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Developmental Exposure to Lead Causes Persistent Immunotoxicity in Fischer 344 Rats

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Lead has been shown to exert toxic effects during early development. In these *in vivo* and *ex vivo* experiments, the effect of lead on the immune system of the developing embryo was assessed. Nine-week-old female Fischer 344 rats were exposed to lead acetate (0, 100, 250, and 500 ppm lead) in their drinking water during breeding and pregnancy (exposure was discontinued at parturition). Offspring received no additional lead treatment after birth. Immune function was assessed in female offspring at 13 weeks of age. Dams in lead-exposed groups were not different from controls with respect to the immune endpoints used in these experiments; however, in the offspring, lead modulated important immune parameters at modest exposure levels. Macrophage cytokine and effector function properties (tumor necrosis factor-α and nitric oxide production) were elevated in the 250 ppm group, while cell-mediated immune function was depressed, as shown by a decrease in delayed-type hypersensitivity reactions in the 250 ppm group. Interferon-γ levels were decreased in the 500 ppm treatment group. Serum levels of IgE were increased in rats exposed to 100 ppm lead. These results indicate that exposure of mothers to moderate levels of lead produces chronic immune modulation in their F344 rat offspring exposed *in utero*. Since the mothers were not susceptible to chronic immune alterations, a developmental bias to the immunotoxic effects of lead is indicated. The differences observed are consistent with the possibility that lead may bias T helper subset development and/or function, resulting in alterations in the balance among type 1 and type 2 immune responses.

The constant differentiation of common progenitor cells from conception through embryonic development makes the fetus particularly susceptible to toxicants. Even minor changes to differentiating cells during this period can have permanent implications. Lead has been identified as one substance of particular concern during early development (Goyer, 1993). Levels of lead in blood as low as 10 to 15 μg/dl in infants can result in cognitive and behavioral deficits (Bellinger *et al.*, 1991; Dietrich *et al.*, 1991). All children appear to be at risk due to the prevalence of lead and the narrow margin of safety associated with lead's low effect level (Rosen, 1995).

Lead also has been shown to modulate various functions of the immune system (Dean and Murray, 1991); exposure may either suppress or enhance immune responses, dependent upon the treatment and the specific parameter in question (Zelikoff *et al.*, 1994). Little information is available concerning the immunological consequences of fetal exposure to lead. Rats exposed to lead *in utero* and for 4 subsequent weeks demonstrated changes in some humoral and cell-mediated immune parameters (Luster *et al.*, 1978; Faith *et al.*, 1979); however, these studies examined neither the differences among *in utero* and perinatal exposure nor the persistence of these effects into adulthood. In addition, the relative susceptibility of various age groups for immunotoxic responses has not been extensively examined.

Comparisons of the relative sensitivity and predictability of immune assessment techniques have shown that as few as two or three specific immune parameters can be informative for prediction of immunotoxic effects in rodents (Luster *et al.*, 1992). The present study was designed to assess a variety of immune endpoints in rats exposed to lead during pregnancy or *in utero*, with the goal of describing possible persistent immunotoxic effects (defined for the purposes of this study as those effects which are present in rats which were exposed either *in utero* and have reached adulthood or during pregnancy and no longer harbor a significant body burden of lead) and the relative sensitivity of rats in these two groups.

METHODS AND MATERIALS

Animals. Seven- to eight-week-old Fischer 344 (F344) rats were purchased from Harlan Sprague–Dawley (Indianapolis, IN). Animals were housed three per polycarbonate cage for a 2-week acclimation period (two per cage during mating). The AIN-93G purified rodent diet (Dyets, Inc., Bethlehem, PA) was fed *ad libitum* to all rats. Weekly feed and water intake and body weights were documented during pregnancy and lactation. A 12-h light/dark cycle was maintained during the entire experiment. Temperature and humidity were maintained between 68 and 75°F and 40–60%, respectively. Protocols

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were approved by the Cornell University Institutional Animal Care and Use Committee and complied with NIH guidelines.

