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Chemotaxis of Mouse Peritoneal Macrophages Following Exposure to Lead

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Lead (Pb) is a toxin with wide environmental distribution. Recently, study of the effects of lead has focused on the immune system (Fichsbein et al. 1993).

The macrbphage is one of the immune cells which undergoes changes when exposed to lead. This involves a reduction in the number of medullary macrophages (Kowolenko et al. 1989). Furthermore, cellular viability of peritoneal macrophages is reduced when exposed *in vitro* to lead (Büchmuller-Rouiller et al. 1989).

The study of the macrophage phagocytosis has revealed contradictory results (Jian et al. 1985; Kowolenko et al. 1988). A decreased capacity to kill intracellular pathogens (Lawrence 1981) can be confirmed, whereas phagocyte activity, such as antigen presentation is lessened (Kowolenko et al. 1988). Alteration of migration and chemotactic factors could explain some of these functional changes brought on by the exposure to lead. For this reason, we have tried to assess the effects of lead on chemotactic activity and spontaneous mobility of resident peritoneal macrophages in a murine model.

MATERIAL AND METHODS

We used 4 week old ± 4 days female BALB/c mice purchased from Charles River. Animals were housed four per cage in stainless steel hanging wire cages and fed a mouse chow (Interfauna Ibérica, Barcelona, Spain) ad *libitum.* Mice were kept in rooms under controlled environmental conditions (22°C and 12 hr light/dark cycle). Following a one-week acclimatization period, 32 mice were divided at random into 4 test groups of eight: a control group and groups exposed to 13, 130, or 1300 parts per million

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(ppm lead), respectively. Exposure to lead (crystallized lead acetate, Panreac, Barcelona, Spain) was administered by their drinking water over a period of 10 weeks. Water was administered *ad libitum.* Weight (expressed in g) and drinking water (measured in ml/day) were recorded 3 times a week throughout the experiment.

Peritoneal lavage was performed using RPMI-1640 medium (Sigma, Chemical Co., St. Louis, U.S.A.),supplemented by 10 mM of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, Sigma, Chemical Co.,St. Louis), and 10 U sodium heparin per mL. The culture medium was composed of RPMI-1640, 10 mM HEPES, and 2% bovine albumin (albumin bovine fraction 5, fatty acid free, Sigma, Chemical Co., St. Louis). The chemotactic substance was prepared from RPMI-1640, 10 mM HEPES, and 3 mg/mL of casein (Sigma, Chemical Co., St. Louis) as per Wilkinson (1986). The culture medium was used to evaluate spontaneous mobility.

We harvested peritoneal macrophages by IP injection of 4 mL of medium. We formed 3 aliquots and counted the number of cells in each with a hemacytometer, thereafter calculating the average from these results. Cytosmears of peritoneal cells were prepared for each animal using the Brummer et al. (1986) modified procedure and we stained slides using Diff-Quick® (Baxter Dade A.G., Dudingen, Switzerland). To assess their composition, we classified 200 cells from four different areas of the smear chosen at random. The percentage of macrophages was multiplied by the number of peritoneal cells so as to obtain the number of macrophages.

Cellular viability was checked using trypan blue dye (Merck, Darmstadt, Germany) at 0.4% (Herscowitz and Cole 1981). Macrophage viability was always greater than 95%.

Chemotaxis was measured according to modifications of the Boyden technique (Lohr and Snyderman 1981). We used three chambers per animal (Neuroprobe, Quimigranel S.A., Madrid, Spain). We added 200 µL of cellular suspension, adapted to a concentration of $2X10^{6}$ macrophages/mL, to the upper compartment. In the lower compartment, we inserted 100 µL chemotactic substance. In the middle, we placed a polycarbonate filter, with a pore size of 5 pm (Nuclepore, Quimigranel S.A., Madrid, Spain). Filters were collected, dried, and stained with Diff-Quick®. The chambers were incubated for four hours at a temperature of 37°C in a humid atmosphere. The chemotactic index measured the cell number (average of 3 filters), which had emigrated to the lower side of the filter, in ten areas chosen at random using 100 X objective and 10 X ocular. Spontaneous mobility was measured in the same way differing only that 100 µL culture medium replaced casein in the lower compartment. Again, we used three

chambers per animal. The spontaneous mobility index calculated the average number of emigrating cells in ten areas chosen at random.

The results from the three exposure groups and the control group were compared using analysis of variance (ANOVA) (Doménech 1990). The results were regarded as significant at $P<0.05$ and the groups producing the statistical significance were identified by means of "a posteriori" orthogonal contrasts, as per the Scheffé test (Doménech 1990). If ANOVA was not possible (under conditions other than normal, and without homogenous variance), the Kruskall-Wallis test was used (Doménech 1990).

RESULTS AND DISCUSSION

Weight gain was statistically similar in the four groups (data not shown) during the trial. Means and standard errors of final body weight were: 19.72±1.19, 19.02±0.39, 20.98±0.40, and 19.13±O.67 g for the control group and groups exposed to 13, 130, and 1300 ppm lead, respectively. Daily water consumption (mL/day) averaged 4.08±0.06, 4.50±0.06, 4.61±0.09, and 4.04±0.10 in the control and groups exposed to 13, 130, and 1300 ppm, respectively. No statistical differences were found in the above parameters. Similar body weight and water consumption among the four groups confirm the animal's good conditions.

The chemotaxis index of animals in the 13 ppm group was 64.8 % of the control group ($P<0.05$) (Table 1). The group exposed to 130 ppm did not differ from the control. The 1300 ppm group was only 63.7 % of that of the control group ($P<0.05$) (Table 1).

Table 1. Chemotaxis and spontaneous mobility index of resident macrophages in mice exposed to lead acetate in drinking water (mean±SE)

Treatment	Chemotactic index	Spontaneous mobility index
Control	41.40±5.85	26.65±5.63
13 mm	26.81±2.60[a]	20.93±3.12
130 ppm	41.86±6.43	18.50±3.23
1300 ppm	26.36±3.84[a]	24.32±2.20

[a] Means (±SE) bearing this superscript are significantly different from controls (ANOVA, $P=0.038$)

Spontaneous mobility did not change with lead exposure ($P>0.05$) (Table 1).

Decreased chemotaxis in neutrophils of humans exposed to lead has been previously revealed (Bergeret et a1.1990; Queiroz et al. 1993). Chemotaxis in mouse peritoneal macrophages seems to be consistent with lead susceptibility, showing inhibition with doses as low as 13 ppm.

Reductions in the chemotactic activity after doses of 13 ppm, a similar value to that in the control group with doses of 130 ppm and subsequent inhibition at doses of 1300 ppm, have not been gleaned from previous studies. Lison et al. (1990) detected a similar alteration in protein kinase C activity of peritoneal macrophages exposed to lead. This enzyme intervenes in signal transduction of chemotaxis, so its modification by lead could explain these results in chemotactic activity.

Lead could reduce cellular orientation caused by chemotactic substance which precedes and is necessary for mobility, as lead interference has been discovered in the assemblage of microtubules, which take part in orientation prior to movement (Roderer and Doenges 1983).

Finally, reduced cellular spreading (Kowolenko et al. 1989), provoked by lead, could explain inhibition in chemotaxis, since correct adherence is necessary for optimum execution of movement (Wilkinson and Haston 1988).

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