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In Vivo and In Vitro Effects of Lead on Humoral and Cell-Mediated Immunity

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The humoral and cell-mediated immune responses of murine lymphocytes exposed to lead in vivo and in vitro were investigated. In vivo Pb was administered via the drinking water (0 to 10 mM) for 1 to 10 weeks. In vivo exposure of the mice to Pb did not alter significantly their plaque-forming cell response to sheep erythrocytes; however, their susceptibility to *Listeria* infection was reduced significantly with Pb dosages of >0.4 mM. Although the in vivo plaque-forming cell responses did not appear to be altered, in vitro assessment of the reactivity of these in vivo Pb-exposed lymphocytes indicated that intermediate doses enhanced, but a high dose (10 mM) was suppressive. The 10 mM in vivo Pb dose suppressed the in vitro plaque-forming cell response, the mixed-lymphocyte culture response, and lipopolysaccharide-induced proliferation, but it did not affect concanavalin A- or phytohemagglutinin-induced proliferation. Interestingly, in vitro Pb exposure (10^{-6} to 10^{-4} M) of murine spleen cells caused an enhancement of most activities even though these in vitro concentrations of Pb were slightly above the in vivo concentrations. Direct in vitro Pb effects on the lymphocytes could be measured, and Pb consistently enhanced humoral and cell-mediated immunity.

Lead (Pb) compounds are known to exert numerous toxic effects (11); however, the physiological effects of Pb on the immune system are not well documented. Most studies have attempted to assess the direct mechanisms of Pb toxicity and, therefore, have been pathological investigations. A limited number of toxicity studies have examined the indirect effects of Pb-induced toxicity by evaluating the effects of Pb on the immune system (34). Since the immune system maintains the integrity of self and protects the host from pathogens, Pb alteration of the immune system could upset homeostatic mechanisms and natural and acquired resistance to invading organisms.

Acute and chronic exposure of experimental animals to low levels of Pb have been shown to alter the immune system and enhance the host's susceptibility to bacterial (3, 13, 30) and viral (8, 9) infections. Although Pb has been shown to enhance morbidity and mortality (4, 34), the mechanisms involved have not been delineated. In addition, the ability of Pb to influence a bacterial infection classically considered to be controlled by T-cells, such as a *Listeria* infection, has not been assessed. Most of the studies to date have investigated only humoral immunity (HI), the production of antibodies. The effects of Pb on T-cell functions are not well documented, and suppression of T-cell function as well as B-cell function could dramatically increase mortality. T-cells not only are responsible for cell-mediated immunity (CMI), but also regulate HI by enhancing or suppressing B-cell proliferation and differentiation.

In vivo, Pb has been shown to inhibit antibody production (16, 19). Since these effects could be due to direct suppression of B-cell, helper T-cell, or macrophage functions or any combination of these, to indirect effects on the pathogens or antigens employed, or to alteration of aspects of innate immunity such as neutrophil function, natural flora, or mucous barriers, the present investigation was undertaken to determine the in vivo and in vitro effects of Pb on HI and CMI.

In this study, the ability of Pb to alter immunological activities was assessed by three different approaches: (i) Pb was administered in vivo, and immune function was assessed in vivo; (ii) Pb was administered in vivo, lymphocytes were removed and washed, and their activities were assessed in vitro; or (iii) lymphocytes were exposed to Pb in vitro, and the in vitro effects of Pb were determined. The results indicate that assessment of the Pb effects on immunity vary, depending on the system employed. The in vivo effects of Pb did not always correlate with the in vitro effects of Pb; however, Pb definitely altered

HI as well as CMI responses, including increased susceptibility to *Listeria* infection.

