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Lead, a Major Environmental Pollutant, Is Immunomodulatory by Its Differential Effects on CD4$^+$ T Cell Subsets

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Lead, a Major Environmental Pollutant, Is Immunomodulatory by Its Differential Effects on CD4$^+$ T Cell Subsets. MCCABE, M. J., JR., AND LAWRENCE, D. A. (1991). *Toxicol. Appl. Pharmacol.* **111,** 13–23. Studies were undertaken to address the necessity of B–T cell contact for the enhancement of B cell differentiation caused by the heavy metal lead (Pb). Membrane segregated cultures were used so that the influences of direct B–T cell contact and T cell factors on B cell differentiation could be independently evaluated. B–T cell contact was not absolutely required for Pb's enhancement of B cell maturation to antibody forming cells (AFCs); however, enhancement of the AFC response by Pb was optimal when B–T cell interactions were allowed. These results were corroborated by use of anti-L3T4 (mouse CD4) to block CD4$^+$ T cell–B cell interaction. Blockade of B–T cell contact with anti-L3T4 did not inhibit the enhancement of the AFC response by Pb. Additional experimentation showed that Pb enhanced the AFC response and Ig production in the presence of antigen-specific T cell help, suggesting that Pb enhances B cell differentiation by augmenting cognate help rather than by inducing a response to Pb-altered-self. In studies employing antigen-specific T cell clones, Pb was found to differentially modulate antigen presentation to T_{H1} versus T_{H2} T cell clones, in that T_{H1} activation was inhibited and T_{H2} activation was enhanced by Pb.

INTRODUCTION

Genetic predisposition and environmental factors influence the induction of autoimmune diseases (Schwartz and Rose, 1986). Although clearly involved, the influence of environmental factors on autoimmunity is not understood. Agents, including pathogens (Watanabe *et al.*, 1983), drugs (Hahn *et al.*, 1972), and chemicals (Pelletier *et al.*, 1986), that disrupt immunohomeostasis may promote autoimmunity.

Heavy metals, such as lead (Pb), induce pathophysiologic changes that affect many organ systems (Goyer, 1986) including the immune system (Lawrence, 1985). Pb exposure can cause hypoimmunity leading to diminished host resistance to pathogens (Lawrence, 1981a). In contrast, Pb also can augment certain immune responses, perhaps contributing to hyperimmunity against self-constituents. Except for a few reports correlating occupational Pb exposure with kidney disease via an immune mechanism (Wedeen *et al.*, 1979; Garcia *et al.*, 1980), the possibility that Pb induces autoimmunity has not been considered despite its immunomodulatory activities. Pb enhances B cell differentiation *in vitro* and *in vivo* to T-cell-dependent antigens such as sheep erythrocytes (SRBC) (Koller *et al.*, 1976; Lawrence, 1981a,b,c, 1983; Warner and Lawrence, 1986a) as well as to the T-cell-independent polyclonal B cell activator, lipopolysaccharide (McCabe and Lawrence, 1990), by an amount comparable to the T-cell-derived cytokine interleukin-5 (Tonkonogy *et al.*, 1989). In

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addition, Pb simulates the activity of the T cell cytokine, interleukin-4, by increasing B cell expression of major histocompatibility complex class-II (Ia) molecules (McCabe and Lawrence, 1990).

For most antigens, B cells require T cell help for differentiation into antibody forming cells (AFCs). Helper ($CD4^+$) T cells act by two distinct mechanisms (Abbas, 1988): cell-to-cell interactions mediated by Ia restricted B–T cell collaboration and factor-mediated help fulfilled by T-cell-derived cytokines including interleukin-2, -4, -5, -6, and interferon-γ (IFN-γ). Furthermore, factor-mediated T cell help has been categorized by the profile of cytokines present in supernatants of various $CD4^+$ T cell clones (Mosmann *et al.*, 1986). Type 1 (T_{H1}) clones produce IL-2 and IFN-γ; whereas, T_{H2} clones produce IL-4, IL-5, and IL-6. T_{H1} and T_{H2}-like cells, termed inflammatory and helper T cells, respectively, may exist *in vivo* (Bottomly *et al.*, 1989). Like their *in vitro* counterparts they differentially regulate immune responses through effector functions attributable to the cytokines that they secrete. Preferential perturbation of either subset may be protective or immunopathologic (Scott *et al.*, 1989; Finkelman *et al.*, 1986).

This study was undertaken to determine which mode of T cell help is required for Pb enhancement of B cell differentiation and whether Pb can enhance B cell differentiation when antigen-specific T cell clones are the sole source of help. These studies suggest that Pb augments factor-mediated T cell help by enhancing factor production by T cells as well as B cell responsiveness to these factors. Furthermore, the results suggest that Pb enhances B cell differentiation by promoting cognate B–T cell interactions via heightened B cell surface Ia density rather than a T cell response to Pb-altered-self. Interestingly, a dichotomy between the influence of Pb on T_{H1} vs T_{H2} activities has been observed that may explain the inverse effects of Pb on host resistance and the posited influence on autoimmunity (Lawrence, 1985).

