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Cadmium-Induced Phagocyte Cytotoxicity[a]

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Introduction

The influence of environmental chemical contaminants on host defense mechanisms is a developing concern in chronic toxicity assessment and in evaluating the biological impact of xenobiotics. One group of environmental contaminants, i.e., heavy metals, particulary lead and cadmium, have been examined for their possible alteration of *in vivo* host resistance and immune response parameters.

The initial observations of an enhanced endotoxin sensitivity in rats (SELYE et al., 1966) and chickens (TRUSCOTT, 1970) treated with lead acetate or in rats which received cadmium acetate (COOK et al., 1974), coupled with a 10X increase in lethality due to *Salmonella typhimurium* in mice treated with 100µg of lead nitrate (HEMPHILL et al., 1971), have been responsible for generating considerable interest in the effect of heavy metals on immunological responses. JONES et al. (1971) demonstrated that passive hemagglutinin titers to human gamma globulin in rats immunized following subcutaneous injection of $CdCl_2$ were signficantly impaired; however, an adjuvant-like response was noted in rats immunized 2 weeks after the last cadmium injection. Also, a 50-75% decrease in pseudorabies virus neutralizing antibody titer was seen in rabbits which received 300 ppm of $CdCl_2$ for 70 days prior to immunization; similar exposure to 2500 ppm of lead acetate reduced the antibody titers 80-90% below control values (KOLLER, 1973). The decreased antibody response following cadmium administration persisted for several months and was more severe than during the administration of the metal (KOLLER et al., 1975). Although a chronic exposure to cadmium elicited a reduction in serum antibody titers, a single oral or intraperitoneal administration of cadmium was reported not to alter the anti-SRBC splenic primary plaque forming cell response (KOLLER et al., 1976). However, the measurement of serum anti-SRBC hemagglutinin titers revealed a distinct trend toward an enhancement in antibody formation following a single administration of cadmium (LOOSE and SILKWORTH, 1977) with a

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concomitant temporal increase in IgG_{2a}, IgG_{2b} and IgM and a dose-related increase in serum IgA.

Since $CdCl_2$ impaired alveolar macrophage metabolic activity (MUSTAFA and CROSS, 1971) and enhanced peritoneal macrophage activity (KOLLER and ROAN, 1977), it would appear that perhaps a selective sensitivity to cadmium may exist for various phagocytic-cell types. To evaluate this possibility the viability of three different phagocytic cell populations was determined following their exposure to different concentrations of cadmiu Also, since a selective cytotoxicity may be a function of solubility of the cadmium salts, cadmium chloride and cadmium acetate, which have different aqueous solubilities, i.e., cadmium chloride being greater than cadmium acetate, were used to delineate the influence of solubility of the metal salts on the viability of the phagocytic cell types.

Materials and Methods

Animals. Male Balb/c mice (18-20 g) were used throughout. Wayne lab diet and water were provided ad libitum and a 12:12 day:night photoperiod was maintained.

Cell Isolation. Peritoneal macrophages were harvested from the peritoneal cavity of mice maintained under light ether anesthesia. Four days prior to cell isolation the mice received an intraperitoneal (ip) injection of 1.0 cc of 6% sodium caseinate to induce a sterile inflammatory response. The cells were harvested by lavage with sterile 0.9% NaCl. Polymorphonucleated neutrophils (PMNs) were harvested from the peritoneal cavity of the Balb/c mice maintained under light ether anesthesia. Eighteen hours prior to cell isolation the mice received an injection of 1.0 cc of 6% sodium caseinate. The inflammatory PMNs were obtained by sterile saline lavage. Pulmonary alveolar macrophages were isolated by sterile saline lavage of the lung. The trachea of mice anesthesized with ether was cannulated using an 18 g needle and the lungs were then lavaged in situ 5X with 1.0 cc of sterile saline.

Cell Viability. The viability of the isolated peritoneal and pulmonary alveolar macrophages and the neutrophils was determined by the ability of the live cells to exclude Trypan blue dye. Dead cells take up the dye and the nucleus stains blue. Cadmium chloride and cadmium acetate were prepared in phosphate buffered Hanks Balanced Salt Solution at pH 7.4 supplemented with 10% fetal calf serum. The isolated cell preparations were

ncubated in the salt solutions for 20 minutes at 37^{0}C. Cellular viability as measured at 20 minutes by counting the percentage of cells excluding rypan blue per 100 cells. A total of 500 cells was counted. Each experiment as conducted in triplicate.

