initially obtained from the Public Health Laboratory Service collection (Oxford, UK) as cells. Ascites and purified antibodies were prepared as described (9). They recognize, respectively, all T cells (10), CD4$^+$ (11), CD8$^-$ (11), IL-2R$^+$ cells (12), MHC class II IA molecules (13), MHC class II IE molecules (14), and CD45R molecules (15). MARK-1 (mouse anti-rat $k$ light chain) mAb was kindly provided by H. Bazin (Louvain, Belgium). B10H2, a mouse anti-thyroglobulin IgGl mAb was kindly provided by Dr. D. Giotz (Hôpital Broussais, Paris, France), and was used as an irrelevant mAb. Cells to be tested were stained by indirect immunofluorescence with a fluoresceinated goat F(ab')$_2$ anti-mouse Ig antibody (Caltag, San Francisco, CA) adsorbed on normal rat serum. All antibodies were used 1:100 diluted.

Preparation of T Cell Lines. T cell lines were obtained from LEW rats injected with HgCl$_2$ for 7 d by cultivating 0.5 × 10$^6$ lymph node cells in 96-well U-bottomed microtiter plates (Nunc), in a 6% CO$_2$ incubator in the presence of normal irradiated (3,000 rad) syngeneic thymocytes (5-10 × 10$^5$/ml), under a volume of 0.2 ml/well in culture medium without Con A supernatant. After 4-6 d, half of the medium was removed and replaced by culture medium containing 10% Con A supernatant. 7-10 d later, cells were stimulated again with thymocytes. Exactly the same procedure was then followed after this second round of stimulation.

To obtain an anti-OVA T cell line (LEW OVA), LEW rats were immunized with 100 μg OVA, and 10 d later lymph node cells were stimulated in the presence OVA (50 μg/ml). Con A supernatant was added after 4-6 d as described above, and a second round of antigenic stimulation was performed 10 d later exactly as the first one. The culture was maintained on a basis of one stimulation every 2 wk. Phenotype of the cells was assessed after three, five, and eight stimulations.

Proliferative Response of T Cell Lines. T cell lines from the HgCl$_2$-injected rats were cultured (5 × 10$^6$ cells under a volume of 200 μl) in 96-well flat-bottomed microculture plates (Costar), alone or in the presence of various stimulator cells (5 × 10$^6$/ml) for 3 d. In some experiments, stimulator cells were preincubated with various mAbs (50 μg/ml) as described in LDA experiments. The proliferative response of the LEW OVA T cell line was assayed by culturing 5 × 10$^6$ cells in the presence of irradiated (3,000 rad) syngeneic thymocytes (10$^6$/ml) and OVA (50 μg/ml) for 3 d. 24 h before the end of the culture, cultures were pulsed with 1 μCi [H]$^3$H]thymidine/well and processed as described (3). Results were expressed as cpm [H]$^3$H]Tdr.

Titration of IL2 in Culture Supernatants. Supernatants were collected after 3 d of culture and assayed 1:4 diluted on triplicate cultures of 10$^5$ IL-2-dependent mouse CTLL-2 cells per well, in 96-well flat-bottomed microculture plates. The CTLL-2 cultures were incubated for 24 h, pulsed during the last 12 h with [H]$^3$H]thymidine, and processed as above. Human rIL-2 (Cetus Corp., Emeryville,
CA) was used as a positive control. Results were expressed as cpm [3H]TDR.

Ig Production In Vitro. Unseparated spleen cells from normal LEW rats were layered over Ficoll-Hypaque and RBC were eliminated by density centrifugation. Cocultures were performed by adding $5 \times 10^6$ purified T cells, preactivated by autologous APC for 3 d, to $2 \times 10^6$ spleen cells under a final volume of 0.2 ml/well in 96-well flat-bottomed microculture plates for 12 d. T cells alone and spleen cells alone were cultured as controls. 12 replicate wells of each culture combination were set up. 3 d after the beginning of the culture, 100 µl of supernatant medium was removed in each well, and was replaced by fresh medium containing 3% Con A supernatant as a source of IL-2. At the end of the culture, supernatants were removed, pooled, and assayed for Ig production by ELISA (18).