Breeding and lead exposure. Following acclimation, 12 females were randomly assigned to each of four treatment groups (0, 100, 250, and 500 ppm lead, as lead acetate, in drinking water) and housed with males for 10 days. Control rats received sodium acetate equivalent in acetate concentration to that of the 500 ppm lead treatment group. Acetic acid (0.00125%) was added to the water to aid in dissolution. The treated water was provided *ad libitum* following acclimation, through mating, and until parturition. At parturition, lead treatment ceased and all rats were switched to normal drinking water. Tapwater was tested for lead contamination and shown to be below laboratory detection limits (<0.001 mg/L). Female offspring were weaned at 21 days, separated by litter, and housed 3 to 4 per cage. At initiation of analyses, dams were 7–8 weeks postpartum, and female offspring were old (a total of 11–14 dams and 12–17 offspring were tested per treatment group); male offspring were not tested in this series of experiments.

Reagents. Monoclonal antibodies for flow cytometry were obtained from Harlan Bioproducts for Science, Inc. (Madison, WI); FITC-, peroxidase-, and alkaline phosphatase-conjugated antibodies were from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Sigma Chemical Company (St. Louis, MO) supplied *Escherichia coli* lipopolysaccharide (LPS), concanavalin A (Con A), Histopaque (1.083 g/ml), *o*-phenylenediamine dihydrochloride (OPD), lactate dehydrogenase (LDH) substrate mixture, bovine serum albumin (BSA), recombinant rat interleukin-2 (rRat IL-2), *p*-nitrophenyl phosphate, and Tween 20. Keyhole limpet hemocyanin (KLH) was purchased from Calbiochem (La Jolla, CA). Monoclonal anti-rat IgE heavy chain and Rat IgE-κ were purchased from Serotec USA (Washington, DC). CTLL-2 target cells were obtained from American Type Culture Collection (Rockville, MD). Rat TNF-α and IFN-γ cytoscreen immunoassay kits were purchased from Biosource International (Camarillo, CA).

Blood collection and lead analysis. Peripheral blood was collected from the neck vein or by cardiac puncture of dams and offspring at various times during and after lead treatment for blood lead determination. Heparin was used as an anticoagulant in all samples. Tibia samples were collected after euthanasia, at the conclusion of experiments. Blood and tibia lead levels were determined by atomic absorption using the platform furnace technique (Pruszkowska *et al.*, 1983). Samples were analyzed with the Perkin–Elmer Zeeman/5000 system and Model AS-40 autosampler at the Cornell Diagnostic Laboratory.

Total leukocyte counts (TLC). Whole blood was diluted 1:20 with acetic acid; leukocytes were counted on a hemacytometer.

Leukocyte subpopulation distribution (flow cytometry). Whole blood (1.5 ml) was transferred to 15-ml siliconized Pyrex tubes. Leukocytes were separated by lysis of erythrocytes and cell surface antigen expression was examined using direct (FITC-conjugated anti-T helper subset, CD4; FITC-conjugated anti-natural killer (NK) cells, NKR-P1 and 3.2.3; FITC-conjugated anti-surface Ig) or indirect (anti-T cytotoxic subset, CD8; anti-macrophage-monocyte, ED1; and anti-Ia antigen, OX-6, with FITC-conjugated anti-mouse IgG, H + L chain) fluorescent staining techniques (Bell *et al.*, 1994). Samples were analyzed with a Coulter EPICS Profile analyzer; a minimum of 10,000 cells per sample was counted.

Natural killer cells. NK cell activity in peripheral blood obtained by cardiac puncture was determined as previously described (Bell *et al.*, 1994). Briefly, leukocytes separated by centrifugation over Histopaque (1.083 g/ml, Sigma) were resuspended in RPMI 1640 medium without phenol red with 1% FBS, loaded into nylon wool columns, and incubated for 1 h at 37°C and 5% CO_2. After incubation, columns were eluted with warm medium. Effector cells were combined with YAC-1 target cells at three E:T dilutions (5:1, 10:1, and 20:1) and incubated for 4 h at 37°C. After incubation, LDH release was assayed and percentage of lysis was determined as described by Korzeniewski and Callewaert (1983).

