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Increased expression of class II major histocompatibility complex molecules on B cells in rats susceptible or resistant to $HgCl_2$-induced autoimmunity

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SUMMARY

Administration of $HgCl_2$ to the susceptible Brown-Norway (BN) rats induces an autoimmune disease characterized by a T-dependent polyclonal activation of B cells responsible for a dramatic increase in serum IgE concentration. The resistant Lewis (LEW) rats injected with $HgCl_2$ do not exhibit such autoimmune manifestations. We show here that, upon $HgCl_2$ injections, major histocompatibility complex (MHC) class II molecule expression is increased very early in lymph nodes and spleen B cells from both strains. So far, it is the earliest marker (day 3) of the effect of $HgCl_2$ on the immune system. In both strains this enhancement is transient, but regulatory mechanisms are much more efficient in the resistant LEW strain than in the susceptible BN strain. In addition, we observed that MHC class II molecule expression on B cells differs according to the organ and the rat strain tested. All these findings are discussed in an attempt to underline the role of MHC class II molecule expression in the occurrence of mercury-induced autoimmunity.

Keywords MHC class II molecule expression B cells Brown-Norway and Lewis rats $HgCl_2$-induced autoimmunity IgE

INTRODUCTION

Mercury-induced autoimmunity in Brown-Norway (BN) rats is characterized by a T-dependent polyclonal activation of B cells (Hirsch *et al.*, 1982). One of the hallmarks of this activation is a dramatic, transient increase in total serum IgE concentration (Sapin *et al.*, 1984). IgG1, IgG2b and IgG2c also increase but to a lesser extent (Pelletier *et al.*, 1988a). In Lewis (LEW) rats, which do not exhibit autoimmune manifestations upon $HgCl_2$ administration, serum IgE concentration is not enhanced (Sapin *et al.*, 1984).

In mice, two different subsets of TH cells (TH1 and TH2) have been well characterized, according to their production of cytokines (Mosmann & Coffman, 1989). Schematically, TH1 cells produce interleukin-2 (IL-2) and gamma interferon (γIFN), whereas TH2 cells produce IL-4, IL-5 and IL-10; IL-4 and IL-5 are responsible for the synthesis of IgE and IgG1 (Finkelman *et al.*, 1986; Del Prete *et al.*, 1988; Snapper, Finkelman & Paul 1988; Pene *et al.*, 1988b; Spiegelberg, 1990) and IgA (Yokota *et al.*, 1987; Coffman *et al.*, 1987). Antagonistic effects between TH1 and TH2 subsets have been described and depend on the production of γIFN (Coffman & Carty, 1986; Mond *et al.*, 1986; Gajewski & Fitch, 1988; Pene *et al.*, 1988a) and IL-10 (Fiorentino, Bond & Mosmann, 1989). Similar T cell subsets have been isolated and functionally characterized in the rat (Spickett *et al.*, 1983; Powrie & Mason, 1990) but not yet cloned.

The increase in serum IgE and IgG1 concentration in the rat mercury model strongly suggests a role for IL-4 and therefore for the TH2-like subset (Goldman, Druet & Gleichmann, 1991). One of the major effects of cytokines produced by TH2 cells (IL-4 and IL-10) is to enhance MHC class II molecule expression on resting B cells (Roehm *et al.*, 1984; Noelle *et al.*, 1984; Mond *et al.*, 1986; Go *et al.*, 1990), while γIFN produced by TH1 cells has no such effect (Mond *et al.*, 1986). In the present study, we investigated sequentially MHC class II molecule expression on B cells from resistant and susceptible strains of rats injected with $HgCl_2$.

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MATERIALS AND METHODS

Animals

Brown-Norway and LEW rats, originating from the CSEAL (CNRS Orléans, La Source, France), were maintained in our animal house. Eight- to 10-week-old (male or female) rats were used in the following experiments.

Monoclonal antibodies (MoAb)

OX6 mouse MoAb was produced in our laboratory from the corresponding antibody producing hybridoma, kindly provided by Dr A. Williams (Oxford, UK) (McMaster & Williams, 1979). OX6 is an IgG1 MoAb that recognizes a monomorphic determinant on rat MHC class II molecules (RT1B). Hybridoma cells were grown and MoAb prepared as previously described (Guery *et al.*, 1990). The purified OX6 MoAb was fluoresceinated as described elsewhere (Sapin, Druet & Druet, 1977). A mouse MoAb B10H2 (thyroglobulin-specific), provided by Dr D. Glotz (Hôpital Broussais, Paris, France), was used as an irrelevant IgG1 MoAb and labelled with biotin succinimide ester (Calbiochem Behring, La Jolla, CA) as described (Goding, 1983). A mouse anti-rat K chain MoAb (MARK1), a gift from Dr H. Bazin (Brussels, Belgium), was biotinylated as aforementioned.