Statistics. Data are presented as mean ± standard error. Statistical ignificance was determined by the Student t test with $p<.05$ considered to e significant.

Results

At equal mEq/l concentrations cadmium acetate was significantly ore cytotoxic than cadmium chloride to peritoneal macrophages. Concentraions of 3.6×10^{-3} or 3.6×10^{-2} mEq/l of cadmium chloride did not alter eritoneal macrophage viability. However, the same concentrations of cadium acetate were signficantly cytotoxic. A reduction in viability of the eritoneal macrophages was observed with 3.6×10^{-1} mEq/l of cadmium hloride (Table I).

Although cadmium acetate was more cytotoxic than cadmium chloride o peritoneal macrophages, the converse was observed with neutrophils, i.e., admium chloride was more cytotoxic than cadmium acetate to polymorphoucleated neutrophils. A significant 11% decrease in viability of the PMNs ncubated in media containing 3.6×10^{-3} mEq/l of cadmium chloride was demontrated, the same amount of cadmium acetate in the media did not alter neurophil integrity as determined by their ability to exclude Trypan blue. At 3.6×10^{-2} mEq/l both the acetate and chloride salts of cadmium were cytooxic to the PMNs.

No preferential cytoxocity to pulmonary alveolar macrophages was anifested by either cadmium acetate or cadmium chloride. At 3.6×10^{-3} Eq/l of either cadmium chloride or cadmium acetate no alteration in viaility of the alveolar macrophages was observed. However, at 3.6×10^{-2} Eq/l a 21% and 9% reduction in viability of cells incubated in media conaining cadmium chloride and cadmium acetate, respectively, was demonstrated.

Within the cadmium chloride treatment group, it was observed that MNs were more sensitive to the cytotoxic effects of $CdCl_2$ than alveolar acrophages and that peritoneal macrophages were the least sensitive phagoytic cell type to the toxic influence of the $CdCl_2$ (Table II). The most ensitive cell type to the cytotoxic influence of cadmium acetate was the

Table 1. Cadmium Chloride Induced Phagocyte Cytotoxicity

Cadmium chloride (meq Cadmium/ℓ)	% Viable Cells[a]		
	Peritoneal Macrophages	PMNs	Alveolar Macrophages
0	98 ± 1.2	99 ± 0.3	92 ± 1.2
3.6×10^{-3}	92 ± 2.3	89 ± 1.2*	86 ± 3.5
3.6×10^{-2}	76 ± 9.1	85 ± 1.7*	73 ± 2.3*
3.6×10^{-1}	65 ± 9.2*	71 ± 2.3*	63 ± 0.5*
3.6×10	56 ± 6.0*	2 ± 0.9*	53 ± 6.7*

[a]Cytoxicity was evaluated 20 minutes following incubation of the cells with the cadmium salt at 37°C. Viability was determined by Trypan blue dye exclusion. Data is presented as mean ± standard error with * indicating signficance at $p<.05$; $n = 3$ in all groups.

Table II. Cadmium Acetate Induced Phagocyte Cytotoxicity

Cadmium acetate (meq/ Cadmium/ℓ)	% Viable Cells[a]		
	Peritoneal Macrophages	PMNs	Alveolar Macrophages
0	100 ± 0.0	99 ± 0.3	95 ± 1.3
3.6×10^{-3}	97 ± 0.7*	97 ± 1.2	93 ± 0.9
3.6×10^{-2}	91 ± 0.9*	97 ± 0.9*	88 ± 1.7*
3.6×10^{-1}	82 ± 1.5*	80 ± 2.3*	70 ± 0.6*
3.6×10	66 ± 1.5*	54 ± 2.1*	65 ± 0.3*

[a]Cytotoxicity was evaluated 20 minutes following incubation of the cells with the cadmium salt at 37°C. Viability was determined by Trypan blue dye exclusi Data is presented as mean ± standard error with * indicating significance at $p<.05$; $n = 3$ in all groups.

peritoneal macrophage. The PMNs and pulmonary alveolar macrophages manifested a comparable response to the cadmium acetate (Table II). The decrease in viability observed with either cadmium chloride or cadmium acetate was maximal at 20 minutes and no further significant alterations were seen past this time point. The loss of viability was not a function of pH shift in the media since it was unaltered from the starting pH of 7.4.

