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Immune responses of rats chronically fed subclinical doses of lead

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SUMMARY

The effects of chronic feeding of low levels of lead on various cells of the rat immune system have been investigated. In the presence of Con A and PHA, lead appears to increase blastogenesis up to approximately 5 weeks with activity approaching that of the unleaded control cells at 14 weeks. B cells responded with increased mitogenicity in the presence of LPS up to the time of termination of the experiment (14 weeks). Macrophage activity and number in the lead-fed rat showed consistent decrease and eventual morphological impairment. It is hypothesized that lead has mitogen-like activity but disruption of macrophage–T cell interaction occurs with time.

INTRODUCTION

Lead is a ubiquitous heavy metal. It is found naturally occurring at very low levels in food, water, air and soil. As a result of centuries of man's mining and smelting activities one finds much higher levels of environmental lead (over 100 times higher) than in prehistoric times. Poisoning from lead has increased significantly with the advent of the automobile and the incorporation of lead pigments into paint (Settle & Patterson, 1980). Effects of lead poisoning are most noticeable clinically in man's haemopoietic, nervous and excretory systems (EPA report, 1977). Anaemia caused by plumbism is the result of both increased erythrocyte destruction and decreased haemoglobin synthesis. Fatal encephalopathy is associated with high lead exposure (Greengard, Adams & Birman, 1965) while low levels of lead have been implicated in behavioural changes and disruption of cognitive functions (Reiter *et al.*, 1975). Excessive lead exposure can result in acute as well as chronic renal tissue injury eventually resulting in death from renal failure in man (Cramer *et al.*, 1974).

The effect of lead on the immune system appears to be dose-dependent. Selye, Tuchweber & Bertok (1966) reported that a single large dose of lead (1 mg/100 g body weight) increased rat susceptibility to *E. coli* endotoxin 100,000 times above controls. Low concentrations of lead (0·348 mg/dl) in mice have been shown to reduce resistance to bacterial infections (Hemphill, Kaeberle & Buck, 1971). Williams, Caraway & de Young (1964) reported that lead binds to antibodies *in vitro* causing inactivation of the antibodies. However, low levels of lead have been reported to enhance antibody synthesis (Koller, Exon & Roan, 1976). Electron micrographs of lung tissue after rats had inhaled particulate lead oxide (200 $\mu g/m^3$) for 14 days showed ultrastructural damage to alveolar macrophages (Bingham *et al.*, 1968).

Since varying concentrations of lead *in vitro* can increase immune activity (Lawrence, 1980), several studies have been concerned with either lymphocyte or macrophage responses to various

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levels of lead for short periods of time. This study attempts to correlate changes in the integration of immune functions with exposure of rats to subclinical levels of dietary lead over a 14-week period.

MATERIALS AND METHODS

Animals. Two groups of male Sprague–Dawley rats (Camm, Wayne, New Jersey), lead-fed and control, were monitored with 12 animals in each group. Initial animal weights were approximately 150 g. The lead-fed rats were given 1% lead acetate (Fischer Scientific, Springfield, NewJersey) w/w mixed into Purina rat chow and both groups were given tap water and food *ad libitum*. Food, water consumption and body weight were measured on a weekly basis. The blood lead levels of each group were measured using atomic absorption spectrophotometry (Joselow & Bogden, 1972).

Tissues used. Individual spleens from dead rats were asceptically removed and immediately placed in sterile 60 × 15 mm petri dishes (Falcon, Oxnard, California) containing 5 ml of MEM–Earles base supplemented with 5% bovine serum, 1% L-glutamine (200 mg), 1% penicillin–streptomycin solution containing 10,000 μg/ml streptomycin and 10,000 units/ml penicillin and 3% of a 7·5% sodium bicarbonate solution (GIBCO, Grand Island, New York). The spleens were gently pressed through a sterile stainless-steel mesh screen, 100 wires/inch (541-100 R, Cistron Corp., Elmsford, New York). Cell suspensions were transferred to 12 × 75 mm tubes and centrifuged at 1,500 r.p.m. for 10 min in a Sorvall centrifuge. The pellet was resuspended in fresh media, centrifuged and washed a third time. Isolated spleen cells (500,000/0·1 ml of supplemented MEM) were placed into individual wells of flat-bottomed 96-well tissue culture plates (Microtest II, Falcon Plastics. Oxnard, California).