Experimental procedure

Brown-Norway and LEW rats were subcutaneously injected thrice weekly with 0·1 mg $HgCl_2$/100 g body weight (Sapin *et al.*, 1977). Four to six rats of both strains were killed on days 1, 3, 6, 15, 21, 30, 40 and 50 after the first $HgCl_2$ injection; rats killed on day 1 or 3 had been $HgCl_2$-administered once whereas those killed on day 6 had received three $HgCl_2$ injections. Five more BN rats were killed on day 60. The same number of rats was injected with acidic (pH 3·8) H_2O as a control solution and killed on the same day as $HgCl_2$-injected rats. Blood samples, spleen and lymph nodes (LN) (paraaortic and mesenteric LN) were collected from each rat on the day of sacrifice.

Determination of serum IgE concentration

The serum IgE concentration was measured in an ELISA as already described (Sapin *et al.*, 1984).

Analysis of class II molecule expression

Spleen and LN cells were teased apart in Dulbecco's medium (Biochrom, KG, Berlin, Germany). Double immunostaining experiments were carried out as follows: 5×10^5–1×10^6 cells, suspended in 100 μl phosphate-buffered saline (PBS) containing 1% fetal calf serum (FCS) (Intermed, Berlin, Germany) and 0·1% NaN_3, were incubated together with optimal dilutions of fluoresceinated OX6 MoAb and biotinylated MARK1 MoAb for 30 min on ice and washed twice with cold PBS-FCS-NaN_3 before a further 30 min incubation with 10 μl phycoerythrin-conjugated streptavidin on ice (Amersham, Aylesbury, UK) and finally washed twice with cold PBS-NaN_3. Cells were fixed with 1% paraformaldehyde in PBS. Cellular expression of MHC class II molecules was measured on a logarithmic mode by the mean fluorescence intensity (MFI) determined with a FACScan (Becton Dickinson, Sunnyvale, CA). Dead cells were eliminated from the analysis by gating on forward and right-angle light scatter. For spleen cell suspensions, erythrocytes were gated out on the basis of their characteristic forward scatter. Mean fluorescence intensity values of cells from $HgCl_2$-injected rats on a given day were expressed as a percentage of the mean MFI value of cells from at least four H_2O-injected rats. Cells from control and $HgCl_2$-injected rats were stained on the same day. Another group of 10 normal 12-week-old female rats (five BN and five LEW) was used for interstrain comparison on the same day. Whatever the strain and the organ considered, all the data reported deal with the same, small-size, homogeneous cell population selected on the basis of its characteristic forward scatter. MHC class II molecule expression was studied on cells double-stained for MHC class II molecule and immunoglobulin K chain.

Statistical analysis

Comparisons between groups were performed using Mann-Whitney's *U*-test.

RESULTS

Serum IgE concentration

As previously described (Sapin *et al.*, 1984), the mean concentration of circulating IgE in normal LEW rats was significantly lower as compared with normal BN rats (0·9 ± 0·4 μg/ml *versus* 9·9 ± 6·3 μg/ml) and the serum IgE concentration in BN rats injected with $HgCl_2$ increased from day 6, peaked on day 15 and decreased slowly from day 30. In this experiment, the IgE concentration remained significantly higher by day 60 in $HgCl_2$-injected rats when compared with BN rats injected with H_2O (Fig. 1). The serum IgE concentration in LEW rats injected with $HgCl_2$ did not differ from the concentration observed in control LEW rats at any stage of the experiment (not shown).

