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Inflammatory Response and Resistance in Lead-Treated Mice

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The toxicity of lead on animals and man is well established. In the young children, lead poisoning is the major source of brain damage, mental deficiency and serious behaviour problems (Chisolm 1971). Lead is also shown to suppress tissue enzymes, induce anaemia and adverse effects on immune system in laboratory animals (Koller 1973, Luster et al 1978, Faith et al 1979, Blakley and Archer 1981, Caren 1981). Koller and Kovacic (1974) showed decreased antibody formation in mice exposed to lead. Faith and his co-workers (1980) further elucidated in mice a dose of 100 or 200 ug of soluble lead nitrate per d for 30 d resulted in a highly significant increase in susceptibility to a gram-negative bacterial pathogen. Since lead-treated mice show immunodepression to infectious disease the aim of this study was to examine whether lead could also exert a deleterious effect on their phagocyte activities in acute inflammation.

MATERIALS AND METHODS

Animals. Female Swiss albino mice weighing about 12 g were used.They were supplied with mouse pellets and water ad libitum and were divided into test and control groups, each consisting of 12 animals. The mice were allowed 2 wk to acclimatise to the experimental conditions. The test group was treated with lead nitrate in their drinking water at a concentration of 2000 ppm lead and this was renewed every 3 d so that the same concentration of lead in the drinking water was approximately maintained. The body weights of both groups were recorded weekly.

Induction of acute inflammation. Mice were lead-treated as above and were divided into test and control groups. Three mon after the initial exposure to lead nitrate, air pouches were induced using the method previously described (Sin et al 1988). Briefly, mice were

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anaesthetized with ether and their backs were swabbed with 70% alcohol. Five mL of air was subcutaneously injected into the back of the mouse. Three d later, 2.5 mL of air was re-injected into the pouch to keep the pouch open. Six d after the initial injection all animals were injected with 1 mL of 1% sterile carrageenan (kindly provided by Marine Colloid, Springfield, U.S.A.)in physiological saline which was sterilised at 90°C as described in our previous work (Sin et al 1990). Six hr after the injection of carrageenan into the air pouch, mice were anaesthetized with ether and bled to death through jugular vein. One mL of cold physiological saline was injected into the pouch. The exudate was collected and cooled in an ice-bath. The exudate volume was recorded and total leucocytes in the exudate fluid were determined in a Coulter counter. Smears of exudate cells were stained with May-Greenwald-Giemsa stain for cell identification.

The cells in the exudate were washed 3 times with ice-cold tissue culture medium (TC 199; containing 5% fetal calf serum, heat-inactivated at 56°C for 30 min). Cell viability was determined using the trypan blue dye exclusion method. The cells were then used for the, *in vitro*, determination of chemotactic response to N-formyl-methionyl-leucyl-phenylalanine (FMLP;Sigma) by the method described by Boyden (1962).

Organs such as kidney, liver and brain were also removed. They were weighed and processed for lead determination using an Atomic Absorption spectrophotometer.

Immunization and challenge to infectious disease. In these experiments, mice were divided into 4 groups. Group I was a control and without lead treatment. Mice in Group II were similar to Group I except they were injected twice with formalin-killed parasites (*trypanosoma evansi*) in complete Freund adjuvant. Group III was similarly lead-treated. Group IV was also lead-treated but received the same vaccine as in Group II. The vaccine was prepared as follows. Mice were infected experimentally with *T. evansi*; at the height of infection mice were bled by cardiac puncture. The blood was mixed with Alserver's solution (1:9; pH 7.2). Heat-inactivated fetal calf serum (10% by volume) was added to the parasite suspension and the resulting suspension centrifuged at about 1000 rpm for 20 min. The supernatant containing the trypanosomes were washed 3 times in Alserver's solution and after the final washing, the parasite suspension was resuspended to a final concentration of about 200 x 10^6 per mL. The vaccine injection (10 x 10^6 dead parasites in complete Freund adjuvant to make up a final volume of 0.1 mL.) was intraperitoneal and it was given 2 mon after the initial treatment of lead nitrate. Two wk later, mice of group II and group IV were re-injected with the same number of dead parasites but prepared in physiological saline.