Mitogen preparations. Con A (Calbiochem, California), PHA-P (GIBCO) and LPS B (Difco, Detroit, Michigan) were diluted to appropriate final concentrations in supplemented MEM and added to culture wells in 0·1-ml volumes. All mitogen experiments were performed by the method of Thurman & Goldstein (1975).

Culture conditions. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 24 hr after which they were pulsed with 0·5 μCi methyl-^{3}H-thymidine (sp. act. 6·7 Ci/mmol; New England Nuclear Corp., Boston, Massachusetts) in a volume of 50 μl supplemented MEM and incubated for an additional 24 hr. Cells were harvested on a MASH II automated harvester (Microbiological Associates, Bethesda, Maryland). The glass-fibre filter paper discs were added to Liquifluor scintillation fluid (New England Nuclear) and counted in a Beckman liquid scintillation spectrometer.

Macrophage culture preparation. Individual spleen cell suspensions were adjusted to a concentration of 5 × 10^6 cells/4 ml supplemented MEM and 4-ml volumes were added to 60 × 15 mm plastic tissue culture dishes (Falcon). The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 1 hr. The supernatant was removed and the culture dish was washed three times with warmed (37°C) supplemented media. Cultures were divided into three groups: supplemented control cultures with no additions, and cultures with SRBC (concentration 0·5 × 10^6 cells/4 ml supplemented media) (Colorado Serum Company Laboratories, Denver, Colorado) or with latex beads (0·81 μm) (DIFCO) (0·5 × 10^6 beads/4 ml supplemented media). These plates were then incubated for 1 hr, washed three times in supplemented MEM, reincubated for 15 min at 37°C and examined using a Zeiss compound light microscope at a magnification of × 450.

Experimental design. (1) Rats on the lead diet and control groups were examined for immune responses at intervals of 2, 5 and 14 weeks. (2) The spleens of control and lead-fed rats were removed within 1 hr of each other and cultured under equivalent conditions using the same mitogens and phagocytic indicators. Cells used for macrophage culture were from the same pool used for mitogen studies at all times. (3) A minimum of six animals were used for each time interval (three control, three lead-fed) and triplicate studies were performed on cells from each animal.

RESULTS

Both control and lead-fed animals consumed equivalent amounts of food (approx. 28 g/day) over

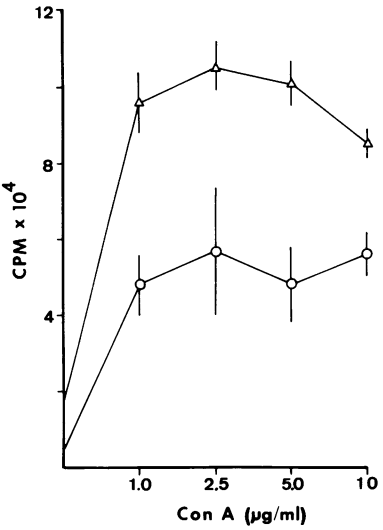

Fig.1

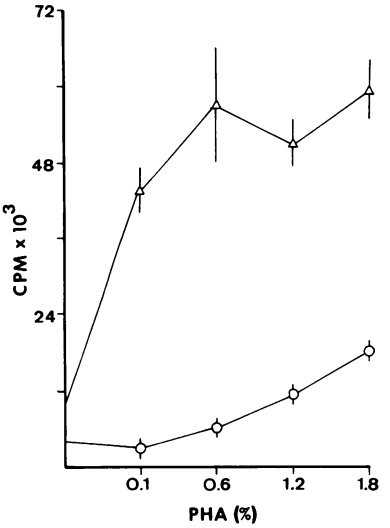

Fig. 2

Fig. 2. Uptake of ^{3}H-thymidine (mean ±s.d.) in response to PHA stimulation of 5 × 10^6 spleen cells from rats fed lead acetate for 2 weeks (▵——▵) as compared to controls (○——○).