MHC class II molecule expression on B cells from control and normal rats

Basal MHC class II molecule expression on B cells from spleen and LN was studied in the 48 control LEW rats and the 45 control BN rats injected with acidic H_2O as described in Materials and Methods. These animals were 2–4 months old and either male (24 BN, 21 LEW) or female (21 BN, 27 LEW). Whatever the day of the experiment (1, 3, 6, 15, 21, 30, 40, 50, 60) and the strain studied, the MFI was significantly higher for LN B cells than for splenic B cells with a LN MFI/spleen MFI ratio ranging from 1·3 to 2·1 (data not shown). In these experiments, the MFI values observed in BN and LEW rats could not be compared since they were not measured on the same day. However, it was striking that MFI values in LEW rats were

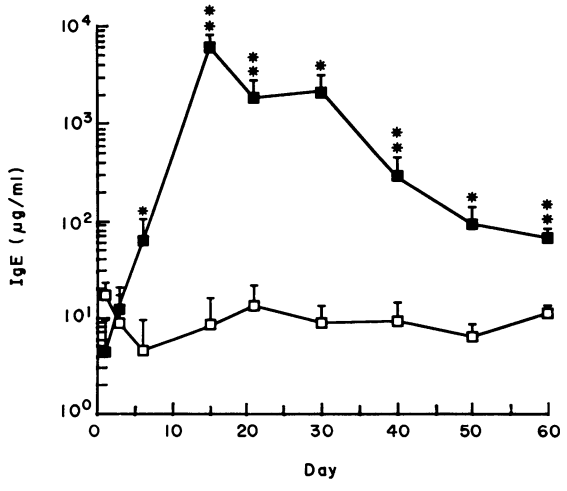

Fig. 1. Kinetics of serum IgE concentration in $HgCl_2$- (■) and H_2O- (□) injected Brown-Norway rats. IgE concentration was measured by ELISA and expressed on a logarithmic scale (mean ± s.d.). Values represent mean of four to six rats. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

always higher than in BN rats for each organ (not shown). For these reasons, 10 normal 12-week-old female BN (five) and LEW (five) rats were studied on the same day. As shown in Table 1, the LN MFI/spleen MFI ratio was confirmed to be higher than 1 in both strains and, moreover, significantly higher in LEW rats than in BN rats (1·5 and 1·8 for BN and LEW respectively, $P<0·02$). In addition, MFI values obtained for LN B cells were significantly higher in LEW than in BN rats ($P<0·01$). The same results were obtained for MFI values of splenic B cells ($P<0·01$). Altogether, these data showed that basal MHC class II molecule expression on B cells from control and normal rats was significantly higher on LN than on spleen B cells and in LEW than in BN rats.

MHC class II molecule expression on B cells from $HgCl_2$-injected rats (Fig. 2)

Lymph node B cells. In $HgCl_2$-injected BN and LEW rats, expression of MHC class II molecules increased by 35 and 30% respectively as early as day 3 ($P<0·05$, when compared with the corresponding control rats). It is of interest that in both strains only one injection of $HgCl_2$ was required to increase MHC class II molecule expression. This enhancement appeared, therefore, between 24 and 72 h following the first $HgCl_2$ injection. In BN rats, the increase was maximal on day 6 ($P<0·05$), lasted until day 15 ($P<0·05$) and was no longer significant from day 21 to day 60. In LEW rats the increase was still significant on day 6 ($P<0·05$) but not from day 15 to day 50.

Splenic B cells. In $HgCl_2$-injected BN rats, an increase in expression of MHC class II molecules was significant from day 6 to day 50, as compared with control rats, with a peak on day 15 ($P<0·05$). No significant enhancement was detected on day 60.

It is interesting to note that the kinetics of MHC class II molecule expression on spleen B cells strikingly paralleled those of the serum IgE concentration until day 60. In $HgCl_2$-injected LEW rats, expression of MHC class II molecules was significantly increased on day 3 only ($P<0·01$).

It remained important to exclude a possible binding of the IgG1 MoAb anti-MHC class II molecules to B cells via Fc receptors. These cells were thus incubated with a biotinylated isotype-matched control MoAb. No staining was observed in this procedure at the acme of the disease.

As shown in Table 1, the basal expression of MHC class II molecules on LN and splenic B cells from BN rats is 67 and 44·1, respectively; the corresponding maximal percentages of increase in MHC class II molecule expression obtained under $HgCl_2$ administration are 65% for LN B cells on day 6 and 61% for splenic B cells on day 15 (Fig. 2); therefore the calculated maximal expression of MHC class II molecules induced by $HgCl_2$ on LN and splenic B cells in BN rats is $67\times(1+0·65)=110·6$ and $44·1\times(1+0·61)=71$, respectively. Interestingly, these values are similar to or even lower than the mean basal values from LEW rats (143 and 78·9 for LN and splenic B cells, respectively) as shown in Table 1.