Transfer Experiments. LEW rats were intravenously injected with $5 \times 10^7$ cells from one of the T cell lines (LEW Hg A) derived from HgCl2-injected LEW rats or from the LEW OVA T cell line, and simultaneously immunized with myelin. Rats transferred with T cells were intraperitoneally injected or not with the OX8 anti-CD8 or B10-H2 mAbs as irrelevant mAbs (0.5 mg/rat per injection on days 0, 10, and 19). It was verified at the time of death that OX8-injected rats were depleted of CD8+ cells (3% of lymph node cells expressed CD8 molecules vs. 18% in LEW rats injected with B10-H2 mAb). Rats have also been transferred with $5 \times 10^7$ LEW OVA cells cultured for 3 d with BP (50 µg/ml) or with the same number of thymocytes cultured in the presence of BP in the same conditions. Rats were examined daily to assess neurological manifestations. These manifestations were scored on a 0–4 scale: 0, no clinical signs; 1, flaccid tail; 2, ataxia; 3, hind limb paralysis; and 4, moribund or dead (6, 7).

Rats were killed on day 21 because paralyzed rats recover at that time. Lymph node cells were prepared as described (2). Cells ($2 \times 10^6$/well) were cultured alone, in the presence of Con A (2.5 µg/ml) for 2 d, in the presence of irradiated (3,000 rad) lymph node cells from normal BN rats (10⁶/ml) for 4 d, or in the presence of BP or PPD (50 µg/ml) for 3 d. Various numbers of lymph node cells from rats transferred with LEW Hg A cells were added at the beginning of the culture, and the percentage of inhibition of the proliferative response was calculated.

The proliferative response was also performed in the presence of $2 \times 10^6$ irradiated (3,000 rad) LEW Hg A or LEW OVA cells, previously activated or not, or in the presence of 20% supernatant obtained after culturing the lines for 3 d in the presence of their antigen, or in the presence of 20% Con A supernatant. Cells were cultured for 4 d in 96-well flat-bottomed microculture plates. [3H]Thymidine was added 24 h before the end of the culture, and thymidine uptake was measured as described above.

The occurrence of manifestations similar to those triggered by HgCl2 in BN rats were looked for in the different groups by measuring serum IgE concentration every 1 wk until death, and by examining kidneys at the time of death for the presence of Ig deposits.

Statistical Analysis. Data were compared by using Student's t or nonparametric Mann Whitney or Wilcoxon tests.

Results

Autoreactive Anti–class II T Cells Are Present in LEW Rats Injected with HgCl2. CD4+ T cells obtained from LEW rats injected with HgCl2 for 4, 6, 14, or 30 d proliferated in the presence of syngeneic B-enriched cells. The frequencies of these autoreactive T cells were not statistically different between days 4 and 30 (Fig. 1). Mean reciprocal frequencies of autoreactive T cells were $34.0 \pm 10.3$, $24.2 \pm 12.5$, $16.0 \pm 4.8$, and $22.2 \pm 3.3 \times 10^3$, at days 4, 6, 14, and 30, respectively (Fig. 1). The ability of B-enriched cells to stimulate autoreactive T cells was similar whether they came from normal LEW rats or from autologous rats exposed to HgCl2 (Fig. 1). T cells did not respond when B cells were preincubated with the OX6 anti-class II mAb (Fig. 1). Preincubation of B cells with the anti-class II mAb, the anti-CD4, or the anti-CD8 mAbs did not modify the frequency of autoreactive T cells (not shown). Normal or autologous T cells were unable to act as stimulator cells (Fig. 1). CD4+ T cells from normal LEW rats did not respond to autologous B cells (Fig. 1) showing that, within the limits of the method, autologous mixed lymphocyte reaction was not observed in normal rats.

CD8+ Cells from LEW Rats Injected with HgCl2 Proliferate in the Presence of Autologous CD4+ Cells. Since non–antigen-specific CD8+ suppressor cells and autoreactive CD4+ cells were found concomitantly in LEW rats injected with HgCl2, a connection between these two populations was sought. To test whether CD8+ cells were stimulated by HgCl2-activated CD4+ T cells, purified CD8+ lymph node cells from LEW rats injected with HgCl2 were cultured in the presence of irradiated autologous purificd CD4+ cells or in the presence of CD4+ cells from normal LEW rats. CD8+ cells from LEW rats injected with HgCl2 for 7 d proliferated in the presence of autologous CD4+ cells but not in the presence of CD4+ cells from normal LEW rats (Fig. 2) or CD8+ cells whether they originated from normal LEW rats injected with HgCl2 for 4, 6, 14, and 30 d proliferated in the presence of syngeneic B cells from autologous (■) or normal (□) rats. CD4+ T cells from LEW rats injected with the control solution were cultured in the presence of normal syngeneic spleen cells (○).
or autologous rats (not shown). The mean reciprocal frequency of these CD8+ cells was 45 ± 12 × 10^3. CD8+ cells from LEW rats injected with HgCl2 for 14 d (Hg14) or 30 d (not shown) were unable to proliferate whatever the stimulator cells tested, although the increase in the number of CD8+ cells was still observed (not shown) as previously reported (4).