MATERIALS AND METHODS

Animals. Female CBA/J and C57BL/6 mice (Jackson Laboratory, Bar Harbor, Maine) were used throughout these studies. Mice were maintained on laboratory chow and acidified, chlorinated water (pH 3.0) ad libitum before being used in the studies. During the individual experimental studies, the above-described water was replaced with distilled, Millipore-filtered water (2-μS; 21°C) with various concentrations of lead acetate (0.08 to 50 mM); 50 mM lead acetate was employed only in the initial 1-week study, because the mice drank only 0.4 ± 0.1 ml per day per mouse during that week as compared to 4.5 ± 1.5 ml per day per mouse for the other groups.

Mitogens, metals, and antigens. The mitogens and their concentrations employed in these studies were as follows: recrystallized concanavalin A (ConA; Miles), 2 μg/ml; purified phytohemagglutinin (PHA; Wellcome Reagents), 2 μg/ml; and *Escherichia coli* O55:B5 lipopolysaccharide (LPS; Difco), 50 μg/ml. All metal salts were obtained from Fisher Scientific at the highest possible purity. Although in the presented results lead acetate and lead chloride were employed for the in vivo and in vitro studies, respectively, the anion did not appear to influence the results. Sheep erythrocytes (SRBC), obtained from Griffin Laboratories, New York State Department of Health, were stored in Alsever solution. The *Listeria monocytogenes* inoculum was prepared and stored as previously described (29).

Preparation of cells. Spleens were aseptically removed from the mice after exsanguination. Spleens were teased and settled to obtain sterile single-cell suspensions as previously described (21). A balanced salt solution was used for the isolation of all cells.

Assessment of in vivo HI to SRBC. Mice were injected intravenously with 10^8 SRBC, and 5 days later their spleens were removed, single-cell suspensions were obtained, and the cell preparations were assayed in the hemolytic plaque assay. All results are expressed as number of plaque-forming cells (PFC) per spleen. The results were relatively comparable to PFC per 10^6 spleen cells.

Assessment of in vivo CMI to *Listeria*. The in vivo immune response to *Listeria* was assessed as previously described (29). Briefly, approximately 10^5 viable *Listeria* organisms were injected intravenously, and 2 to 4 days later the mouse spleens were aseptically removed, homogenized, and plated for enumeration of *Listeria* colonies.

Assay for in vitro HI to SRBC. The in vitro cultures were set up as previously described (21). Briefly, 5×10^6 spleen cells were cultured in 0.5 ml of medium per well by the technique described by Mishell and Dutton (24). The number of direct hemolytic PFC in each culture well was determined at day 5. All results are expressed as the number of PFC per culture, averaged from three cultures. Background responses to SRBC were determined from cultures lacking antigen, and background PFC values were subtracted from the experimental values.

Hemolytic plaque assay. Cells producing antibody specific for SRBC were enumerated by use of a modification (10) of the Jerne plaque technique (15).

Assay for mixed-lymphocyte culture (MLC) responsiveness. Responder lymphocytes were mixed with irradiated (2,000 R) stimulator lymphocytes in various ratios (2:1, 1:1, 1:2), and the proliferative response was measured 5 days later by pulsing with [^{3}H]thymidine from day 4.5 to 5 (12 h). A total of 2×10^6 cells were cultured in 0.2 ml of RPMI 1640 medium supplemented with nonessential amino acids, sodium pyruvate, $NaHCO_3$, penicillin and streptomycin (100 U of each per ml), 5×10^{-5} M 2-mercaptoethanol, and 5% heat-inactivated human type AB serum. A flat-bottom tissue culture microtiter plate was employed (Costar, Cambridge, Mass.).

Assay for in vitro mitogen responsiveness. Single-cell suspensions of spleen cells were cultured at a cell concentration of 2×10^5 in 0.2 ml per well. A minimal essential suspension culture medium + 5% fetal calf serum ± mitogen was employed. Cultures were pulsed with [^{3}H]thymidine for 6 h on day 2, 3.5, or 5.

Statistical analysis. The analysis of variance (7) or the Student's *t* test was employed. For the Student's *t* test, $P < 0.01$ was considered significant.

