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Decreased T cell function in mice exposed to chronic, low levels of lead

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SUMMARY

The immune responsiveness of mice given low levels of lead acetate (1300 p.p.m.) in their drinking water for 8 weeks was determined. Mean percentages of splenic T and B lymphocytes in lead-exposed mice were slightly lower than in controls. Mean mitogen response to phytohaemagglutinin and concanavalin A in mice exposed to lead was significantly decreased compared to controls. The response to lipopolysaccharide on the other hand was not significantly different in the two groups. These data suggest that T lymphocyte function as measured by mitogenic response to phytohaemagglutinin and concanavalin A is impaired in mice exposed chronically to low concentrations of lead.

INTRODUCTION

Chronic exposure to low levels of lead is a potential environmental hazard (Chisolm, 1971). Among the adverse effects reported in animal models are increased susceptibility to infection (Hemphill, Kaeberle & Buck, 1971), carcinogenic effects (Van Esch & Kroes, 1969; Kobayashi & Okamoto, 1974) and the possibility of immunosuppression. Several reports (Koller & Kovacic, 1974; Koller, Exon & Roan, 1976) have suggested that humoral immunity is impaired. Muller *et al.* (1976) have reported that delayed hypersensitivity to sheep red blood cells is diminished in mice chronically exposed to low levels of lead. In the present study we quantitated splenic B and T lymphocytes and evaluated splenic blastogenic responses to both B cell and T cell mitogens in mice exposed to low levels of lead for 8 weeks.

MATERIALS AND METHODS

Forty-eight C57Bl/6 male mice, 4 to 10 weeks old, were studied. Twenty-four mice were given lead acetate (1300 p.p.m.) in their drinking water for 8 weeks while twenty-four controls received deionized lead-free water. At the end of the 8-week period, splenic T and B cells were counted and their response to phytohaemagglutinin (PHA), concanavalin A (Con A), and lipopolysaccharide (LPS) determined. A blood sample was obtained also from each animal for blood lead analysis.

Spleen cell suspensions and response to mitogens. Mice were killed with ether and spleens immediately removed aseptically. Spleen cell suspensions were made (Handwerger & Schwartz, 1974) and treated for 2 minutes with Tris–0·85% ammonium chloride to lyse red blood cells (Boyle, 1968) and washed with medium. The cells (5×10^5) were then cultured in Eagle's MEM containing 10% foetal calf serum, penicillin, streptomycin, glutamine, non-essential amino acids and sodium pyruvate in quadruplicate wells of 3040 microtest II culture plates (Falcon Plastics). Several concentrations of each mitogen [PHA (HA17, Burroughs–Wellcome Company) 0·3, 1·0 and 3·0 μg/ml; Con A (Sigma Chemical Company) 0·3, 1·0 and 3·0 μg/ml; and LPS (*E. coli* 055:B5, Difco Laboratories) 30, 100 and 300 μg/ml] were run in every experiment. Only the results with the optimal mitogen concentration are reported; this was usually 1 μg/ml PHA, 1 μg/ml Con A and 100 μg/ml LPS for lead

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exposed and control mice. After 48 hr of incubation at 37°C in 5% CO_2, 95% humidified air atmosphere, 0·5 μCi of [methyl-^{3}H]thymidine (NET-027 New England Nuclear) were added to each culture. After 16 hr of additional incubation, the plates were harvested on filter papers, using a microharvester (Bellco Glass Incorporation) placed in scintillation fluid and counted in a β-scintillation counter (Packard). The results are expressed as the mean of quadruplicate cultures with mitogen minus mean of quadruplicate cultures without mitogen.

Antisera. Rabbit anti-mouse brain (RAMB) serum was produced in a New Zealand female rabbit by subcutaneous injection of an emulsion of mouse brain in Freund's complete adjuvant (Golub, 1971). The serum was then heat-inactivated for 45 min at 56°C and absorbed exhaustively with PU-5 a murine B lymphoblastoid cell line (Zuckerman, 1977). The T cell specificity of RAMB serum was tested by microtitre, dye exclusion, complement-dependent cytotoxicity and indirect immunofluorescence. The RAMB serum killed and/or stained 100% of BALB/c thymocytes, 25–35% of BALB/c unseparated spleen cells, 76–78% of nylon wool non-adherent spleen cells and 3–7% of nylon wool adherent spleen cells.

Quantitation of splenic B and T cells. Spleen cells (1×10^6) were washed with 2% bovine serum albumin in phosphate-buffered saline and 0·02% sodium azide and incubated with RAMB as described previously (Handwerger & Schwartz, 1974). Goat anti-rabbit IgG was obtained from Cappel Laboratory. For identification of B cells, goat F(ab′)$_2$ anti-mouse IgG (Cappel Laboratory) was added to spleen cells (1×10^6) and incubated for 30 min at 4°C (Handwerger & Schwartz 1974).