Antigen-specific antibody ELISA. Concentrations of IgM and IgG antibodies against KLH antigen were measured using a noncompetitive solid-phase enzyme linked immunosorbent assay (ELISA) similar to the procedure described by Exon and Talcott (1995). Briefly, KLH was bound to a 96-well EIA plate. Plasma samples from rats were serially diluted and added to the plate and incubated for 1 h at 37°C. Peroxidase-conjugated mouse anti-rat IgM or IgG antibodies were added next and incubated for 1 additional h at 37°C. The chromogen OPD was added last, and the absorbance was read at 450 nm. Relative absorbencies of plasma samples were compared with those of positive and negative (pooled) controls.

IgE levels. Total IgE in serum was determined by ELISA using modifications of protocols from Kiely *et al.* (1995) and Negrao-Correa *et al.* (1996). Briefly, EIA plates were coated with monoclonal anti-rat IgE heavy chain and washed with PBS containing Tween 20 and blocked with 0.5% BSA for 30 min at 37°C; samples were added and incubated for 2 h at 37°C (Kiely *et al.*, 1995). After washing, alkaline phosphatase-conjugated purified Fab-fragment mouse anti-rat IgG was added for 1.5 h at 37°C, followed by *p*-nitrophenyl phosphate in diethanolamine buffer for 1 h (Negrao-Correa *et al.*, 1996). Absorbance was read at 405 nm. Results (ng/ml) were calculated from a standard rat IgE-κ curve.

Spleen cell preparation. Spleens were removed on day 15 after KLH administration; single-cell suspensions were prepared using 400 μm sterile nylon mesh. Erythrocytes were lysed with a buffer containing 0.15 M NH_4Cl, 1.0 mM $KHCO_3$, and 0.1 mM EDTA (pH 7.2). Splenocytes were washed twice with HBSS and plated in 24-well tissue culture-treated plates (3×10^6 cells/well). For some experiments, 6×10^6 cells were incubated at 37°C and 5% CO_2 for 2 h to isolate adherent cells. When adherent cell monolayers were stimulated to produce metabolites, duplicate unstimulated wells were analyzed for protein content to ensure similar adherent cell numbers, using the bicinchoninic acid (BCA) binding method (Pierce Biochemical, Rockford, IL).

Tumor necrosis factor-α (TNF-α). Adherent spleen cells in RPMI 1640 medium containing 2% FBS were stimulated with 10 ng/ml LPS during a 16-h incubation. Supernatants were frozen at −70°C until TNF-α was analyzed by ELISA.

Nitric oxide (NO). Adherent spleen cells in RPMI 1640 medium with 2% FBS were stimulated with 0, 1, 10, or 100 ng/ml LPS during a 24-h incubation. NO production was evaluated by measuring the accumulation of the more stable end product, nitrite, by the Griess reaction (Green *et al.*, 1982).

Superoxide (SO). Superoxide anion was measured by a modification of the procedure described by Golemboski *et al.* (1990). Briefly, adherent spleen cells were exposed to 0 or 10 ng/ml LPS during a 24-h incubation. Monolayers were then incubated with 2 μg/ml PMA and 80 μM ferricytochrome C, with or without 133 U/ml superoxide dismutase. After 1 h of incubation at 37°C and 5% CO_2, absorbances were read at 550 nm with a slitwidth of 1 nm on a microplate reader (Biotek EL312).

Interferon-γ (IFN-γ). Unseparated splenocytes were incubated in RPMI 1640 medium with 5% FBS and 0 or 5 μg/ml Con A for 72 h. Supernatants were frozen at −70°C until analyzed for IFN-γ by ELISA.

Interleukin-2. Unseparated splenocytes were incubated for 24 h with 5 μg/ml of Con A, and the supernatants frozen for later testing of IL-2 activity. Each cell-free supernatant was serially diluted in a 96-well flat-bottom microtiter plate. The CTLL-2 target cells (5×10^3 cells in 100 μl) were added to the diluted supernatants and incubated for 24 h in 5% CO_2 at 37°C (Gillis *et al.*, 1978). Proliferation was measured using MTT reduction (Mosmann, 1983). Standard curves were created with known units of rRat IL-2.