RESULTS

In vivo Pb effects on HI and CMI. Mice were fed lead acetate in their drinking water in concentrations ranging from 0.08 to 50 mM (16 to 10,400 ppm of Pb) for 1 to 10 weeks. After 1, 2, 4 (Fig. 1), 8, or 10 weeks, the mice were immunized intravenously with 10^8 SRBC, and the primary HI response to SRBC was assessed 5 days later by enumeration of the SRBC-specific PFC per spleen. As shown in Fig. 1, Pb did not significantly affect the response to SRBC. Pb did not significantly alter the in vivo HI response to SRBC in any of the groups tested (fed Pb for 1 to 10 weeks) (data not shown).

CMI was assessed in mice fed Pb for 4 weeks by determining their resistance to *Listeria*. In contrast to HI, CMI to *Listeria* appeared to be suppressed. As shown in Fig. 2, 10 mM lead acetate solutions significantly reduced resistance to *Listeria* as apparent from the enhanced number of viable *Listeria* per spleen. The 0.08 to 2 mM lead acetate solutions did not significantly alter the number of viable *Listeria* per spleen; however, 0.4 to 10 mM solutions enhanced the mortality rate of the *Listeria*-infected mice. Ten days after infection, all 0.08 mM and control mice were alive, but the mice in the 2 and 10 mM groups were all dead by day 3 to 4, and the 0.4 mM mice were all dead by day 7. Similar results were obtained with mice fed Pb for only 2 weeks (data not shown).

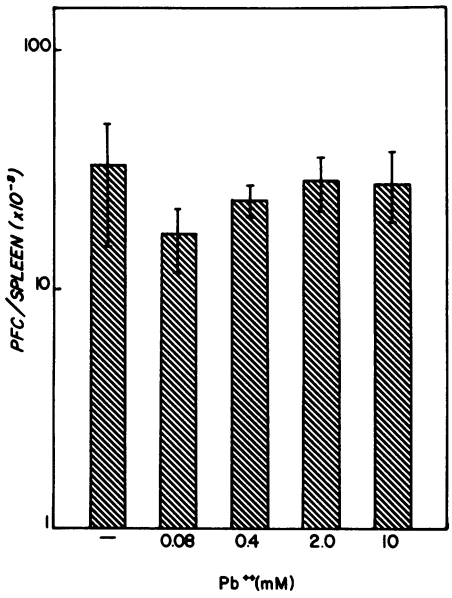

FIG. 1. *In vivo assessment of the influence of Pb (4 weeks) on the primary HI response of CBA/J mice to SRBC. Mice drank water (control, —) or various amounts of Pb acetate (0.08 to 10 mM) for 4 weeks before intravenous immunization with 10^8 SRBC. The PFC per spleen were enumerated 5 days later. Brackets represent the mean ± standard deviation of five mice per group.*

In vitro assessment of in vivo Pb-treated lymphocytes. The ability of splenic lymphocytes from Pb-fed mice to produce a primary in vitro HI response to SRBC was assessed. Unlike the in vivo HI responses, substantial differences were observed in vitro. Lymphocytes isolated from mice drinking 0.08 to 0.4 mM lead acetate for 4 weeks showed a twofold increase in the number of SRBC-specific PFC when challenged in vitro (Table 1). On the other hand, lymphocytes from the 2 mM group did not produce more PFC than the control group, and the lymphocytes from the 10 mM group were suppressed in that only 30% of the control response was obtained. Similar results were obtained with mice fed Pb for 10 weeks.

The in vitro assessment of T-lymphocyte activity also differed as compared to the in vivo assessment of CMI (*Listeria* resistance). In vitro responsiveness of T-cells from Pb-fed mice was investigated by MLC reactivity. Mice fed Pb for 4 weeks were assessed (Table 2). As in the in vitro assessment of HI, the 0.08 mM and 0.4 mM groups had enhanced activities, the 2 mM group was not affected, and the activity of the 10 mM group was slightly suppressed (10 to 24% inhibition).