Discussion

LUCKEY et al. (1973) suggested that mammalian systems detoxify metal ions by sequestering them in erythrocytes and leucocytes. The lysosomes of the macrophages may then accumulate the insoluble and colloidal particles of the metal compounds and excrete them as residual vacuoles. When the body burden of the toxic metals increases, new proteins such as metallothionins and Cd-binding proteins are then synthesized in the liver and kidney.

In support of this hypothesis, GRANTA et al. (1970) have demonstrated that $CdSO_4$ at 0.5 mg/ml of blood resulted in in vitro cytophagy, i.e. an enhanced phagocytosis of erythrocytes and thrombocytes by leucocytes. It was proposed that the increased erythrophagocytosis was perhaps due to an alteration in the erythrocyte resulting from the accumulation of Cd^{++} bound to SH-containing ligands of the membrane and other cell constituents.

Although phagocytic cells may be integral components in the detoxification of metal ions, the direct effects of the metals on phagocytic cell function have been more amply studied than the detoxifying processes. Since certain macrophage functions have been shown to be altered by cadmium (CROSS et al., 1970; MUSTAFA and CROSS, 1971), then it would be logical to assume that perhaps the detoxifying process may also be impaired. Such an impairment would accentuate the metal toxicity.

As demonstrated in the present study, cadmium is directly cytotoxic to phagocytic cells which may explain certain of the observed in vivo alterations of host defense induced by cadmium. An obvious selective phagocytic cell cytotoxicity exists depending on the cadmium salt used, i.e. cadmium chloride was more cytotoxic to PMNs than it was to alveolar or peritoneal macrophages; indeed, it was least cytotoxic to the peritoneal macrophages. However, cadmium acetate was more cytotoxic to the peritoneal macrophages than to PMNs or alveolar macrophages. Since cadmium chloride and cadmium acetate elicited a significant reduction in alveolar macrophage viability at

the same mEq/l concentrations, i.e. 3.6 x 10^{-2} mEq/l, it would suggest that solubility is not a signficant contributing factor in the cadmium-induced *in vitro* manifestation of cytotoxicity to the alveolar macrophages. Similarly, if solubility of the cadmium salts was the primary contributing factor to cytotoxicity, it could not explain the decreased viability of the peritoneal macrophages exposed to cadmium acetate as compared to the greater viability of the cells incubated with cadmium chloride. Therefore, the selective cytotoxicity of the cadmium salt is suggested to be a function of differences *per se* in the metabolic and biochemical characteristics of the three different cell types and their distinct and different responses to the two salts of cadmium.

KARNOVSKY et al. (1970) reported significant differences in respirat rates of peritoneal macrophages, PMNs and alveolar macrophages. The peritoneal macrophages have a 3X greater respiratory rate than PMNs and that this function was again 3X greater in alveolar macrophages, i.e. PMNs - 2.5, peritoneal macrophages - 7.0 and alveolar macrophages - 21.0µ 10_2/mgP/hr. Also, alveolar macrophages had a greater capacity to oxidize glucose than the PMNs or peritoneal macrophages. ALLEN and LOOSE (1976) have recently shown that the chemiluminescent (CL) response of PMNs is approximately 3-orders of magnitude greater than alveolar macrophages and 1-order of magnitude greater than peritoneal macrophagès. Inhibition of chemiluminescence by superoxide dismutatase (SOD) was more profound in the PMNs than with either alveolar or peritoneal macrophages. However, when Na^+ benzoate was used to inhibit CL, the least inhibition was observed in the alveolar macrophages. It is interesting to note that alveolar macrophages, which use primarily aerobic glycolysis for energy, contain five times more SOD activity than PMNs (RISTER and BAEHNER, 1976). The metabolic, biochemical and cytochemical (BRAUNSTAINER and SCHMALZL, 1970) differences of PMNs, alveolar and peritoneal macrophages do not allow a general statement as to their unique responsiveness to the cadmium salts. However, it may be speculated that the effect of the cadmium salts on the various phagocytic cells studied may be a result of either a difference in plasma membrane fluidity or permeability and/or a reflection of the metabolic differences between the cell types.

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