DISCUSSION

In the present study we found (i) that $HgCl_2$ induces an early increase in MHC class II molecule expression on splenic and LN B cells from BN and LEW rats; (ii) that the kinetics of this $HgCl_2$-induced MHC class II molecule expression vary depending upon the strain with a prompter down-regulation in the resistant LEW rats than in the susceptible BN rats and (iii) that

Table 1. Expression of MHC class II molecules on lymph node (LN) and spleen B cells from normal Brown-Norway (BN) and Lewis (LEW) rats

		MHC class II molecule expression (MFI)		MFI ratio
Rat strain	Rat No.	Lymph node	Spleen	Lymph node/spleen
BN	1	67·9	45·7	1·5
	2	67·3	43·3	1·5
	3	75·0	44·9	1·7
	4	60·0	45·7	1·3
	5	64·9	41·0	1·6
	Mean ± s.d.	67·0 ± 5·4*‡	44·1 ± 2*§	1·5 ± 0·1‖
LEW	1	147·2	82·8	1·8
	2	142·0	77·0	1·8
	3	151·2	83·5	1·8
	4	128·6	77·7	1·6
	5	145·9	73·6	2·0
	Mean ± s.d.	143·0 ± 8·7†‡	78·9 ± 4·2†§	1·8 ± 0·1‖

Expression of MHC class II molecules is measured as mean fluorescence intensity (MFI).
* Comparison between LN and spleen MFI from BN rats $P<0·01$.
† Comparison between LN and spleen MFI from LEW rats $P<0·01$.
‡ Comparison between BN and LEW rat LN MFI $P<0·01$.
§ Comparison between BN and LEW rat spleen MFI $P<0·01$.
‖ Comparison between BN and LEW rat MFI ratio $P<0·02$.

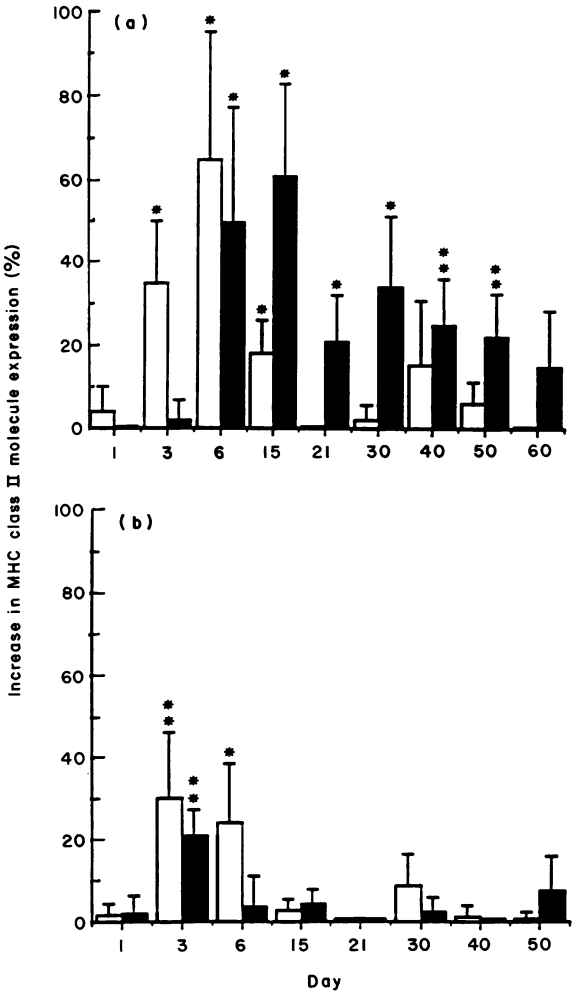

Fig. 2. Kinetics of the $HgCl_2$-induced increase in MHC class II molecule expression. Spleen (■) and lymph node (□) cells were double stained with FITC-conjugated anti-MHC class II molecules (OX6) and biotinylated anti-κ chain (MARK I). FACScan analysis was performed. Mean fluorescence intensity (MFI) values of MHC class II molecule positive B cells from $HgCl_2$-injected (a) Brown-Norway or (b) Lewis rats are expressed as the percentage (mean ± s.d.) of the mean MFI value of cells from control rats. $*P < 0.05$, $**P < 0.01$.

the basal MHC class II molecule expression is different according to the lymphoid organ considered and, more important, to the strain tested.