The mitogenic responsiveness of the in vivo Pb-treated (4 weeks) splenic lymphocytes also was determined (Table 3). The spleen cell preparations from the Pb groups showed responses to PHA that did not significantly differ from the control cells, with the exception of the 0.08 mM dose, which did cause significant enhancement. The ConA-induced response was significantly enhanced in the 0.4 mM group only. The LPS-

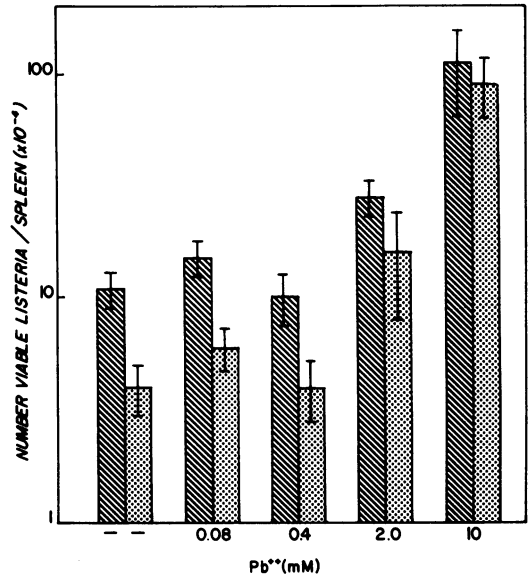

FIG. 2. *In vivo assessment of the influence of Pb (4 weeks) on the CMI response of CBA/J mice to L. monocytogenes. Mice drank water (control, —) or various amounts of lead acetate (0.08 to 10 mM) for 4 weeks before infection with 1.52×10^5 viable Listeria. The log numbers of viable Listeria per spleen were enumerated 48 (hatched bars) and 72 (dotted bars) h after infection. Bars represent the mean ± standard deviation of five mice per group.*

TABLE 1. *Effect of lead (4 weeks) on in vitro primary HI response to SRBC*[a]

Lead dose[b] (mM)	PFC per culture[c]	% of control
None	378 ± 36	100
0.08	768 ± 81	203
0.4	725 ± 71	192
2.0	473 ± 51	125
10.0	112 ± 62	30

[a] The in vitro primary HI response (day 5) to SRBC was assessed with cultures established with 5×10^6 spleen cells (from mice treated with various doses of Pb) plus 2×10^6 SRBC.

[b] Groups of four mice were fed various doses of lead acetate (0.08 to 10.0 mM) in their drinking water.

[c] Mean number of PFC from triplicate cultures of the spleen cells from four mice per group ± standard deviation.

induced response was significantly suppressed in the 2 mM and 10 mM groups. This suggests that intermediate in vivo doses of Pb enhance T-cell proliferation and that higher in vivo doses of Pb inhibit proliferation of LPS-induced B-cells. In addition, spleen cells from the 0.08 to 2 mM Pb-treated groups had significantly higher background responses (medium; no mitogens).

In vitro Pb treatment. Pb enhanced the in vitro primary HI response to SRBC (Fig. 3). Metal chloride concentrations ranging from 10^{-4} to 10^{-7} M were employed, and the effects of Pb, Ca, Ni, and Hg were compared. Ca, which was employed as a control cation, since it was used within its normal basal levels, produced no significant effect on the response, whereas Hg inhibited the response and Pb and Ni enhanced the response. The ability of Pb and Ni to enhance the in vitro HI response is being investigated more extensively and will be presented in another report. The ability of Pb to potentiate the PFC response to SRBC in vitro correlates with the enhanced activity of in vivo Pb-treated spleen cells tested in vitro (Table 1).