MATERIALS AND METHODS

Mice. Female BCF_1 and BALB/c mice were obtained from Taconic Farms (Germantown, NY). C3H/HeJ, DBA/2J, C57BL/6 and CBA/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) whereas, CBA/N mice were obtained from the National Cancer Institute (Frederick, MD). Mice were housed in the AMC animal facility under SPF conditions and maintained on mouse chow and acidified water *ad libitum* until they were euthanized at 6–10 weeks of age.

Medium and reagents. M199 with Hank's BSS (M.A. Bioproducts, Walkersville, MD) supplemented with 5% FBS (Hyclone Laboratories, Logan, UT), 1 mM L-glutamine, 1 mM Na-pyruvate, 0.1 mM nonessential amino acids, 25 μg/ml gentamycin, 50 μM 2-mercaptoethanol, and $NaHCO_3$ was used in all cultures.

A stock solution of 10 mM $PbCl_2$ (Fisher Scientific, Rochester, NY) was prepared in physiological saline and sterile filtered prior to dilution and addition to culture. Sheep erythrocytes, purchased from the Colorado Serum Co. (Boulder, CO) were stored in Alsever's preservative and washed 3× in BSS prior to use. Stock solutions of conalbumin (CA, Sigma Chemical Co., St. Louis, MO), keyhole limpet hemocyanin (KLH; Calbiochem, La Jolla, CA) and rabbit γ globulin (RGG; Sigma) were prepared in physiological saline and sterile filtered prior to addition to culture. Anti-Ig (Rabbit $F(ab')_2$ anti-mouse $F(ab')_2$) was purchased from Jackson ImmunoResearch (West Grove, PA).

Monoclonal antibodies. The following hybridoma cell lines were obtained from the American Type Culture Collection (Rockville, MD): HO-13.4 (anti-Thy1.2, a mouse IgM to all T cells); GK1.5 (anti-L3T4a, a rat IgG anti-CD4) and 53-6.72 (anti-Lyt2, a rat IgG anti-CD8). Cells were cultured as recommended by ATCC, supernatants were harvested, precipitated with $(NH_4)_2SO_4$, dialyzed extensively into PBS pH 7.4, and sterile filtered. Each preparation was characterized by either titering for optimal staining as assessed by flow cytometry or cytotoxicity prior to use.

B cell preparation. B cells were enriched by treating splenocyte suspensions with two cycles anti-Thy1.2 (HO-13.4, 1:10) plus rabbit complement to remove T cells. B cells obtained in this manner were routinely 90–95% sIg^+ and <1% $Ly\text{-}1^+$, and they did not respond to Concanavalin A (Con A) indicating that T cell contamination was functionally insignificant. Resting B cells were obtained by centrifugation of the B cell preparations into a discontinuous Percoll gradient with Percoll (Sigma) concentrations of 50, 60, 70, and 75%. The fraction of cells that layered at the 70% Percoll interface (sp grav 1.087 g/cm^3) was verified as resting by size (forward angle light scatter, FALS) and cell cycle analysis (acridine orange staining) by flow cytometry. "Unseparated B cells" are defined as those B cells that were not further fractionated by the Percoll gradient separation, whereas "activated B cells" are defined

as those that layered above 60% Percoll, indicating blast-like status. The AFC responses per 10^6 unseparated B cells and resting B cells were <50 and 0, respectively.

Primary in vitro AFC assay. Spleens were aseptically removed and a single cell suspension was made by pressing the spleens between the frosted ends of two sterile microscope slides. Clumps were allowed to settle and the cell suspension was washed 1× in BSS. Each spleen equivalent was resuspended in 5 ml of PBS + 10% FBS + 0.1% NaN_3, layered onto a 4-ml Ficoll–metrizoate density gradient, and centrifuged at 3000 rpm for 15 min at 22°C to remove erythrocytes, granulocytes, and dead cells. The lymphocyte-rich interface was collected, washed 3× in BSS and cultured as described (Mishell, 1967). Briefly, 5×10^6 spleen cells/0.5 ml/well, ± $PbCl_2$ ± 25 μl of 1% SRBC were cultured in 24-well cluster plates for 5 days in a special gas composed of 10% CO_2, 7% O_2, and 83% N_2. On Day 5, cells of triplicate cultures were harvested and pooled, and the number of AFCs were enumerated by a slide modification of the Jerne plaque assay (Jerne and Nordin, 1963). Only direct plaques were enumerated.

Membrane segregated cultures were designed as described (Eastman and Lawrence, 1984). A 1.0-μm Nucleopore membrane was used to separate B cells at a density of 2.5×10^6 cells/upper chamber from splenocytes (approximately 50% B cells) at a density of 5×10^6 cells/lower chamber. Both chambers contained 1% SRBC ± 100 μM $PbCl_2$ or Pb-pretreated B cells (Pb-B cells; 100 μM $PbCl_2$, 45 min, 37°C, washed 3× prior to culture).