Characterization of Autoreactive T Cell Lines Derived from LEW Rats Injected with HgCl2. We have studied the possibility that cells activating the CD8+ suppressor cells were HgCl2-induced autoreactive anti-class II T cells. To test this hypothesis, we developed autoreactive T cell lines from HgCl2-treated LEW rats. Seven autoreactive T cell lines were derived from seven rats (LEW Hg A through LEW Hg G). After three stimulations, all the lines had the same phenotype, i.e., W3/13+ CD4+ CD8- Ig- CD45R- IL-2R+. This phenotype remained stable after 10 mo of culture. These cell lines produced IL-2 after stimulation with irradiated syngeneic thymocytes (Table 1; and not shown). As a control, a T cell line was obtained from LEW rats immunized with OVA (LEW OVA) that had the same phenotype and also produced IL-2 when stimulated with OVA (Table 1).

Table 1. IL-2 Production by LEW Hg and LEW OVA T Cell Lines

| CTLL-2 | Supernatant from: | cpm |
|--------|------------------|-----|
| +      | -                | 310 ± 115 |
| +      | LEW Hg A         | 18,795 ± 380* |
| +      | LEW Hg B         | 15,425 ± 875* |
| +      | LEW Hg C         | 14,695 ± 1,218* |
| +      | LEW OVA          | 17,880 ± 1,112* |
| +      | rIL-2*           | 26,545 ± 9,754* |

Supernatant from T cell lines was added to CTLL-2 cells, and thymidine incorporation was measured after 24 h.

*p < 0.001 when compared with control culture.

rIL-2 was added at a concentration of 5 U/ml.

![Figure 2](image-url)  
**Figure 2.** Proliferative response of CD8+ lymph node cells from LEW rats injected with HgCl2 for 7 (Hg7) or 14 d (Hg14) in the presence of irradiated CD4+ cells from normal (n) or HgCl2-injected (Hg7 or Hg14) rats. Results are mean of five experiments.

![Figure 3](image-url)  
**Figure 3.** Specificity of the LEW Hg A T cell line. (a) Proliferative response of LEW Hg A T cells cultured alone (■) or in the presence of APC (□). Cultures were also performed in the presence of syngeneic APC preincubated with the OX6, anti-class II IA, or the OX17, anti-class II IE mAb. (b) Proliferative response of LEW OVA T cells cultured alone (■), in the presence of APC (□), or in the presence of OVA (50 μg/ml) and APC (□), preincubated or not with the OX6 mAb. Results are the mean of five experiments.

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concentration: 115 ± 25 ng/ml in cocultures vs. 95 ± 40 ng/ml in cultures containing spleen cells only). Similar results were obtained with LEW Hg B and C T cells.

All seven attempts to derive autoreactive anti-class II T cell lines from normal LEW rats failed. This might be related to the very low frequency of such cells in normal rats as shown by LDA.

The Autoreactive Anti-class II LEW Hg A T Cell Line Protects LEW Rats from EAE. It has been previously shown that HgCl2-induced CD8+ suppressor cells were able to protect LEW rats from the appearance of autoimmune diseases such as EAE (7). To test the role of anti-class II T cells in the initiation of the suppressor circuit, we transferred the LEW Hg A T cell line in rats immunized with myelin. As shown in Table 2, all the LEW rats immunized with myelin developed a severe EAE, while the immunized rats transferred with preactivated LEW Hg A cells were protected. Rats were not protected when they received nonactivated LEW Hg A T cells. The protection was completely abolished after treatment of the recipients with the OX8 anti-CD8 mAb. Injections of the irrelevant B10-H2 mAb did not abrogate LEW Hg A-mediated suppression (Table 2). LEW OVA cells had no effect on the course of EAE whether they were cultured in the presence of OVA or BP before transfer. Injection of thymocytes alone cultured in the presence of BP did not protect recipients from EAE (Table 2), showing that LEW Hg A-induced suppression was not due to contaminating thymocytes.