TABLE 2. *Effect of lead (4 weeks) on MLC response of CBA/J mice to C57BL/6 mice*

Lead dose to CBA/J responders (mM)	cpm of stimulator cells[a]		SI[b]	% of control
	CBA/J	C57BL/6		
None	20,685 ± 1,211	70,983 ± 6,284	3.43	100
0.08	20,463 ± 5,180	91,116 ± 3,198	4.45	130
0.4	14,023 ± 365	79,485 ± 4,785	5.67	165
2.0	22,025 ± 2,825	72,928 ± 2,775	3.31	97
10.0	28,887 ± 3,435	74,696 ± 4,644	2.59	76

[a] Stimulator cells were the syngeneic CBA/J spleen cells or the allogeneic C57BL/6 spleen cells. A ratio of two stimulators to one responder was used, and the stimulator cells were irradiated with 2,000 R before addition to the MLC. Data represent the mean from triplicate cultures ± standard error.

[b] Si, Stimulation index: [counts per minute (cpm) of C57BL/6:CBA/J cultures]/[cpm of CBA/J:CBA/J cultures].

Likewise, the T-cell activities tested in MLC responses were enhanced by in vitro Pb treatment (Table 4), which correlates with the enhanced MLC reactivity of in vivo Pb-treated cells (Table 2). The MLC reactivity in both cases was inhibited by the highest concentration of Pb (0.5 mM, in vitro; 10 mM, in vivo).

In vitro, Pb itself induced limited proliferation, but it produced significant enhancement of the ConA- and PHA-induced responses with high doses of Pb (Table 5). The LPS-induced response was significantly enhanced by in vitro Pb treatment with the highest concentration (10^{-4} M), whereas in vivo Pb-treated spleen cells had LPS responses lower than those of the control cells (Table 3). Higher concentrations of $PbCl_2$ ($>5 \times 10^{-4}$ M) were not used because they were toxic. It should be noted that an in vitro concentration of 5×10^{-4} M would be approximately 100-fold greater than the blood Pb concentration in Pb-poisoned animals.

In vitro macrophage function was not significantly affected by 10^{-4} M $PbCl_2$, although there was a slight increase in the uptake of SRBC preincubated with Pb (Table 6). Furthermore, in one experiment Pb caused an increase in phagocytosis and a concomitant decrease in the percentage of rosetted cells. Since macrophage processing of SRBC did not appear to be substantially altered, the Pb effects on in vitro PFC and MLC responses may be due to a direct effect on B-cells and T-cells, respectively.

DISCUSSION

Numerous reports have indicated that heavy metals can react with cells of the immune system and alter HI (13, 16, 19, 30, 34) and CMI (26, 34) responses. However, many aspects of these reports are conflicting, and the types of cells affected and the mechanisms involved are unresolved. Since, to date, no studies have attempted to dissect the cellular components of the immune system, the effects of Pb and other heavy metals on B-cell and T-cell activities are un-

TABLE 3. *Effect of in vivo lead treatment on the in vitro mitogen reactivity of spleen cells*[a]

Mitogen	Mitogenic response (cpm per culture)[b] at Pb dose[c]:				
	None	0.08 mM	0.4 mM	2.0 mM	10 mM
None	479 ± 144	842 ± 235	1,164 ± 262	1,136 ± 259	658 ± 88
ConA	37,361 ± 1,264	39,664 ± 4,866	54,238 ± 2,486	42,838 ± 3,329	36,335 ± 2,720
PHA	38,549 ± 3,361	48,726 ± 3,486	39,096 ± 3,053	36,727 ± 3,259	32,309 ± 2,905
LPS	41,061 ± 2,361	43,164 ± 1,168	44,659 ± 1,819	26,417 ± 1,283	29,782 ± 1,072

[a] The mitogenic response of 2×10^5 CBA/J spleen cells from the experimental and control groups was determined with the mitogens on day 2 by pulsing for 6 h with [^{3}H]thymidine.

[b] Mean counts per minute (cpm) ± standard error for triplicate cultures with the spleen cells of three mice per group.

[c] Concentrations of lead acetate fed to the mice for 4 weeks.