Generation of a SRBC specific T cell line (SRBC'T). CBA/J mice were immunized intravenously with 5×10^6 SRBC. After 7 days, the spleens from these immunized mice were removed and T cells were obtained by nylon wool nonadherence (Julius *et al.*, 1973). T cells (10^7) were cultured with 10^7 irradiated (3300 rads) splenocyte feeders + 0.5% SRBC and 50 units/ml human IL-2 (Calbiochem) in 2 ml of medium in a six-well plate. The SRBC'T cells were maintained in culture under these conditions with weekly passage for 3 months before termination. SRBC'T proliferated (as measured by [^{3}H]thymidine incorporation) in the presence of feeder cells and SRBC independent of the addition of exogenous IL-2. Furthermore, SRBC'T were specific for the eliciting antigen, SRBC, since they supported B cell differentiation into SRBC-specific AFCs but did not support AFC generation of B cells cultured with horse RBC or chicken RBC.

T cell clones. The T cell clones D10.G4.1 (Kaye *et al.*, 1983), M264-37 (Mosmann *et al.*, 1986), and HDK-1 (Cherwinski *et al.*, 1987) were obtained from Dr. T. Mossman, DNAX Research Institute (Palo Alto, CA). D1.6 (Kurt-Jones *et al.*, 1987) and CDC 25 (Tony *et al.*, 1985) were obtained from Dr. R. Noelle, Dartmouth Medical School (Hanover, NH). T cell clones were maintained and passaged as recommended by the suppliers and each clone was routinely assayed for mycoplasma contamination by the use of the Mycotect Test Kit, GIBCO (Grand Island, NY). Clones were maintained in medium plus 50 units/ml human IL-2 without antigen or feeders for 7 to 14 days prior to use.

Measurement of Ig in culture supernatants. Immulon 1, 96-well, flat-bottomed, ELISA plates (Dynatech Laboratories, Alexandria, VA) were used as the solid phase for the ELISAs. For the conalbumin-specific ELISA, 30 μg/0.1 ml/well conalbumin in 0.1 M HCO_3 buffer, pH 8.6, was used as capture reagent. Supernatants were diluted 1:4 in binding buffer (PBS + 0.1 mM EDTA, 0.25% BSA, 0.05% Tween 20, pH 7.0–7.4). Biotinylated goat anti-mouse IgM (Zymed Laboratories, San Francisco, CA) followed by alkaline phosphatase-conjugated streptavidin (Tago Immunologicals, Burlingame, CA) was used according to the suppliers specifications as developing reagents. Paranitrophenyl phosphate (Sigma) was used as substrate. The methodology for the polyclonal total IgM ELISA was essentially the same as the conalbumin-specific ELISA except that goat anti-mouse IgM (Zymed) was used as capture reagent and absorbance units were converted into IgM concentration by extrapolation from standard curves using mouse reference serum (ICN Immuno-Biologicals, Lisle, IL) of known IgM concentration.

Antigen presentation assays. Triplicate cultures containing the T cell clones (10^4/well) and appropriate antigen and antigen presenting cells (irradiated B cells or splenocytes, 5×10^4/well) were set up in 96-well round bottom plates ± $PbCl_2$. Culture wells, pulsed with 1 μCi [^{3}H]-thymidine for the final 24 hr of culture, were harvested (Skatron, Lierbyen, Norway) 72 hr after initiation, and radioactivity was counted by liquid scintillation spectroscopy.

Statistical analysis. Differences between various treatment and control groups were evaluated for by Student's *t* test.

RESULTS

Pb enhances both factor-mediated and cognate T cell help for AFC generation. The independent influences of B–T cell contact and soluble T cell factors on B cell differentiation were evaluated using membrane segregated cultures. As shown in Table 1, $PbCl_2$ significantly increased the number of SRBC-specific AFCs from both the upper and lower chambers of membrane segregated cultures. Optimal enhancement (sixfold) of the AFC response of upper chamber B cells was obtained when $PbCl_2$ was added to both chambers (Table 1, Row 1); however, the addition of Pb-pretreated B cells to the upper (Row 2) or lower (Row 3) chambers yielded two- and threefold increases respectively in the AFC response of

TABLE 1

EFFECT OF Pb ON AFC GENERATION IN THE PRESENCE AND ABSENCE OF B–T CELL CONTACT

Row	Culture conditions[a]		AFC response[b]	
	Low chamber	Top chamber	Low chamber	Top chamber
1	Splenocytes plus B cells plus Pb	B cells plus Pb	3.56 ± 0.76*	6.58 ± 0.16*
2	Splenocytes plus B cells	Pb-B cells	1.39 ± 0.27	1.94 ± 0.42**
3	Splenocyte plus Pb-B cells	B cells	3.02 ± 0.76**	3.45 ± 0.43*
4	Splenocytes plus Resting B cells plus Pb	Resting B cells plus Pb	11.40 ± 2.16**	38.43 ± 8.39**
5	Splenocytes plus Activated B cells plus Pb	Activated B cells plus Pb	5.36 ± 0.11*	3.75 ± 0.28*

[a] Double-chamber culture wells were employed to prevent B cell contact with T cells as described under Materials and Methods. Both chambers contained SRBC.