Delayed-type hypersensitivity (DTH). Injections of KLH were conducted as described by Exon *et al.* (1990). Briefly, 200 μl of 5 mg/ml KLH in sterile, deionized water was injected into the caudal tail fold on days 1 and 8. Control rats received 200 μl sterile water. On day 14, 100 μl of 20 mg/ml heat-aggregated (80°C for 1 h) KLH in saline was injected into the right footpad and 100 μl of sterile saline was injected into the left footpad. DTH reactions were measured 24 h after the heat-aggregated KLH injections using spring-loaded calipers (Dyer, Model 304). Results were reported as the percent difference between the right footpad (KLH) and the left footpad (sterile saline).

TABLE 1
Blood Lead Values in Dams at Various Time Points

Time point	Exposure	*n*	Mean	SE mean
Pregnancy	Control	5	0.0	0.0
	100 ppm	3	39.4	6.7
	250 ppm	12	70.8[a]	8.2
	500 ppm	7	112.0[a]	19.9
Lactation	Control	5	4.8	1.8
	100 ppm	2	30.2	1.1
	250 ppm	12	74.1[b]	11.4
	500 ppm	16	86.5[b]	12.6
Euthanization	Control	8	0.9	0.6
	100 ppm	8	10.1	3.6
	250 ppm	8	13.0	4.1
	500 ppm	8	12.5	4.4

[a,b] $p < 0.05$ when compared to controls.

TABLE 2
Tibia Lead (μg/g) Accumulation at Euthanasia

	Treatment group	*n*	Mean	SE mean
Offspring	Control	4	2.44	0.55
	100 ppm	4	6.30	1.32
	250 ppm	8	7.71[a]	0.30
	500 ppm	8	10 93[a]	1.77
Dams	Control	2	1.3	0.1
	100 ppm	2	222.0	70.0
	250 ppm	6	491.3[b]	45.2
	500 ppm	6	564.7[b]	46.0

[a,b] $p < 0.05$ when compared to controls.

Statistical analyses. One-way analysis of variance (ANOVA) tested for differences among the four treatment groups in this study. Post ANOVA (multiple comparison) tests determined which groups were statistically different when the overall ANOVA showed differences among the groups. Two different multiple comparison tests were employed. Fisher's LSD provided confidence intervals for all pairwise differences between treatment means, and Dunnett's test provided a confidence interval for the difference between each treatment mean and the associated control mean (Minitab Statistical Software, Minitab Inc., State College, PA). A p value of <0.05 was used to determine statistical significance.

RESULTS

Blood lead levels (BLL). During pregnancy and lactation, dam BLL in the 250 and 500 ppm treatment groups differed significantly from controls for the same time period ($p < 0.01$) (Table 1). Samples collected from the 100 ppm group during pregnancy and lactation did not differ from controls. No significant differences in BLL were noted among groups at the time of euthanization. Offspring blood lead levels at the time of euthanization (9–10 weeks post exposure) were low and variable and were not statistically different (range 0.68–2.63 μg/dl; data not shown).

Tibia lead accumulation. Offspring (13–14 weeks old) and dams (8–9 weeks postpartum) showed dose-dependent increases in bone lead accumulation across groups, as seen in Table 2. In both dams and offspring, bone lead levels in the 250 and 500 ppm groups were significantly ($p < 0.05$) higher than controls. Accumulation in dams was much greater than that in offspring.

Growth rates. Growth rates were determined by weighing offspring and dams at various time points and dividing by the number of days for the growth period. Offspring weights were started on the day of weaning and completed at the time of euthanasia. No differences were noted for offspring or dams (data not shown).

Total leukocyte counts. Offspring TLCs were decreased significantly ($p < 0.02$) in the 250 and 500 ppm lead groups when compared with controls (Fig. 1). Dam TLCs in the lead-exposed groups were not different from those of controls.

Leukocyte subpopulation distribution. Flow cytometry data was assessed by analyzing both percentage of positive cells and mean fluorescence for each monoclonal antibody. No significant differences were noted in either category, in data from offspring or from dams. Control values for dams were as follows: CD4, 53.6%+; CD8, 31.8%+; NK, 3.6%+; macrophage/monocyte, 1.6%+; IA, 9.8%+; SIg, 12.4%+. Control values for offspring were: CD4, 48.6%+; CD8, 38.0%+; NK, 4.2%+; macrophage/monocyte, 2.9%+; IA, 17.3%+; SIg, 30.2%+.