Fig. 2. Uptake of ^{3}H-thymidine (mean ±s.d.) in response to PHA stimulation of 5 × 10^6 spleen cells from rats fed lead acetate for 2 weeks (▵——▵) as compared to controls (○——○).

the 14-week period. However, weights of the lead-fed animals averaged approximately 25% less over the duration of the experiment. Average weight gain of control rats was 21 g/week whereas the lead-fed animals averaged 11 g/week.

Blood lead levels of the control rats averaged 2·47 μg/dl over the 14-week period. After 2 weeks on the lead diet, the blood lead level of these rats was 46·29 μg/dl and averaged 56·65 μg/ml throughout the experiment.

Cells from 2-week lead-fed rats exposed to Con A showed a two-fold increase in mitogenic

Table 1. Mitogen stimulation of spleen cells from control and lead-fed rats (c.p.m./5 × 10^6 spleen cells/0·5 μCi ^{3}H-TdR)

Weeks on diet		Unstimulated cells		Con A (2·5 μg/ml)		PHA (1·8%)		LPS (25 μg/ml)	
		x̄ c.p.m.	s.d.	x̄ c.p.m.	s.d.	x̄ c.p.m.	s.d.	x̄ c.p.m.	s.d.
2	Control	3,783	593	55,895	15,191	20,064	759	21,795	3,717
	Pb-fed	9,641	3,407	103,981	7,508	59,483	6,425	37,382	6,546
	P value	<0·05		<0·05		<0·05		<0·5	
5	Control	5,326	716	75,286	10,398	53,184	7,800	15,834	4,064
	Pb-fed	5,364	1,057	92,822	985	54,774	5,106	26,816	2,804
	P value	>0·05		<0·05		>0·05		<0·5	
14	Control	9,166	1,417	84,454	3,107	64,178	3,479	25,342	476
	Pb-fed	10,564	860	96,118	9,522	56,386	1,153	30,547	2,337
	P value	>0·05		>0·05		>0·05		<0·05	

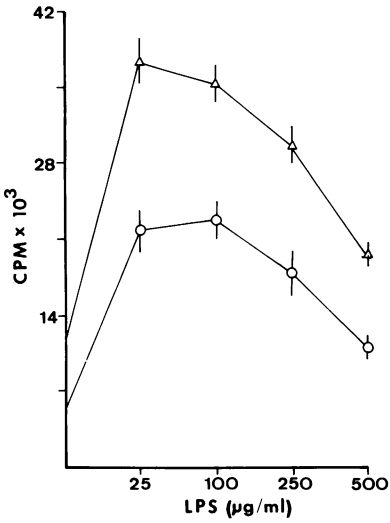

Fig. 3. Uptake of ^{3}H-thymidine (mean ± s.d.) in response to LPS stimulation of 5 × 10^6 spleen cells from rats fed lead acetate for 2 weeks (▵——▵) as compared to controls (○——○).

activity as compared to controls at all levels of mitogen concentration (Fig. 1). Following feeding of lead for 14 weeks the T cells from both groups showed no significant difference in the blastogenic response ($P > 0{\cdot}05$) (Table 1). Similarly, spleen cells from 2-week lead fed rats showed a greater mitogenic response to all concentrations of PHA tested as compared to control cells. After 14 weeks of the lead acetate diet no significant difference was seen between control and lead-fed animal mitogen responses (Fig. 2, Table 1).

LPS stimulation of B cells also increased two-fold over controls during the first 2 weeks of lead feeding and remained elevated to significant levels ($P < 0{\cdot}05$) throughout the experimental period (Fig. 3, Table 1). At no time during any of the experiments did lead feeding cause suppression of blastogenesis.