[b] After 5 days in culture, cells from both chambers were harvested and AFCs were enumerated. Values represent the ratio of the number of AFC detected in the indicated chambers compared to the number of AFC detected in identical control cultures without any Pb treatment. AFC/10^6 recovered cells for the controls were: Row 1–3, splenocytes plus B cells in the bottom (low) chamber (760 ± 151, n = 4) plus B cells in the upper (top) chamber (1094 ± 107, n = 4); Row 4, splenocytes plus resting B cells in the low chamber (336 ± 50, n = 3) with resting B cells in the top chamber (98 ± 15, n = 3); Row 5, splenocytes plus activated B cells in the low chamber (777 ± 116, n = 3) with activated B cells in the top chamber (2553 ± 383, n = 3). Student's t test was employed to examine statistically significant differences between the Pb treatments and the controls.

* Indicates $p \leq 0.001$.

** Indicates $p \leq 0.01$.

upper chamber B cells. Pb-pretreated B cells placed in the upper chambers did not significantly modify the AFC response of splenocytes in the lower chambers suggesting that the Pb did not dissociate from the upper chamber B cells and modulate the lower chamber response. Overall, the data suggest that Pb augments the AFC response by enhancing the production of putative helper T-cell-derived factors from lower chamber cells as well as the responsiveness of B cells to these factors. In addition, it is likely that Pb enhances the production of T-cell-derived factors by potentiating B–T cell interaction (i.e., cognate help). The magnitude of the enhancement in the upper chambers correlated with the activation stage of the responding B cells; that is, Pb increased the AFC response of resting B cells (Row 4) to a greater extent than unseparated B cells (Row 1) or activated B cells (Row 5).

To substantiate that Pb enhanced B cell differentiation by cognate T cell help, splenocytes obtained from CBA/N mice which require B–T interaction for B cell differentiation (Sher, 1982) were studied. As with splenocytes from "normal" mouse strains, including CBA/J, DBA/2J, BALB/c, BCF1, and C3H/HeJ, Pb increased the AFC response of CBA/N splenocytes (Table 2).

To further elucidate whether B–T cell contact was required for Pb's enhancement of B cell differentiation into AFCs, the ability of Pb to enhance the AFC response under conditions where B–T cell contact was prevented by the use of monoclonal antibodies with specificity for T cell surface molecules that are involved in B–T cell interaction was examined. As demonstrated in Fig. 1, anti-L3T4 reduced the control AFC response by 75% but insignificantly lowered the AFC response in the presence of Pb. In contrast, anti-Lyt2, which blocks suppressor cell activity, enhanced the AFC response in both the presence and absence of Pb. By reducing B cell–$CD4^+$ T cell interactions, anti-L3T4 reduces the activation of helper T cells; however, in the presence of Pb,

TABLE 2

EFFECT OF Pb ON THE AFC RESPONSE OF VARIOUS MOUSE STRAINS

Mouse strain	AFC/10^6 recovered cells	
	−Pb	+Pb
BALB/c	39	55
BCF1	545	1331
C3H/HeJ	215	487
CBA/J	212	734
CBA/N	35	218
DBA/2J	271	680

Note. Splenocytes from the indicated mouse strains were cultured at 5×10^6 cells/well plus SRBC in the presence or absence of 100 μM $PbCl_2$. After 5 days, the cells were harvested and AFCs were enumerated. The values are the mean of triplicate wells (SD < 15%) and are representative of four separate experiments.

despite the reduced cognate interaction, factor-mediated help remained enhanced. Hence, the effect of diminished cognate help by the anti-L3T4 is negated. Furthermore, the efficiency of the anti-L3T4 blockade could be diminished since Pb increases the density of the CD4 ligand (i.e., Ia) on B cells (McCabe and Lawrence, 1990). Enhancement of the AFC response by Pb in the presence of anti-Lyt2 supports the view that Pb immunopotentiation is due to the enhancement of helper T cell activities or direct effects on B cells and not due to the inhibition of suppressor T cell functions.

The effect of Pb on Ig production in the presence of antigen specific T cell help. The nature of the effect of Pb on B–T cell interaction was unclear. Conceivably, Pb could alter the B cell surface (i.e., create a neoantigen) so that T cells bearing the appropriate clonotypic T cell antigen-specific receptors could then recognize Pb-altered-self antigens and respond accordingly. Considering this possibility, the influence of Pb on B cell differentiation in cultures containing B cells, antigen, and antigen-specific T cell clones as the sole source of T cell help was tested. In contrast to the heterogeneous polyclonal repertoire of T cell receptor specificities of splenic T cells (potentially including those with specificity for a possible Pb-altered-self antigen), T cell clones having unique antigen specificities were used to cir-