Natural killer cells. No lead treatment related effects were noted with respect to NK cell mediated lysis; however, interexperiment variability was significant for this bioassay (data not shown).

Antigen-specific ELISA. IgG and IgM antibody titers to the KLH antigen were compared by lead treatment group. Figure 2 shows IgG titers for offspring showing an upward trend in IgG production with increased lead dose. These results are not statistically significant. αKLH IgM titers were highly variable and did not differ significantly among groups (data not shown).

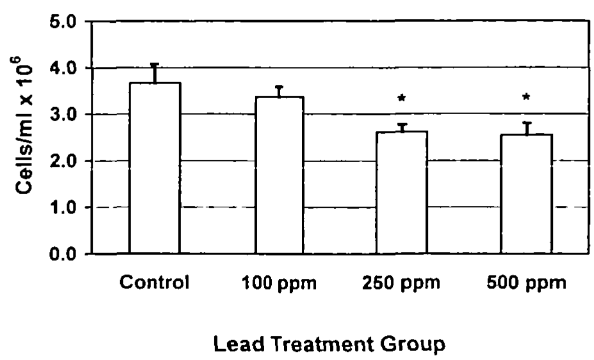

FIG. 1. Offspring total blood leukocyte counts were counted with a hemacytometer. Data were analyzed by ANOVA ($p < 0.02$). *Significant difference when compared with controls.

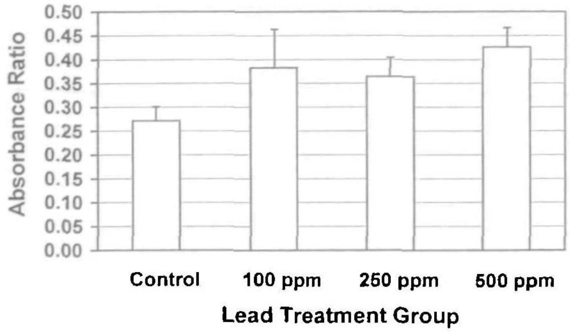

FIG. 2. KLH-specific IgG titers were measured in offspring by ELISA. Results are expressed as relative absorbance of plasma samples compared to both positive and negative controls.

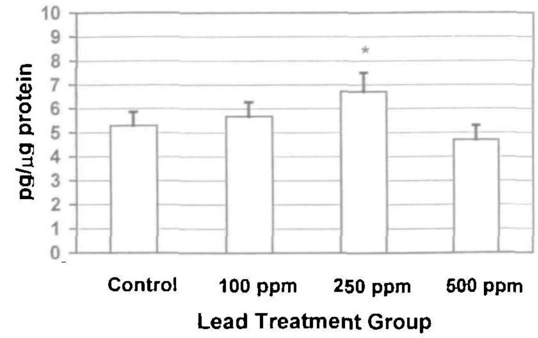

FIG. 4. Offspring tumor necrosis factor-α (TNF-α) was measured by ELISA in LPS-stimulated splenocyte supernatants. Data were analyzed by ANOVA ($p < 0.05$). *Significant difference when compared with controls.

IgE levels. Serum IgE levels were increased ($p < 0.05$) in samples from offspring of the 100 ppm rats compared with those from control rats (Fig. 3). IgE levels in samples from offspring of the 250 ppm group were not significantly different from control samples. Samples from offspring of the 500 ppm group were not evaluated for IgE levels, nor were those from dams.

Tumor necrosis factor-α. TNF-α production by 16-h adherent splenocyte cultures (with 0.01 μg/ml LPS) was elevated ($p < 0.05$) in offspring of the 250 ppm treatment group compared with that from control offspring splenocytes (Fig. 4). Dams exhibited consistent TNF-α production among treatment groups (6.73 pg/μg protein for control rats vs 6.18, 5.90, and 6.54 in 100, 250 and 500 ppm lead groups, respectively).