Macrophage morphological changes were observed using light microscopy over the length of the experiment. After 2 weeks, the adherent cells of the control animal appeared ameboid in shape and adhered to the bottom of the plastic petri dish. The concentration of lead at 2 weeks appeared to have little morphological effect on the adherent cells. After 5 weeks on the lead diet, the macrophages were decreased in number and less ameboid, assuming a small cigar-shaped form. Phagocytic ingestion of both sheep red blood cells and latex beads was also diminished by 5 weeks on the lead diet (Table 2) and remained suppressed throughout the remainder of the experiment.

Table 2. Macrophage cell cultures

	Controls		Lead diet	
	2 weeks	5 weeks	2 weeks	5 weeks
Morphology	Ameboid	Ameboid	Ameboid	Small cigar-shaped
Response to SRBC*	2–3	2–3	2–3	1
Response to latex beads*	7–10	7–10	7–10	1–2

* Cells or beads engulfed.

DISCUSSION

In the past, lead has been considered an immunosuppressive agent. Selye *et al.* (1966) found that a single intravenous injection of lead increased the sensitivity of rats to endotoxins and Luster, Faith & Kimmel (1978) found that the rat antibody response to SRBC was depressed while the B cell response to LPS was not altered when low levels of lead were fed to rats over a period of 7 weeks. In one case of a child who died from lead poisoning, the finding of bacteria septicaemia was considered indicative of lead-induced immune suppression (Williams *et al.*, 1964). High levels of lead were shown to be suppressive in adult mouse and rabbit antibody production (Koller, 1973; Koller & Kovacic, 1974). Gaworski & Sharma (1978) showed that *in vivo* exposure to high concentrations of lead for 30 days resulted in a decreased response to PHA.

The data presented here indicate a two-fold increase in the population of T cells responding to Con A, following exposure to lead, as compared to non-exposed controls. Both Gaworski & Sharma (1978) and Lawrence (1980) showed an increased mitogenic response when lymphocytes were exposed to lead *in vitro*. Holt & Keast (1977) found hyperactivity of splenic lymphocytes following *in vivo* exposure of rats to air pollutants. As manifested by an increased response to LPS, B cells from lead-fed rats in our study showed increased mitotic activity throughout the 14-week period. With higher levels of lead exposure, Luster *et al.* (1978) found that B cells responded normally to LPS stimulation despite T cell suppression. Similarly, Lawrence (1980) found that lead could not only increase B cell proliferation but could also overcome cyclic AMP-induced immune suppression. He also reported that cyclic GMP and lead synergistically enhanced B cell proliferation. These blastogenic changes are dependent upon the extracellular concentration of various ions, such as calcium and potassium (Hart, 1979), Since divalent lead may act in a manner similar to calcium, or in place of calcium (Settle & Patterson, 1980), the stimulatory effects we found with low levels of lead may be linked to the calcium-like activity of the heavy metal.

The present finding of decreased macrophage number and phagocytic ability as well as morphological changes is in accordance with the results of Bingham *et al.* (1968) who found decreased phagocytic ability of alveolar macrophages after lead exposure. Trejo *et al.* (1972) also found a depression in rat intravascular macrophage phagocytosis following exposure to lead acetate.

T cells in the presence of lead showed marked proliferation during the initial weeks of the experiment. This blastogenic activity began to decline towards control levels after 2 to 5 weeks of lead exposure. It would therefore appear that the T cell dependence on macrophage integrity begins to manifest itself as macrophage injury becomes evident.

In summary, therefore, we find that low doses of lead when fed to rats as a dietary supplement for a period of 14 weeks resemble mitogens, having a blastogenic effect on T and B cells. Macrophages, on the other hand, are suppressed after short-term exposure to lead. In time, then, one would postulate that this level of lead would lead to the suppression of a major portion of the immune system.

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