The Autoreactive Anti-class II LEW Hg A T Cell Line Triggers Non-Antigen-specific CD8+ Suppressor Cells. Responses to BP or PPD, but also to the Con A mitogen or to allogeneic antigens of lymph node cells from rats transferred with the LEW Hg A T cell line, were inhibited when compared with the responses of LEW rats immunized with myelin alone (Fig. 4). This suppression was CD8 mediated since in vivo depletion of CD8+ cells restored, at least in part, all the responses. Treatment with an irrelevant mAb did not modify

Table 2. Effect of Transfer of T Cell Lines on Actively Induced EAE

| Cells transferred challenge with: | mAb | Clinical score |
|---------------------------------|-----|---------------|
|                                 | in vitro | mAb | mAb | in vivo | Clinical score |
|                                 | - | - | - | 2, 2, 3, 4, 4 |
| LEW Hg A | APC | - | 0, 0, 0, 0, 0, 0 |
| LEW Hg A | APC | - | 1, 2, 2, 3, 3 |
| LEW Hg A | APC, OX8 | - | 1, 2, 3, 3 |
| LEW Hg A | APC, B10-H2 | - | 1, 0, 0, 0, 0 |
| LEW OVA | BP | - | 3, 2, 3, 3 |
| LEW OVA | OVA | - | 3, 4, 2, 4 |
| APC | BP | - | 2, 2, 3, 4 |

APC are syngeneic thymocytes. OX8 is an anti-CD8 mAb; B10-H2 was used as an irrelevant mAb. Cells transferred were activated for 3 d with the appropriate stimulus.

* In this culture, LEW Hg A cells were kept for 7 d after the end of stimulation in medium containing Con A as a source of IL-2.

† Rats were transferred with thymocytes incubated with BP (50 μg/ml) as a source of myelin-bound APC.

Figure 4. Non-antigen-specific, CD8-dependent immunosuppression induced by transfer of the LEW Hg A T cell line in normal LEW rats. Proliferative responses to BP (a), PPD (b), Con A (c), or lymph node cells from BN rats as an alloantigen (d) of lymph node cells from LEW rats. LEW rats were normal (I), immunized with myelin ([]), immunized with myelin and transferred with 5 x 10^7 LEW Hg A T cells whether they were treated with an anti-CD8 mAb (■), an irrelevant mAb (■), or not (■), or immunized with myelin and transferred with 5 x 10^7 LEW OVA T cells preactivated with OVA (■) or BP (■). Stimulation index = cpm of stimulated cells/cpm of unstimulated cells. Results are mean of six experiments.

Figure 5. Inhibition of the proliferative response to BP of lymph node cells from LEW rats immunized with myelin by various numbers of heterologous lymph node cells. Lymph node cells assessed for suppression came from LEW rats immunized with myelin and transferred with the LEW Hg A T cell line (■), injected in addition with an anti-CD8 mAb (■), or with an irrelevant mAb (■), or from rats transferred with the LEW OVA T cell line (■).
T cells described here in LEW rats recognized self-class II molecules as in BN rats; their frequency was similar in both strains. Because these CD4+ T cells were unable to promote autoimmunity in LEW rats, even when CD8 depleted, they probably differ at the functional level from those observed in BN rats. Two different CD4+ T cell subsets have been described in mice according to the cytokines produced: Th1 cells produce IL-2 and IFN-γ, and are mainly involved in delayed-type hypersensitivity, while Th2 cells produce IL-4, IL-5, and IL-10, and are mainly involved in B cell help (20).

There are convincing arguments supporting a role for Th2 cells in HgCl2-induced autoimmunity in rats (21) and mice (22). The autoreactive T cell lines obtained in LEW rats produced IL-2 and were unable to help B cells in vitro for Ig production, suggesting that they belong to the Th1-like subtype. It has been demonstrated that human Th1 clones may

Table 3. Proliferative Responses of Lymph Node Cells from Rats Transferred with the LEW Hg A or the LEW OVA T Cell Line