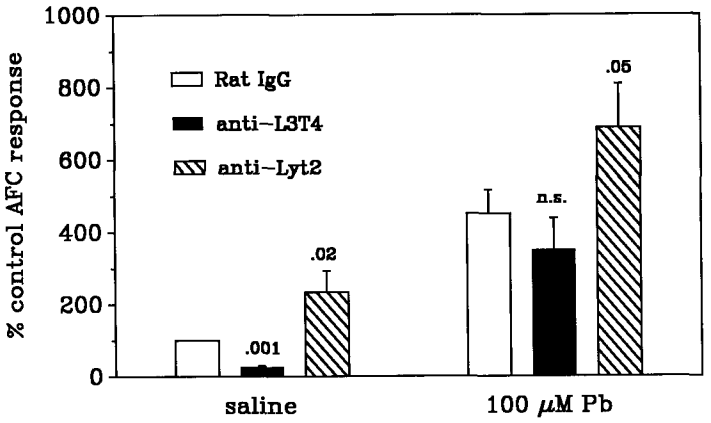

FIG. 1. Blockade of "associative recognition" does not inhibit Pb enhancement of the AFC response. Splenocytes were cultured at 5×10^6 cells/well plus SRBC ± $PbCl_2$. Monoclonal anti-L3T4 and anti-Lyt2 at 2.5 μg/ml or 5 μg/ml of nonspecific Rat IgG were added to individual wells at culture initiation. After 5 days, the cells were harvested and AFCs were enumerated. The results represent the mean ± SEM from three experiments (actual AFC/10^6 cells for the saline Rat IgG control was 108 ± 24). The values above the bars represent p values (t tests) for comparisons of AFC responses in the presence of blocking antibodies (anti-L3T4 or anti-Lyt2) ± Pb versus the AFC responses in the presence of Rat IgG ± Pb.

cumvent the complication of Pb potentially activating putative Pb-altered-self reactive T cells.

Initially, a SRBC primed T cell line was tested in cocultures containing B cells and SRBC ± 100 μM $PbCl_2$. Pb significantly enhanced (twofold) the AFC response in these cultures (Fig. 2) arguing against a Pb-altered-self phenomenon. To further substantiate this claim, other antigen-specific T cell clones were utilized. The T_{H2} clone, D10.G4.1, which is specific for either CA/H-2^k or an allo-reactive epitope of H-2^b, was cultured ± 10 or 100 μM $PbCl_2$ with either C3H/HeJ (H-2^k) B cells plus CA or C57BL/6 (H-2^b) B cells plus anti-Ig. In the CA/H-2^k B cell system, optimal help for CA-specific IgM production was obtained with 10^4 D10.G4.1 cells per culture. At this cell density, 10 μM Pb significantly increased CA-specific IgM production (Fig. 3a). This result has been corroborated using an additional T_{H2} cell clone, CDC 25, which has specificity for RGG in association with H-2^d (data not shown). As with the CA/H-2^k-specific stimulation of the D10.G4.1 cells, Pb enhanced polyclonal IgM production in the allo-stimulated system (Fig. 3b). In contrast to this influence on allo-stimulated polyclonal T_{H2} activity, Pb inhibited the polyclonal IgM response of BALB/c (H-2^d) B cells cultured with anti-Ig and the H-2^d allo-specific T_{H1} clone M264-37 (Fig. 3c).

Pb differentially affects antigen presentation to T_{H1} and T_{H2} T cell clones. In a previous study, Pb (noncytotoxic doses) has been shown to inhibit antigen presentation (as measured by IL-2 production) to the ovalbumin-reactive T cell hybridoma, DO11.10 (Smith and Lawrence, 1988). The DO11.10 resembles a T_{H1}-like cell and similarly to the T_{H1} clones (Fig. 3c) its activity was suppressed by Pb. In contrast to the inhibition of T_{H1} activation, our present results show that Pb enhances T_{H2} cell activity and the accompanying B cell responses (Figs. 3a and 3b). To further substantiate these selective and diverse effects of Pb on helper T cell activities, the effects of Pb on several T_{H1} and T_{H2} clones were tested in antigen presentation assays. Pb inhibited antigen presentation by B cells to the KLH/H-2^d-restricted T_{H1} clone HDK-1 (Fig. 4a). The opposite result was obtained with D10.G4.1 (T_{H2} clone), in that Pb enhanced its antigen-specific (CA/H-2^k) response (Fig. 4b). Using additional T_{H1} and T_{H2} clones, D1.6 and CDC25, respectively, which are both specific for RGG associated with H-2^d, Pb inhibited the T_{H1} proliferative response induced by RGG presented by splenocytes (Fig. 5a) and B cells (Fig. 5b), but Pb enhanced the T_{H2} proliferative response (Fig. 5c).