The role of IL-4 in IgE production is well documented in mice and humans (Coffman & Carty, 1986; Finkelman *et al.*, 1986; Del Prete *et al.*, 1988; Pene *et al.*, 1988a; Snapper *et al.*, 1988; Spiegelberg, 1990) but not in rats. Data reported herein show that, in BN rats, there is a striking increase in MHC class II molecule expression on LN and spleen B cells preceding the increase in serum IgE concentration. The enhancement of MHC class II molecule expression is a well-known marker for IL-4 in mice (Roehm *et al.*, 1984; Noelle *et al.*, 1984; Abramowicz *et al.*, 1990). Richter, Blankenstein & Diamantstein (1990) recently demonstrated that rat IL-4 increased MHC class II molecule expression on spleen cells. Therefore, our results are compatible with a role of IL-4 in the increase in serum IgE concentration in the mercury model. In agreement with this hypothesis, Ochel *et al.* (1991) have reported that the increase in serum IgE concentration in mice injected with $HgCl_2$ was abrogated following treatment with a rat anti-mouse IL-4 MoAb. However, two recent results must be taken into account. First, McCabe & Lawrence (1990) recently demonstrated that mercury was able to induce *in vitro* a T-independent increase in MHC class II molecule expression on mice spleen B cells. It has been clearly established that $HgCl_2$-induced autoimmunity, including the increase in serum IgE concentration, is T cell-dependent (Pelletier *et al.*, 1988b). It is conceivable that $HgCl_2$-induced enhancement of MHC class II molecule expression is initially T cell-independent and later T cell-dependent. Second, Go *et al.* (1990) showed that IL-10 produced by TH2 cells is also able to increase MHC class II molecule expression on mice splenic B cells. A clear demonstration of the role of IL-4 in $HgCl_2$-induced increase in MHC class II molecule expression and serum IgE concentration in the rat would require treatment with an anti-IL-4 MoAb, which is not yet available. Whatever the mechanisms, it is important to stress that the augmentation of MHC class II molecule expression on B cells occurs as early as day 3 and is so far the foremost effect of $HgCl_2$ on the immune system which testifies to B cell activation.

In BN rats, the increased MHC class II molecule expression was transient suggesting a down-modulation of IL-4 production possibly by other cytokines such as γIFN. That TH1 cells are able to down-regulate TH2 cells in mice through γIFN is well documented (Coffman & Carty, 1986; Pene *et al.*, 1988a; Gajewski & Fitch, 1988). Furthermore, Aten *et al.* (personal communication) detected γIFN in serum and plasma from $HgCl_2$-injected BN rats from day 10 and not at day 3 or 6. Therefore, during the course of this mercury-induced autoimmune disorder, different cytokines are produced, which could explain the spontaneous regulation observed. Interestingly, in resistant LEW rats our results demonstrate an increment in MHC class II molecule expression on LN B cells at day 3 of similar magnitude as compared with susceptible BN rats. However, this increase is much more transient, probably because of more efficient regulatory mechanisms.

Finally, we report two interesting findings in normal rats. First, MHC class II molecule expression was shown to be always significantly higher on LN B cells than on splenic B cells, whatever the strain considered. These differences cannot be attributed to variations in the size of the cells, since only the homogeneous, small-size, cell population was analysed. Similar results have been reported in mice (Mond *et al.*, 1980). These discrepancies may distinguish between B cells at different stages of maturation or between different B cell subpopulations. Alternatively, such discordances may be due to a different distribution of cells producing various cytokines, as suggested by Killar *et al.* (1989) after IL-1 treatment. They may also be related to the higher percentage of T cells in the LN than in the spleen and/or to the fact that the LN studies were preferentially exposed to exogenous antigenic stimulation. Second, the level of MHC class II molecule expression on normal LN and splenic B cells was significantly higher in LEW rats than in BN rats, a finding that is in agreement with previous reports (McMaster & Williams, 1979). The reason is so far unknown but these results support the hypothesis that the basal level of MHC class II molecule expression may be important in determining the susceptibility to autoimmune disorders as previously assumed

by Janeway *et al.* (1984). Our results also suggest that, besides the basal level of MHC class II molecule expression, the gradient of MHC class II molecule expression induced by $HgCl_2$ may also be an important factor in the appearance of autoimmunity.

To conclude, in BN as well as in LEW rats, $HgCl_2$ modulates MHC class II molecule expression on B cells and this effect is the earliest so far reported. As far as the $HgCl_2$-induced increase in MHC class II molecule expression on B cells is concerned, susceptible BN rats and resistant LEW rats are different in that, in the former, the increase is higher and in the latter, the down-regulation is more efficient. The precise role of the various cytokines in the modulation of MHC class II molecule expression on B cells in $HgCl_2$-injected BN and LEW rats remains to be elucidated.

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