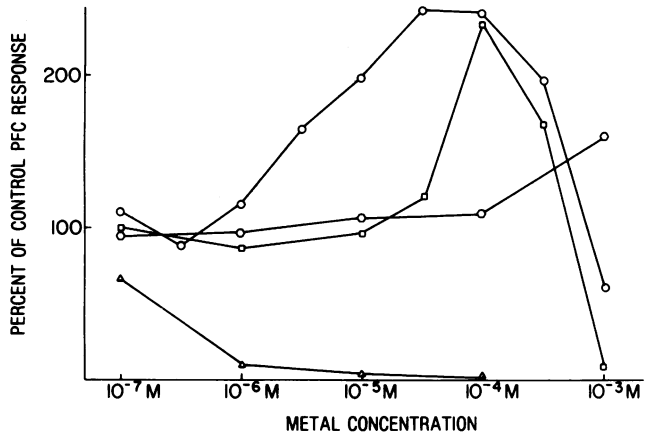

FIG. 3. *In vitro assessment of the influence of metals on the HI response to SRBC. Cultures of 5 × 10^6 CBA/J spleen cells + 2 × 10^6 SRBC were initiated with various concentrations of Pb, Ca, Ni, or Hg, and the development of SRBC-specific PFC was determined 5 days later. Each point was calculated from the mean number of PFC per culture from triplicate cultures. The percent of control value was determined by dividing the mean experimental values by the mean control (cultures without addition of metal chlorides) values. The control cultures had 553 PFC per culture. The results are representative of numerous experiments.*

TABLE 4. *In vitro effects of lead on one-way MLC response*[a]

Lead dose (M)	cpm[b] at stimulator:responder ratio:		
	1:2	1:1	2:1
None	24,700 ± 2,561	46,800 ± 3,718	100,164 ± 9,974
10^{-6}	36,345 ± 4,968 (135)	50,736 ± 6,713 (102)	133,213 ± 14,016 (130)
10^{-5}	40,556 ± 3,876 (148)	68,584 ± 6,793 (138)	135,215 ± 12,489 (131)
10^{-4}	40,550 ± 3,891 (150)	44,448 ± 4,971 (111)	131,710 ± 12,799 (128)
5 × 10^{-4}	19,716 ± 2,316 (80)	3,986 ± 1,115 (9)	10,196 ± 1,358 (10)

[a] CBA/J responder spleen cells were mixed with 2,000 R-irradiated C57BL/6 stimulator spleen cells in ratios of 1:2, 1:1, and 2:1. The MLC were set up with various concentrations of $PbCl_2$.

[b] The results represent the mean counts per minute (cpm) of triplicate cultures ± standard deviation. The control (no Pb) stimulation indices were 1.9 (1:2), 3.6 (1:1), and 7.7 (2:1). The numbers in parentheses indicate percent of the corresponding control stimulation index.

TABLE 5. *Effect of in vitro lead treatment on the mitogen reactivity of spleen cells*[a]

Mitogen	Mitogenic response (cpm/culture)[b] at Pb dose[c]:			
	None	10^{-6} M	10^{-5} M	10^{-4} M
None	477 ± 110	646 ± 181	799 ± 99	946 ± 192
ConA	52,005 ± 2,849	57,489 ± 1,059	56,214 ± 2,301	60,843 ± 7,020
PHA	44,702 ± 2,229	43,818 ± 2,404	47,754 ± 2,329	62,983 ± 4,706
LPS	71,064 ± 1,189	76,142 ± 713	85,461 ± 3,895	91,306 ± 2,161

[a] The mitogenic response of 2 × 10^5 CBA/J spleen cells incubated with various concentrations of Pb + mitogen was determined on day 2 by pulsing for 6 h with [^{3}H]thymidine.

[b] Mean counts per minute (cpm) ± standard error for triplicate cultures.