Three mon after the initial treatment of lead nitrate mice of the all 4 groups were challenged with $1x10^3$ live parasites which were obtained from a heavily parasite-infected mouse. All mice of the 4 groups were then returned to their cages and mortality was monitored and recorded daily.
All the data were analyzed by Student's-t-test. A value of $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The concentration of lead nitrate used in these experiments was within the range used in studies on the immunosuppressive effects of lead in mice (Gaworski and Sharma 1978).The present study showed that mice which were exposed to lead nitrate in their drinking water at a concentration of 2000 ppm lead for 3 mon did not exhibit any adverse effect on their growth (Fig.1). The

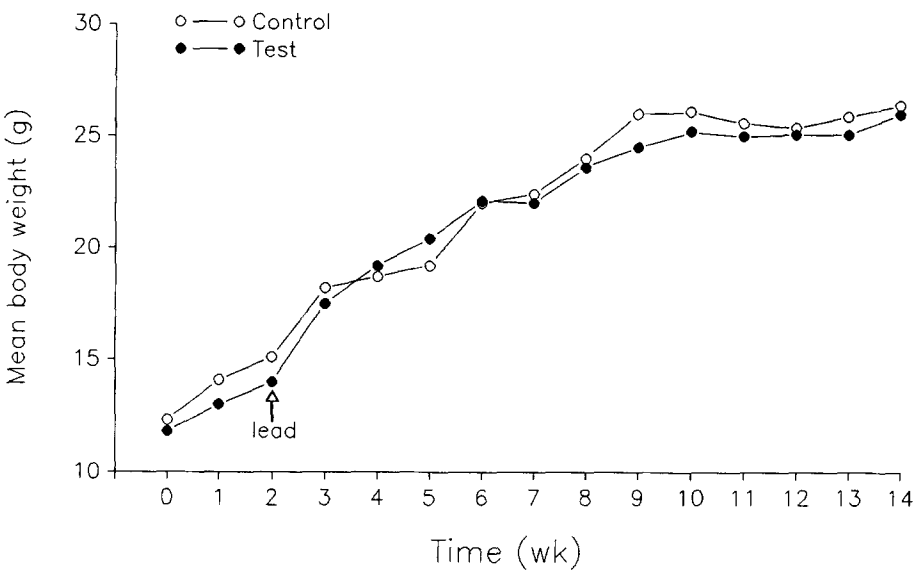

Figure 1. The growth rate of lead (test) and non-lead-treated (control) mice. n = 12

body weights of the lead-treated (test) group were not significantly different ($p > 0.05$) from the naive (control) group at any time throughout the 3 mon of the experiment. No obvious behaviourial changes were observed in the test group.
However, lead-treated mice did not respond (protective immunity) to a trypanosome vaccine (Table 1). Mice from

Table 1. The survival rate of test (lead-treated) and control (non-lead-treated) immune mice when challenged with trypanosomes.

Group (Treatment)	Survival(%) Days after trypanosome challenge 0	4	5	6	7	8	9	12	30
I Control (non-immune)	100	100	0	-	-	-	-	-	-
II Control (immune)	100	100	100	100	83	83	75	58	58
III Test (non-immune)	100	100	0	-	-	-	-	-	-
IV Test (immune)	100	100	75	75	75	50	0	-	-

n = 12

the lead and non-lead treated naive (control) group died within 5 d after the challenge. Mice of lead-treated but immunized with the parasite vaccine lived longer but they all died within 9 d. However, the non-lead-treated mice immunized with the vaccine had about 50% survival when the experiments were terminated 1 mon after the parasite challenge. The result is in agreement with the findings of others that lead exerts an immunodepressive effect in animal disease resistance (Hemphill et al 1971, Cook et al 1975, Faith et al 1980, Caren 1981). It is interesting to note that tissue lead appears not to have cytotoxic effects on the parasites. This observation is different from the mercury which was found to cause cytotoxic effect on the same parasites (Ryan et al 1990). This discrepancy could be attributed to either the higher cytotoxic effect of the mercury or the lower amount of lead accumulated in the various tissues and organs of the lead-treated mice (Table 2).