Nitric oxide. Nitric oxide production by macrophages from pups increased with lead treatment up to 250 ppm (Fig. 5). Levels in the 250 ppm group were significantly ($p < 0.05$) increased compared to controls; however, NO production returned to control levels in the 500 ppm group. Nitrite levels in splenocyte cultures from dams averaged 58.65 μM/mg protein in control animals; no differences were noted among the four lead treatment groups (data not shown). Protein levels in adherent monolayers used for both NO and SO production did not differ among treatment groups (data not shown).

Superoxide anion. No differences in SO anion production were noted among the treatment groups, in cells from offspring or from dams (data not shown).

Interferon-γ. IFN-γ measured in 72 h Con A-stimulated culture supernatants from offspring was significantly lower in the 500 ppm group when compared with supernatants from either controls or 100 ppm lead group ($p < 0.05$) (Fig. 6). IFN-γ production from dam splenocyte cultures (mean 1074.6 pg/ml from controls) did not differ among the treatment groups (data not shown).

Interleukin-2. IL-2 production by Con A-stimulated splenocytes, as assayed by proliferation of CTLL-2 cells followed by MTT reduction, did not differ among treatment groups, either for dams (controls, 34.7 $\pm$ 6 U/ml, overall mean 43.0) or for offspring (controls, 7.1 $\pm$ 2 U/ml, overall mean 5.1).

Delayed-type hypersensitivity. In the female offspring, the reaction to KLH in the 250 ppm lead-exposed group was significantly decreased when compared with controls ($p < 0.05$) (Fig. 7). Dams did not differ significantly in DTH reactions among the four treatment groups (control dams had a 33.6% difference between footpads, while the 100, 250, and 500 ppm groups differed by 45.9, 49.4, and 41.6%, respectively).

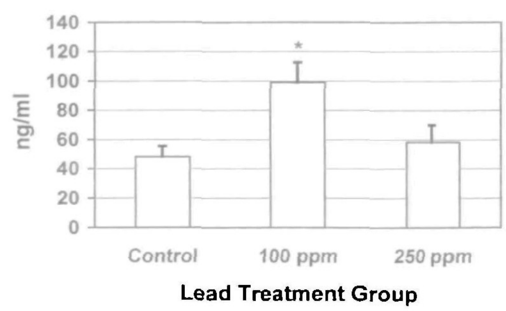

FIG. 3. Offspring serum IgE was determined by ELISA. Results were analyzed by ANOVA ($p < 0.05$). Samples from the 500 ppm treatment group were not tested. *Significant difference when compared with controls.

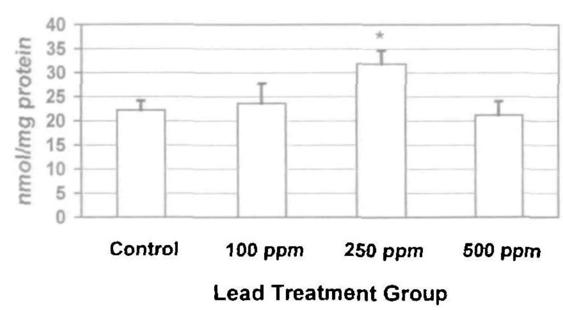

FIG. 5. Offspring macrophage nitrite accumulation was measured in adherent splenocyte cultures stimulated with LPS for 18 h. Results were analyzed by ANOVA ($p < 0.05$). *Significant difference when compared with controls.

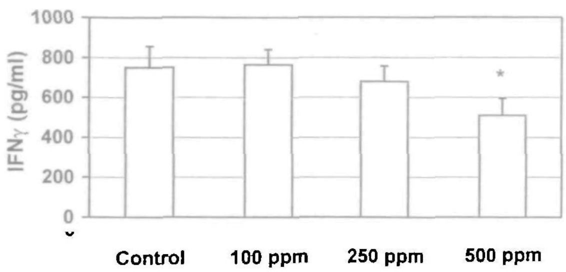

FIG. 6. Offspring interferon-γ (IFN-γ) production was measured in unseparated splenocyte cultures stimulated with Con A for 72 h. Results were analyzed by ANOVA ($p < 0.05$). *Significant difference when compared with controls.