[c] Final concentrations of lead chloride in culture.

known. The ability to resist infection and control neoplasia rests on the functional integrity of the immune system. In this investigation, the ability of Pb to alter HI and CMI has been correlated with the in vivo and in vitro effects of Pb on B-cell, T-cell, and macrophage function. T- and B-cells and, to a lesser extent, macrophage functions were altered by Pb.

The differences between the in vivo and in vitro Pb effects on B-cell activity may relate to the fact that in vitro exposure was acute and in vivo exposure was relatively chronic. This suggests that an acute exposure to Pb during HI response enhances, as shown in our in vitro study and in the in vivo acute Pb exposure study (18), but chronic in vivo exposure to high doses

(>2 mM) of Pb can suppress (16, 19; Table 1). In vivo and in vitro Pb treatments clearly produced differential effects on lymphocyte activation by LPS, a selective murine B-cell mitogen (12). Long-term in vivo exposure to Pb may alter normal B-lymphocyte differentiation and, thus, alter the HI response. Chronic in vivo Pb exposure has been reported to alter the percentage of B-cells with C3b receptors (17). B-lymphocytes lacking C3b receptors exist very early or late in the ontogenic developmental pathway of stem cell to B-cell to plasma cell. Pb alteration of the ontogenic development of the B-cell also would explain why our in vivo and in vitro studies indicated different Pb effects on LPS-induced B-cell proliferation. Pb could differentially affect the development of the subpopulations of B-cells. Numerous reports have shown that Pb reduces in vivo resistance to endotoxin (3, 5, 13, 30, 31), which is believed to be controlled by HI, whereas, in this study, suppression of in vivo HI responses to SRBC was not apparent although LPS-induced proliferation of in vivo Pb-treated lymphocytes was inhibited. Endotoxin (LPS) is a T-independent antigen, and SRBC is a T-dependent antigen. It has been suggested that T-independent antigens (and possibly some T-dependent antigens) stimulate different subpopulations of B-lymphocytes (25). Pb may differentially affect a subpopulation of B-cells or alter the lineage of B-cells in vivo, which would account for the ability of Pb to inhibit LPS-responsive cells and decrease resistance to LPS (3, 13, 30).

T-cell activities also were differentially affected by Pb treatment. A central feature of CMI is the interaction of soluble mediators (lymphokines) from an activated subset of T-lymphocytes with macrophages to enhance their nonspecific bactericidal (27, 32) or bacteriostatic (6) activity. The enhancement of macrophage bactericidal activity has been shown to be mediated by T-cells (28). Challenge with the facultative intracellular bacterium *L. monocytogenes* has commonly been employed to monitor macrophage activation (1). Regulation of immunity to *Listeria* has been correlated with a cell that carries the Thy-1 alloantigen (T-cell), and treatment of immune spleen cells with anti-Thy-1 serum and complement abrogates the transference of adoptive immunity to *Listeria* (28) and macrophage mobilization at the sites of infection (28). Since activation of macrophages is a central feature of CMI (22), investigation of an increase or decrease in the number of *Listeria* cells recovered from Pb-treated mice can provide evidence for evaluating the regulatory effects of Pb on CMI. Pb significantly reduced in vivo resistance to *Listeria*, which indicates that

TABLE 6. *In vitro effect of lead on macrophage binding and phagocytosis*[a]

Cultures	% Rosettes[b]		% Phagocytosis[b]	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Mϕ + SRBC	0	0	0.5	1.0
Mϕ + SRBC + Pb	5.5	0	1.0	2.0
Mϕ + Pb-SRBC	ND	2.0	ND	3.5
Mϕ + AbSRBC	19.0	0	60.0	77.0
Mϕ + AbSRBC + Pb	0	1.0	69.0	70.0
Mϕ + Pb-AbSRBC	ND	0	ND	75.0

[a] A volume of 5×10^4 noninduced, adherent peritoneal cells (macrophages; Mϕ) was cultured for 1 h in the presence of SRBC, anti-SRBC:SRBC complexes (AbSRBC), SRBC precinbuated with 10^{-4} M $PbCl_2$ (Pb-SRBC), or AbSRBC preincubated with 10^{-4} M $PbCl_2$. The percentage of macrophages rosetting with ≥3 SRBC or AbSRBC or phagocytosing ≥3 SRBC or AbSRBC was calculated as previously described (23).