| Cells transferred | Treatment with anti-CD8 mAb | Stimulus | cpm |
|-------------------|----------------------------|----------|-----|
| LEW Hg A          | -                          | LEW Hg A | 225 ± 157 |
| LEW Hg A          | -                          | LEW Hg A | 17,568 ± 9,236 |
| LEW Hg A          | -                          | LEW Hg A | 4,790 ± 3,678 |
| LEW Hg A          | -                          | Sn LEW Hg A | 523 ± 145 |
| LEW Hg A          | -                          | Sn Con A | 986 ± 163 |
| LEW Hg A          | -                          | LEW nl | 722 ± 251 |
| LEW Hg A          | -                          | LEW nl | 8,892 ± 270 |
| LEW Hg A          | -                          | LEW OVA | 5,655 ± 2,436 |
| LEW Hg A          | +                          | LEW OVA | 498 ± 242 |
| LEW Hg A          | +                          | LEW Hg A | 356 ± 175 |
| LEW Hg A          | +                          | LEW Hg A | 3,442 ± 1,364 |
| LEW Hg A          | +                          | LEW Hg A | 1,021 ± 498 |
| LEW Hg A          | +                          | Sn LEW Hg A | 778 ± 298 |
| LEW Hg A          | +                          | Sn Con A | 1,225 ± 625 |
| LEW Hg A          | +                          | LEW OVA | 1,798 ± 293 |
| LEW Hg A          | +                          | LEW OVA | 512 ± 131 |
| LEW OVA           | -                          | LEW OVA | 945 ± 198 |
| LEW OVA           | -                          | LEW Hg A | 3,223 ± 354 |
| LEW OVA           | -                          | LEW Hg A | 1,045 ± 512 |
| LEW OVA           | -                          | LEW OVA | 8,990 ± 1,693 |
| LEW OVA           | -                          | LEW OVA | 3,629 ± 355 |

Sn, supernatant.
* Cell lines were activated for 3 d with the appropriate stimulus before they were used as stimulator cells.
† Stimulator cells were used 7 d after the last stimulation.
§ Stimulator cells were normal LEW lymph node cells (LEW nl) stimulated for 2 d with Con A.

Discussion

This work shows that HgCl2 induces in LEW rats the appearance of anti-class II autoreactive CD4+ T cells and of allogeneic antigens as compared to the responses of rats immunized with myelin alone (Fig. 4).

Lymph node cells from rats transferred with LEW Hg A T cells suppressed in a dose-dependent manner the proliferative responses to BP (Fig. 5) and to PPD (not shown) of lymph node cells from LEW rats immunized with myelin alone. Treatment with the OX8 mAb of LEW rats transferred with LEW Hg A cells inhibited this suppressive effect while treatment with the irrelevant B10H2 mAb did not. Lymph node cells from rats transferred with LEW OVA cells had no effect on these aforementioned responses.

Lymph Node Cells from LEW Hg A Recipient Rats Recognize Activated T Cells. To assess the specificity of cells from rats transferred with the LEW Hg A T cell line, lymph node cells were cultured in the presence of various stimulus, 21 d after the transfer. They proliferated significantly in the presence of the activated LEW Hg A T cell line (p < 0.005) and also, but to a lesser extent, in the presence of the activated LEW OVA T cell line (p < 0.01), (Table 3), but not in the presence of autologous APC (not shown), when compared with lymph node cells cultured alone. The proliferative response was greatly reduced when LEW Hg A stimulator cells were not activated, and was abolished when nonactivated LEW OVA cells were used (Table 3). LEW spleen cells activated by Con A, but not normal spleen cells, can also stimulate cells from rats transferred with the LEW Hg A T cell line. Supernatant from Con A-activated spleen cells or from LEW Hg A-activated cells only induced a marginal proliferative response (Table 3). Lymph node cells from rats transferred with the LEW Hg A T cell line and treated with the anti-CD8 mAb exhibited the same pattern of responses but with much less intensity. Lymph node cells from rats transferred with the LEW OVA T cell line responded to activated LEW OVA T cells, and less to activated LEW Hg A T cells or resting LEW OVA T cells, and not at all to resting LEW Hg A cells. They did not respond to OVA (not shown).

None of the LEW rats transferred with the LEW Hg A T cell line, whether or not they were immunized with myelin and whether or not they were treated with the OX8 mAb, exhibited an increase in serum IgE concentration or renal immune deposits (not shown). These data show that the LEW Hg A T cell line fails to promote autoimmunity.

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have a cytotoxic effect on APC (23) that could induce suppression. We have verified that the LEW Hg A T cell line had no such cytotoxic effect (not shown).