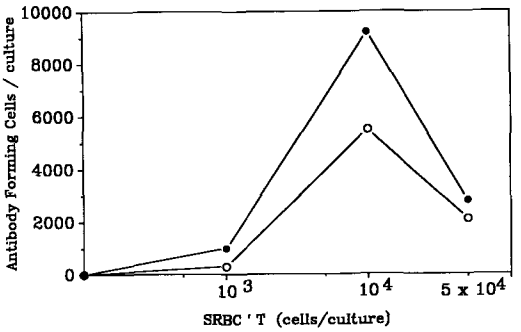

FIG. 2. Pb enhancement of SRBC-specific T cell help. The indicated number of SRBC T cells were cultured with 3×10^6 B cells plus SRBC in the presence (closed symbols) or absence (open symbols) of 100 μM $PbCl_2$. After 5 days, the cells were harvested and AFCs were enumerated. The data points are the mean of triplicate wells (SD < 10%) and are representative of three separate experiments.

DISCUSSION

The present study was undertaken to determine the mechanisms by which the heavy metal Pb enhances T-cell-dependent B cell differentiation. Although Pb is known to enhance the *in vitro* production of antibody (Lawrence, 1981a,b; Warner and Lawrence, 1986a) and directly activate B cells (McCabe and Lawrence, 1990), the influence of Pb on B–T cell interactions has not been clearly delineated. It was discovered (Table 1) that Pb can potentiate B cell differentiation, as measured by AFC formation, under conditions whereby

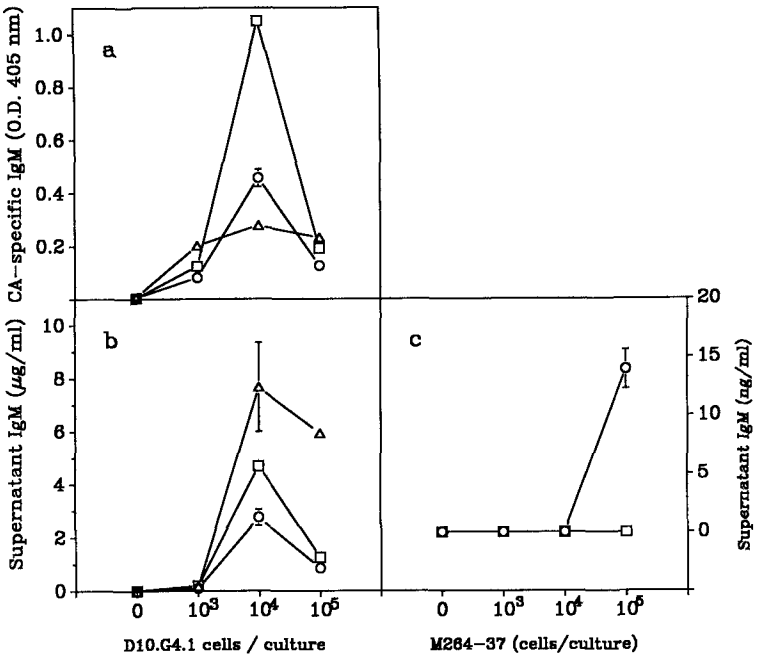

FIG. 3. Pb enhances T_{H2}-mediated help but inhibits T_{H1}-mediated help for IgM production. (a) Resting T_{H2} cells (D10.G4.1) were cultured with 5×10^5 resting C3H/HeJ B cells and 50 μg/ml conalbumin (CA), in the absence (circles) or presence of 10 μM $PbCl_2$ (squares) or 100 μM $PbCl_2$ (triangles). Supernatants were collected on Day 7, and CA-specific IgM was quantitated by ELISA. Data points represent the mean OD at 405 nm ± SD. The data is representative of three separate experiments. No CA-specific IgM was detected from cultures without CA. (b) Resting T_{H2} cells (D10.G4.1) were cultured with 10^6 resting, allogeneic C57BL/6 B cells, 20 μg/ml anti-Ig, ± $PbCl_2$ (symbols as in a); (c) resting T_{H1} cells (M264-37) were cultured with 10^6 resting, allogeneic, BALB/c B cells, 20 μg/ml anti-Ig, ± 10 μM $PbCl_2$ (symbols as in a). In (b) and (c), supernatants were collected on Day 7 and total IgM was quantitated by ELISA.

direct B–T cell contact was not permitted, although optimal enhancement of the AFC response by Pb occurred when B–T cell contact was permitted. Hence, it appears that Pb enhances both cognate helper T cell activity as well as factor-mediated T cell help. Furthermore, with regard to the effects of Pb on factor-mediated T cell help, it appears that Pb influences the production of factors by T cells, which likely is related to the effect of Pb on cognate interactions and subsequent T cell activation. Presently, the identities of the T cell factors, which may be any of the well-characterized interleukins or combinations thereof, are not known. It has been previously reported that Pb can enhance *in vitro* generation of lymphokines by T cells (Warner and Lawrence, 1988). Pb also appears to enhance B cell responsiveness to the T cell helper factors, in that the upper chamber Pb-pretreated B cell response (in the absence of cognate T cell help) was enhanced. This suggests that Pb may influence B cell expression of surface receptors for particular interleukins. At present, this is a matter of conjecture since we have not measured Pb effects on interleukin receptor density on B cells. It is conceivable that Pb could modulate the surface density of interleukin receptors on B cells since Pb has been shown to modulate the cell surface density of other important molecules on B cells such as Ia, CD23, and sIgD (McCabe and Lawrence, 1990). The ability of Pb to increase B cell surface Ia density is consistent with the interpre-