DISCUSSION

The immunotoxic effects of lead have been examined in some detail (Kerkvliet and Baecher-Steppan, 1982; Exon *et al.*, 1985; McCabe and Lawrence, 1991), but few studies have included developmental status as a variable (Luster *et al.*, 1978; Faith *et al.*, 1979). Therefore, the potential for immunotoxicity during development, as well as the relative risks to the mother and fetus, required further examination.

The present study demonstrated that *in utero* lead exposure in rats can modulate the immune system of the offspring and, additionally, provided a direct comparison of relative lead immunotoxicity risk to developing offspring vs pregnant dams. Since the offspring were examined as adults, the altered function in the progeny of lead-treated dams reflects persistent effects on the immune system. After a similar time interval, no differences were noted in maternal immune function, suggesting that any effects of the same exposure in adults were transient. In a study which examined acute effects of lead exposure using adult mice, animals with blood lead levels similar to maternal levels in this study (38 μg/dl) exhibited increases in Th2-dependent immune functions, including IgE and IL-4 production (Heo *et al.*, 1996).

It is important to note that no alterations in offspring growth rate were observed. This is consistent with the observations of Hammond *et al.* (1993) that lead-induced growth alterations were usually transitory and suggests that chronic immune alterations can result from *in utero* lead exposures which do not affect overall growth.

Lead exposures were restricted to the period of *in utero* development, but lead remobilized from maternal bone is transported in rat milk (Palminger and Oskarsson, 1995) and could have contributed to some postpartum exposure during lactation. However, recent studies using the same F344 *in utero* exposure system detected insufficient lead concentrations in maternal milk to produce significant oral exposure to the nursing pup (Chen *et al.*, unpublished observations). Therefore, the observed immune alterations in the present study can be attributed to embryonic exposure.

Blood lead concentrations in dams during pregnancy were relatively high, ranging from 39.4 ± 6.7 to 112 ± 19.9 μg/dl. These levels are higher than those reported to affect behavioral function in young children (15–25 μg/dl) (Goyer, 1993). Offspring blood and bone lead levels at the time of immune assessment were remarkably low, suggesting that significant lead storage need not occur in the embryo for the induction of persistent immune alterations. Based on these results, if there are key periods of early immune development which are particularly susceptible to persistent lead-induced alterations, it is possible that even short-term exposure of pregnant females, in rodents or in other species including humans, could compromise offspring immune function.

The specific immune alterations observed in the present study support the hypothesis presented by other investigators that lead is capable of altering the balance of T helper cell (Th1 vs Th2) activity (McCabe and Lawrence, 1991; Heo *et al.*, 1996). Because Th1 vs Th2 activities promote different immune functions resulting in different host defense processes (Kuchroo *et al.*, 1995; Surcel *et al.*, 1994; Kawakami and Parker, 1992; Clerici *et al.*, 1993), significant shifts in the balance of these functions could alter disease resistance of the host. In studies using adult mice *in/ex vivo* and murine *in vitro* models, Lawrence and colleagues found lead chloride-induced elevation of IL-4 and IgE levels with a significant reduction of IFN-γ levels in exposed Balb/c mice (McCabe and Lawrence, 1991; Heo *et al.*, 1996).

In the present study, antigen-specific cell mediated immune function (delayed type hypersensitivity) associated with a Th1 response was depressed in the 250 ppm treatment group. In contrast, IgE production, a Th2-dependent response (Mosmann and Coffman, 1989) was significantly elevated in the 100 ppm lead-exposed group (specific IgG production to KLH was unaltered). The findings suggest that embryonic exposure to lead decreased Th1 function while elevating some Th2-dependent activities. These results are consistent with several prior neonatal and adult exposure studies in which antibody response was either unaltered or elevated in contrast to lead-induced depression of cell-mediated immunity (Lawrence, 1981; Kim-