Since the immunodepression was shown to occur in mice exposed to lead nitrate it would be interesting to examine the effect of lead on the efficacy of phagocyte response to an inflammatory agent; a process known to play an important role in eliminating and preventing dissimilation of the foreign agents. In the present study we used an air pouch model to quantify phagocytes in the cavity after the injection of the irritant, carrageenan, into the air pouch (Sin and Ang 1990, Sin and Chio 1990). Table 3 showed that the number of total phagocytes in the exudate fluid of the test (lead-treated) group was $9.04 \pm 1.05 \times 10^6$ which was significantly reduced ($p < 0.05$)

Table 2. Concentration of tissue lead in various organs of test (lead-treated) and control (non-lead-treated) mice.

Organs	Mean ± S.E.(ug Pb^{2+}/g. F.Wt.) Control	Test
Liver	0.42 ± 0.09	2.41 ± 0.32*
Kidney	1.32 ± 0.03	11.71 ± 0.73*
Brain	0.93 ± 0.04	2.24 ± 0.23

*$p < 0.05$ significantly different from controls
n = 6
F.Wt.: fresh weight

Table 3. Effect of lead on exudate leucocytes during acute inflammation.

Group	Exudate fluid (mL)	Exudate leucocytes Total No. (1×10^6)	Chemotactic activity (%)
Control	0.98 ± 0.08	24.94 ± 1.76	103.27 ± 4.08
Test	0.88 ± 0.07	9.04 ± 1.05*	7.18 ± 0.71*

*$p < 0.05$ significantly different from controls
n = 12

as compared to the control group ($24.94 \pm 1.76 \times 10^6$). However, no significant difference was found in exudate volume between the test and the control group. This indicates that lead does not have a direct effect on the vascular permeability in the acute inflammation. Therefore, the slight decrease of the exudate fluid volume in the lead-treated mice could be the result of the significant decrease ($p < 0.05$) of exudate leucocytes. The majority of the exudate leucocytes from the carrageenan-induced inflammatory pouch were neutrophilic polymorphonuclear leucocytes (PMNs). This tends to support the work of Wedmore and William (1981) that neutrophilic PMNs are involved in the increased vascular permeability during acute inflammation. It has to be pointed out that there was also no significant difference in peripheral blood leucocyte count between control and lead-treated mice (unpublished data). Hence, the slight decrease of the vascular permeability in the lead-treated mice is most likely the result of significant reduction in neutrophilic PMN emigration from the blood circulation into the inflammatory cavity.

The results of the, in vitro, study of the exudate leucocytes from lead-treated group (Table 3) showed that the chemotactic response of the neutrophilic PMNs to the chemical attractant (FMLP) was significantly reduced ($p < 0.05$). This might explain the significant reduction in total number of exudate leucocytes in the lead-treated mice. However, one cannot ignore a possibility that lead also decreases the efficacy of phagocytosis in neutrophilic PMNs as shown in mice treated with gold compound during carrageenan-induced acute inflammation (Sin and Wong 1991) ; thus lowers the degree of inflammatory reaction since a number of pro-inflammatory chemicals were known to be released during phagocytosis of inflammatory irritants (Higgs and Youlten 1972).
In conclusion, the findings of the present study showed that lead produces a deleterious effect on the defense mechanisms of the mice by reducing the chemotactic response of neutrophilic PMNs to inflammatory irritant. It is also highly possible that lead could similarly affect the mononuclear phagocytes. All these factors might render the animals more vulnerable to infectious disease. However, whether lead would exert similar effects in children who have ingested excessive lead, usually from paint chips (Bogden et al 1974) remains to be verified.

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