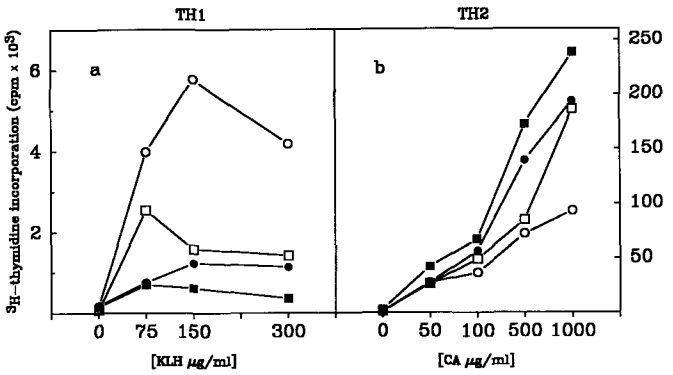

FIG. 4. Effect of Pb on the activation of T_{H1} vs T_{H2} clones. Resting T cell clones were cultured at 10^4 cells/well in round-bottomed wells with 5×10^4 irradiated (3300 rads), resting, syngeneic B cells (open symbols) or 5×10^4 irradiated, syngeneic splenocytes (filled symbols) in the presence (squares) or absence (circles) of 10 μM $PbCl_2$. Culture wells, pulsed with 1 μCi [^{3}H]thymidine for the final 24 hr of culture, were harvested 72 hr after initiation, and radioactivity was counted by liquid scintillation spectroscopy. The data points are the mean cpm of triplicate wells (SD < 10%), and the results are representative of two separate experiments. (a) T_{H1} clone, HDK-1, plus BALB/c B cells or splenocytes. (b) T_{H2} clone, D10.G4.1, plus C3H/HeJ B cells or splenocytes.

tation that Pb promotes cognate T cell help, in that Ia molecules mediate B–T cell interactions (Katz *et al.*, 1975) and increased Ia expression has been indicated to enhance the immune response (Matis *et al.*, 1983). Experiments to determine whether factor–receptor systems are influenced by Pb should be undertaken to shed more light on how Pb enhances the responsiveness of the B cell component of the immune system. The present understanding of how B cell differentiation is controlled by endogenous factors and normal cellular interactions, let alone how environmental factors such as Pb may influence these processes, is far from clear. However, it has become clear that after antigen-specific stimulation of the appropriate B cell and T cell clones, the ensuing immune response leading to antibody production is controlled by an intricate network of nonspecific factors and cellular interactions. Our data suggest that environmental factors such as Pb can influence the immune response by modulating these nonspecific regulatory components rather than creating neoantigens that may be recognized in a specific way by the appropriate lymphocytes. This influence by environmental factors on the regulation of the immune response perhaps is more difficult to envision than a direct modification of "self," but it is likely to be of paramount importance with respect to the influence of toxicants on autoimmune disease and host resistance to pathogens and neoplasia. Furthermore, such modulations may be more ubiquitous, in that they may result from modification of similar biochemical processes in different organ systems.

It seems odd that a highly toxic metal enhances humoral immunity. Nonetheless, enhancement of inappropriate immune responses certainly are not beneficial since they may progress to autoimmune disease. Furthermore, enhancement of particular responses may result in dysregulation of the appropriate responses or may prevent them from occurring altogether. This is especially interesting in the cases of immune responses dominated by one or the other helper T cell subset (T_{H1} and T_{H2} cells). Modulation of T_{H1} vs T_{H2} activity *in vivo* has been implicated in various immunopathological states, most strikingly with protozoal and helminth infections (Scott *et al.*, 1989) or the nematode *Nippostrongylus brasiliensis* (Finkelman *et al.*, 1986). In ad-