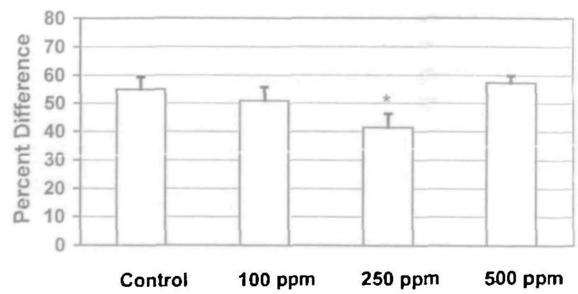

FIG. 7. Delayed-type hypersensitivity was measured in offspring as percentage of difference between control- and KLH-injected footpads. Results were analyzed by ANOVA ($p < 0.05$). *Significant difference when compared with controls.

ber *et al.*, 1986; McCabe and Lawrence, 1990; Redig *et al.*, 1991; Horiguchi *et al.*, 1992).

Modulations of various immune endpoints were observed at different lead exposure concentrations in these experiments. A number of parameters (TNF-α, NO, IgE, and DTH) were altered in one of the lower treatment groups (100 or 250 ppm) and then returned to control levels at a higher dose (250 or 500 ppm). Similar results were described in Heo *et al.* (1996) where IL-4 production *in vitro* was increased by exposure to 25 μM Pb but not by 50 μM Pb treatment. These data suggest the possibility that embryonic lymphocyte populations may differ in sensitivity to lead-induced modulation.

A potential neonatal bias to Th2 vs Th1 function has implications for subsequent host resistance to disease. For example, Th2 hyperresponsiveness can increase the susceptibility of the host to infectious challenges and could predispose the host to allergic atopic manifestations (Romagnani, 1995) and autoimmunity (Pelletier *et al.*, 1994). Increased Th2-type responses with concomitant suppression of IL-2 production has been reported to cause severe immune dysfunction in NMRI mice (Faxvaag *et al.*, 1995).

Shirakawa *et al.* (1997) have shown an inverse correlation between Th2-mediated atopy (asthma, rhinitis, eczema) and childhood conversion to positive tuberculin status. They suggest that the presence of a strong Th1-inducing stimulus, such as *Mycobacterium tuberculosis* exposure, could inhibit Th2 cytokines to an extent sufficient to decrease subsequent atopy. Applying the same hypothesis to the developing rat immune system, it is conceivable that a stimulus such as lead, which may increase Th2 cytokine levels could influence fetal immune development to persistently suppress Th1 responses.

In the present study, adult offspring exposed to lead *in utero* also exhibited altered macrophage function (nitric oxide production). While such changes could be associated with the shifts in T cell functional capacities and concomitant changes in cytokine production (e.g., IFN-γ production), other studies have reported direct lead-induced changes in macrophage function *in vitro* (Tian and Lawrence, 1996; Chen *et al.*, 1997). However, the *in utero* exposure at 250 ppm resulted in enhanced TNF-α and NO production by rat splenocytes, in contrast with *in vitro* studies which suggest that lead can directly suppress NO production. The elevated TNF-α production seen in the present study is consistent with the observation of Guo *et al.* (1996) that exposure to lead chloride elevates TNF-α production by LPS-stimulated human peripheral blood monocytes. Since TNF-α is an autocrine factor for macrophage activation (Witsell and Schook, 1992), this could explain some of the effects of lead on NO production. TNF-α also plays a role in airway hypersensitivity and bronchoalveolar inflammatory cell accumulation (Renzetti *et al.*, 1996). Based on these results, if lead induces increases in Th2 cytokine levels, these compounds could act in concert with lead-induced elevations in TNF-α to increase airway hypersensitivity.

Recent increases in both the prevalence and the severity of childhood asthma have prompted a search for potential environmental risk factors. Lead-induced Th1-Th2 immunomodulation (McCabe and Lawrence, 1991; Heo *et al.*, 1996, 1997), the known role of other heavy metals in facilitating autoimmune reactions (Kiely *et al.*, 1995), the relationship among elevated IgE levels and airway reactivity (Sears *et al.*, 1991) and the present embryonic exposure results implicate lead exposure as a possible risk factor for the development of Th2-dependent chronic diseases. Exposure to lead concentrations which produce no (or only transient) maternal immunomodulation may increase the propensity for allergic disease and/or autoimmunity in the exposed fetus through persistent shifts in Th1 vs Th2 activity.

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