[b] Each number represents the mean percentage of duplicate cultures in which >100 cells were counted. ND, Not done.

CMI (or T-cell activity) was inhibited; 0.4 to 10 mM Pb treatment enhanced *Listeria*-mediated mortality. However, by another criterion for the assessment of T-lymphocyte activity, the MLC response, T-cell activity was enhanced by in vivo treatment with 0.08 to 0.4 mM and only slightly suppressed by 10 mM Pb (Table 2). Although the 10 mM in vivo dose slightly suppressed the reactivity of the T-cells in the MLC response, no in vivo Pb dose suppressed the mitogenic response of the T-cells to ConA or PHA, selective T-cell mitogens (12). All in vitro doses of $<5 \times 10^{-4}$ M enhanced MLC reactivity. Therefore, Pb suppression of host resistance to *Listeria* cannot be adequately accounted for by a direct inhibitory effect on T-cells. However, T-cells are heterogeneous. At least three subpopulations exist (Ly-1, Ly-2,3, and Ly-1,2,3) (2), and the T-cell responsible for macrophage activation in resistance to *Listeria* may reside in a T-cell subpopulation affected by Pb, whereas the majority of T-cells are unaffected.

The differences in B-cell as well as T-cell activity resulting from in vivo versus in vitro assessment could be due to the involvement of different subpopulations of lymphocytes or to in vivo effects on the development of the subpopulations. Furthermore, the fact that Pb had no effect on in vivo HI but enhanced in vitro HI could be due to Pb effects on accessory cells. Pb has been shown to alter hepatic clearance of foreign matter (33), and the phagocytic Kupffer cells of the liver were shown to be morphologically altered by Pb (14). Pb alteration of antigen in vitro (SRBC or *Listeria*) clearance could

indirectly alter HI and CMI. However, Pb did not appear to significantly alter macrophage capture of antigen (Table 6), but it may affect macrophage processing of antigen (20), which could influence lymphocyte activation as well as the effector phase of activated lymphocytes. Pb reduction of hepatic clearance of endotoxin has been suggested as the cause of Pb enhancement of endotoxin-induced shock (31, 25) and may be the cause of the ability of Pb to increase susceptibility to pyrogenic bacteria (3, 13) as well as to *Listeria*. These indirect effects would not be applicable in in vitro assessment of lymphocyte activities. In vitro assessment should render a more accurate assessment of the direct effects of Pb on B- and T-cell functions. In vitro, Pb only enhanced B- and T-cell activities.

The three different approaches employed in this study have shown that the methods employed to determine the influence Pb may have on the immune system can affect the conclusions. Pb did produce different effects on the immune system, and the use of different experimental systems can be manipulated to answer various questions about the ability of Pb to alter B- and T-cell activities. Evaluation of the Pb effects on the immune system must correlate the in vitro and in vivo Pb effects with the subpopulations of lymphocytes actually being assessed in the assays employed. It is important to note that in vivo exposure to Pb can increase susceptibility to listerial infections; however, in general, the reactivity of T-cells did not appear to be substantially reduced by Pb. On the other hand, with higher doses of Pb (10 mM), in vivo B-cell reactivity was depressed. Therefore, further studies are required to determine whether resistance to *Listeria* was reduced by Pb because of reduction(s) of in vivo B-cell or macrophage activities, since T-cell activities did not appear to be hindered. In vitro, Pb enhanced T- and B-cell activities and did not appear to alter macrophage activities, which suggests that the ability of Pb to increase morbidity and mortality in mice with bacterial infections may be due to mechanisms other than direct inhibition of lymphocyte activation.

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