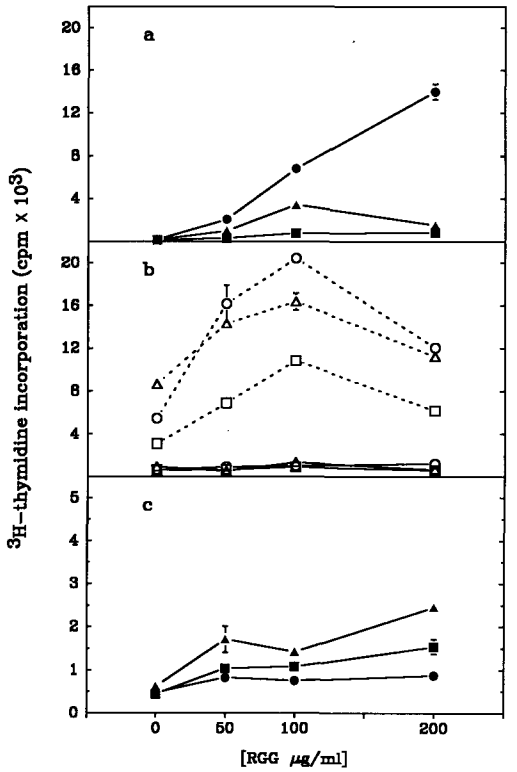

FIG. 5. Effect of Pb on the activation of T_{H1} vs T_{H2} clones having the same antigen specificity and the same haplotype restriction. Resting T cell clones were cultured at 10^4 cells/well with 5×10^4 irradiated (3300 rads), resting, syngeneic, BALB/c B cells (open symbols) or 5×10^4 irradiated (3300 rads), syngeneic, BALB/c splenocytes (filled symbols) in the absence (circles) or presence of 10 μM $PbCl_2$ (squares) or 100 μM $PbCl_2$ (triangles). Culture wells were pulsed and harvested as indicated in the legend to Fig. 3. The data points are the mean cpm of triplicate wells (SD < 10%), and the results are representative of two separate experiments. (a) T_{H1} clone, D1.6, plus splenocytes. (b) T_{H1} clone, D1.6 (dotted lines), plus B cells; T_{H2} clone, CDC 25 (solid lines), plus B cells. (c) T_{H2} clone, CDC 25, plus splenocytes.

dition to the categorization of T_{H1} and T_{H2} cells based on their interleukin activities (Mosmann *et al.*, 1986), these $CD4^+$ T cell subsets differ in several other important aspects. Recent information indicates that the biochemistry of T_{H1} cell activation differs from that of T_{H2} cells (Gajewski *et al.*, 1990; Munoz *et al.*, 1990; Betz and Fox, 1991) which may be mechanistically related to the differential effects found with Pb. The differential effects of Pb on the $CD4^+$ T cell subsets may involve cAMP since these subsets have been reported to have differential sensitivities to cAMP (Munoz *et al.*, 1990), and Pb and cAMP have been reported to synergistically modulate lymphocyte activity (Lawrence, 1981c). Additional studies are needed to elucidate the biochemical mechanism(s) whereby Pb affects lymphocytes and T_{H1} and T_{H2} cells in particular. The observed differential effects of Pb on the T_{H1} and T_{H2} clones may provide some clues about the interleukin activities that Pb influences in regard to the enhancement of antibody production. Since T_{H2} clones are considered to be the optimal helpers for B cell responses (Janeway *et al.*, 1988), it is not surprising that Pb enhances T_{H2} activity and B cell differentiation to AFC formation and Ig production. T_{H1} clones and their associated cytokines can influence B cells, in that they can to a limited extent support polyclonal but not antigen-specific B cell differentiation. Pb did not impart on T_{H1} clones the capacity to support antigen-specific B cell differentiation (data not shown). Furthermore, in contrast to its influence on allo-reactive polyclonal T_{H2} activity, Pb inhibited the limited polyclonal B cell response supported by the T_{H1} clone, M264-37, a result consistent with the fact that T_{H1} cells, which aid only at a high cell density, are not efficient helpers of B cell differentiation. In light of the differential effects of Pb on the helper T cell subsets, it is interesting to note that T_{H1} cells require a higher density of "processed antigen" to be presented for their activation (Janeway *et al.*, 1988).

The differential influence of Pb on T_{H1} vs T_{H2} cells may explain, in part, the diverse *in vivo* effects of Pb, e.g., suppression of host resistance to *Listeria monocytogenes* (Lawrence, 1981a) and other pathogens (Lawrence, 1985) yet the postulated ability of Pb to promote autoimmunity by its immunopotentiating effects. *Listeria,* an intracellular pathogen, is controlled mainly by T_{H1} cells *in vivo.* If Pb decreases T_{H1} activity *in vivo* less macrophage stimulatory factors (e.g., IFN-γ) would be

produced. In addition to impairing macrophage activation, Pb has been shown to directly inhibit macrophage development (Kowolenko *et al.,* 1989). On the other hand, Pb can directly activate B cells (McCabe and Lawrence, 1990; Lawrence, 1981c), and enhanced B cell: T_{H2} cell interactions might lead to dysregulated B cell responsiveness and autoimmunity. Features of autoimmune disease induction include aberrant expression of Ia and cytokines as well as lymphoid infiltration of target tissues due to altered interactions between the lymphoid subsets (Schwartz and Rose, 1986). Autoimmune disease incidence increases with aging. Interestingly, age-related changes in cytokine production by T helper cell subsets recently have been reported (Kubo and Cinader, 1990). Pb involvement in immune-mediated nephritis has been suggested (Wedeen *et al.,* 1979; Garcia *et al.,* 1980); however, data directly implicating Pb in autoimmune disease is not available. Autoimmune disease induced by mercury has been documented (Hirsch *et al.,* 1982; Pelletier *et al.,* 1985). Our data suggest that Pb can promote immune dysfunctions characteristic of autoimmune disease induction. Inasmuch as little is known about the etiology of autoimmune diseases, a role for toxic agents such as Pb warrants consideration.

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