Blood lead level determination. Blood samples, in 3-ml glass reaction vessels, were wet-ashed using perchloric and nitric acids. Lead was then extracted from the ashed blood samples and a series of standards using dithizone reagent and buffer (Jones & Szutka, 1966). Extracts were analysed for lead using a Varian AA175B atomic absorption spectrophotometer equipped with graphite furnace.

Statistical analysis. Student's unpaired *t*-test was used to analyse the statistical significance of the results.

TABLE 1. Percentage of splenic T and B cells in mice exposed to lead acetate for 8 weeks

Group	Weight (g)	Blood lead level (μg/100 ml)	Per cent T cells*	Per cent B cells†
Controls (n = 22)	31·8±0·3	17·5±0·7	27·8±0·2	52·8±0·6
Lead-exposed (n = 24)	31·7±0·2	69·9±1·5	25·2±0·2	49·6±0·6
P value		< 0·001	< 0·001	< 0·001

All values are mean±s.e.m.

* Cells staining by indirect immunofluorescence with rabbit anti-mouse brain serum.

† Cells staining by direct fluorescence with fluorescein-conjugated goat F(ab′)$_2$ anti-mouse IgG.

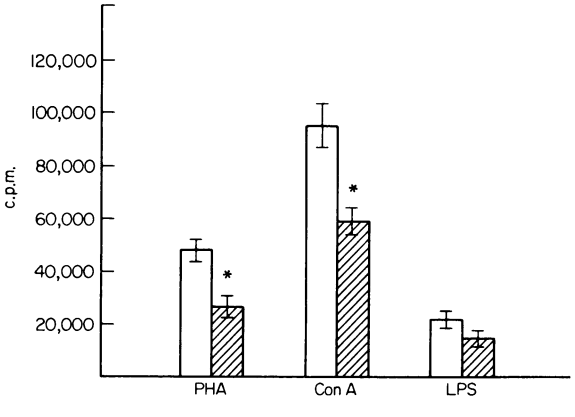

FIG. 1. Mean ^{3}H-thymidine incorporation in response to Con A, PHA and LPS in lead-exposed (▨) and control (□) mice. n = 24 in each group, brackets = s.e.m. * = P< 0·001.

RESULTS

Administration of 1300 p.p.m. lead acetate in the drinking water resulted in a significantly higher blood lead level but did not affect body weight gain of the mice (Table 1). A minimal, but statistically significant decrease in percentages of splenic T and B cells was observed in mice exposed to lead (Table 1).

In the lead-exposed mice mean response to PHA (27,007±3652 c.p.m.) was significantly lower than in controls (47,503±4318 c.p.m.) (Fig. 1). The mean response to Con A was also significantly decreased in the mice exposed to lead (59,021±5066 c.p.m.) as compared to controls (95,348±8423 c.p.m.) (Fig. 1). The mean mitogenic response to LPS in the lead-treated group (15,317±2911 c.p.m.) was slightly lower than mean response of controls (21,456±3205 c.p.m.), but this difference was not statistically significant (Fig. 1).

DISCUSSION

Low level lead exposure in animal models is associated with abnormal immune responses. Alteration of humoral immune responses appears to be dependent on the duration of exposure and route of administration. In mice given lead orally for 8 weeks both *in vivo* 19S and 7S plaque-forming cells are decreased (Koller & Kovacic, 1974). However, when the lead is given as a single oral or intraperitoneal dose, 19S plaque-forming cells are increased, while 7S plaque-forming cells are still decreased. The decrease in 7S plaque-forming cells was greater in the mice injected intraperitoneally (Koller *et al.*, 1976). Cell-mediated immunity may also be affected since delayed hypersensitivity as measured by footpad swelling to sheep red blood cells is decreased in mice given lead intraperitoneally (Muller *et al.*, 1976).

Most studies investigating the effect of lead on immune responses have been done in experimental animals. One report, however, has evaluated humoral immunity in children with low lead exposure (Reigart & Graber, 1976). No differences in complement or immunoglobulin levels, or in the anamnestic response to tetanus toxoid were found.

In our study the decreased number of splenic B and T cells in lead-exposed mice is modest and probably not biologically significant. The lower number of splenic B lymphocytes is consistent with a previous report of slightly decreased splenic B cells in mice given lead orally for 10 weeks (Koller & Brauner, 1977). The markedly diminished mitogenic response of lead-exposed mice to PHA and Con A but not to LPS, suggests that T lymphocyte function is impaired primarily.

These altered immune responses could be important to the host's ability to resist certain diseases. Mice exposed to low concentrations of lead have an increased susceptibility to bacterial infection (Hemphill *et al.*, 1971). A variety of malignancies have been observed in experimental animals exposed to lead (Van Esch & Kroes, 1969; Kobayashi & Okamoto, 1974). In addition, the increased incidence of cancer in certain industries suggests that lead may be co-carcinogenic (Cooper, 1976).

Our data suggest that T lymphocyte function in mice exposed chronically to low levels of lead is impaired. Further investigation of cell-mediated immune responses including assays of T cell-mediated cytotoxicity are indicated.

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