The characteristics of our autoreactive lines were very similar to those of the autoreactive cells detected in HgCl₂-injected LEW rats. They were IA but not IE restricted. In HgCl₂-injected LEW rats, autoreactive T cells and suppressor T cells appeared concomitantly, and CD8⁺ cells were able to proliferate in the presence of autologous CD4⁺ cells but not in the presence of CD8⁺ cells. The LEW Hg A T cell line also induced, after transfer, the appearance of CD8⁺ suppressor cells, which proliferated in the presence of the transferred CD4⁺ line. In HgCl₂-injected, as in transferred, rats, CD8⁺ cells were able to downmodulate various T cell functions and to prevent EAE (7). One apparent difference between suppression induced by HgCl₂ or by transfer of the LEW Hg A T cell line concerns the appearance of contrasuppressor cells, which were probably responsible for the partial protection towards EAE in HgCl₂-injected LEW rats while all the rats transferred with the line were protected. Studies are in progress to look for the presence of such cells in rats transferred with the LEW Hg A T cell line and to establish their possible connection with the suppressor cells.

The CD8⁺ suppressor cells induced by the transfer of the line or after HgCl₂ injections proliferated in the presence of CD4⁺ T cells. In the latter situation, as reported in another system (24), APC were not required; whether APC are important or not in the former situation is presently under study. Results obtained in rats transferred with the LEW Hg A T cell line showed that lymph node cells from these animals recognized far better this T cell line when preactivated. They also recognized, although less, the activated, irrelevant, anti-OVA T cell line or Con A-activated spleen cells but not resting cells. According to Lohse et al. (25), these data demonstrate that the LEW Hg A T cell line triggers CD8⁺ antiergotypic cells, defined as cells that recognize a T cell activation marker. Therefore, the induction of antiergotypic cells is not restricted to T cell vaccination (25). CD8⁻ depleted lymph node cells from rats transferred with the LEW Hg A T cell line still proliferated to the same stimuli as undepleted lymph node cells, but with much less intensity; this residual proliferation could be due to the remaining CD8⁻ cells, or to CD8⁻ antiergotypic cells (25). LEW Hg A T cell line-induced antiergotypic cells did not proliferate either in the presence of culture supernatant from activated LEW Hg A cells, or from Con A-activated cells, which suggests that these cells did not respond to lymphokines only but to a membrane antigen. The nature of this antigen remains to be determined. It could be an adhesion molecule (25) or peptides derived from the trimolecular complex (class II molecule, TCR, and peptide), expressed in the context of class I molecule as proposed by Janeway (26). The fact that lymph node cells from rats transferred with the LEW Hg A T cell line proliferated far better in response to activated LEW Hg A cells than to the other activated cells tested suggests either that activated LEW Hg A cells are better stimulators or that LEW Hg A-induced suppressor cells are heterogeneous. This is reminiscent of T cell vaccination where antidiotypeic (27–32) and, to a lesser degree, antiergotypic (25) suppressor T cells are able to protect against T cell-mediated diseases.

The way the CD8⁺ suppressor cells act in our model remains to be determined. A cytotoxic effect on T cells or APC is unlikely since lymph node cells from rats transferred with the LEW Hg A T cell line are unable to kill LEW Hg A cells, autologous spleen cells, or lymph node cells from normal LEW rats immunized with myelin and challenged in vitro (not shown). They could interfere with signaling pathways of encephalitogenic effector cells (33) or by releasing cytokines such as IL-4 (34) or TGF-β (35). This latter hypothesis could explain the non-antigen-specific immunosuppression as shown for a suppressor line obtained from a nonobese diabetic mouse that was diabetes resistant (36). Works are in progress to obtain suppressor T cell lines to answer these questions.

It has already been reported that anti-class II T cells, able to polyclonally activate B cells, might trigger the appearance of suppressor cells able to suppress immune responses to several exogenous antigens (8). These results, as ours, could be explained by the emergence of antiergotypic and/or antidiotypeic cells. It could also explain results concerning the control of autoreactive anti-class II T cells by suppressor cells (37–39). Anti-class II T cells, initially described as a consequence of an immune response directed against nominal antigens (40), also exist as normal elements of the immune system (37, 39). Anti-class II T cells activated during an immune response would trigger suppressor cells that downmodulate in turn every autoreactive activated cell, whatever their specificity. Thus, HgCl₂-induced suppressor cells could represent an exacerbation of a physiological process controlling autoreactive cells that escape tolerance in periphery.

To seek chemicals that mimic mercury effects in LEW rats could be an interesting approach for therapeutics of multiple sclerosis in humans. Indeed, this demyelinating disease, of which EAE is the best experimental model, has been shown to be associated with a defect of suppressor inducer cells (41). Such treatments, by restoring normal regulatory circuits, would be more interesting than T cell vaccination since they do not require heavy technology, and since they could be applied to different autoimmune diseases.

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