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Influence of Cadmium on the Phagocytic and Microbicidal Activity of Murine Peritoneal Macrophages, Pulmonary Alveolar Macrophages, and Polymorphonuclear Neutrophils

LELAND D. LOOSE*, JAY B. SILKWORTH, AND DIANE W. SIMPSON

Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York 12208

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A significant depression in the phagocytic capacity of elicited peritoneal macrophages, pulmonary alveolar macrophages, and elicited peritoneal polymorphonucleated neutrophils was manifested when the cells were incubated in medium containing cadmium chloride. With the exception of the neutrophils, a similar influence was observed when the cells were exposed to cadmium acetate. The impaired phagocytic capacity was related to the concentration of the cadmium in the medium. Peritoneal macrophages and neutrophils did not demonstrate any alteration in their microbicidal activity (percentage of ingested yeast which were killed) in the presence of the cadmium salts. However, a significant suppression in the intracellular microbicidal activity of alveolar macrophages was observed when the cells were incubated in medium containing either cadmium chloride or cadmium acetate. This unique response to Cd^{2+} may be related to general metabolic characteristics of these cells living at an elevated O_2 tension.

Heavy metal-induced alterations of immune responses and impaired host resistance to infectious agents is a critical variable in toxicity assessment and in evaluating the biological influence of environmental chemical contaminants. The effect of heavy metals on host defense mechanisms was, perhaps, first observed by Selye et al. (11), who described an increase (×100,000) in bacterial endotoxin sensitivity in rats treated with lead acetate. This increased senitivity to endotoxin was postulated to be due to a reticuloendothelial blockade. Filkins (5) has subsequently shown that endotoxin detoxification is a macrophage (primarily hepatic)-mediated event which is profoundly impaired by lead (12). Truscott (13) observed a similar effect in chickens which received lead acetate, and Cook et al. (3, 4) reported that rats which received cadmium acetate also manifested an enhanced endotoxin sensitivity. In addition to the increase in endotoxin sensitivity observed in animals treated with lead or cadmium, an increased lethality due to bacterial challenge has also been reported to occur in mice (6) and rats (3) administered heavy metals. Biozzi et al. (2) reported that an enhanced phagocytic function of the reticuloendothelial cells is a primary mechanism of host defense against gram-negative and gram-positive bacteria. The composite results of an enhanced endotoxin sensitivity and an increased lethality due to bacterial challenge in animals treated with heavy metals in conjunction with the known macrophage involvement in these two entities has suggested that macrophage dysfunction may be a contributing factor in heavy metal-induced host defense alterations.

Previous results have demonstrated that cadmium (chloride or acetate) deceases the respiratory burst in phagocytic cells (7). Because the respiratory burst is a concomitant of phagocytosis (9), the phagocytic step may be the event which is impaired by cadmium. The present studies were conducted, therefore, to evaluate the influence of cadmium on the phagocytic uptake and intracellular microbicidal activity of isolated phagocytic cells.

MATERIALS AND METHODS

Animals. Male BALB/c mice (Charles River Breeding Laboratories, Inc.) weighing 18 to 20 g were used throughout. The mice received Wayne lab diet and water ad libitum and a 12:12 day:night photoperiod was maintained.

Cell isolation. Elicited peritoneal macrophages were obtained from the peritoneal cavity by saline lavage 4 days after an intraperitoneal injection of 1.0 ml of 6% Na^+ caseinate in saline. The alveolar macrophages were harvested by pulmonary lavage by using physiological saline administered via a trachea cannula. Peritoneal polymorphonuclear neutrophils (PMNs) were elicited by an intraperitoneal injection of 1.0 ml of Na^+ caseinate in saline and were harvested from the peritoneal cavity 18 h later by saline lavage. The cell suspensions were centrifuged at 312 × *g* for

6 min at 4°C. The cell pellet was resuspended in Hanks balanced salt solution (Grand Island Biological Co., 402S) without phenol red. Total cell counts were obtained by using a hemacytometer technique, and differential counts were used as an index of purity. The peritoneal exudate cell suspension obtained 4 days after Na^+ caseinate injection contained 80 to 85% macrophages (as determined by eosin Y ingestion), and about 5% PMNs and lymphocytes. The exudate obtained from the peritoneal cavity 18 h after Na^+ caseinate administration was about 90% PMNs and 5% macrophages and lymphocytes. The alveolar lavage fluid contained 95% macrophages.

Phagocytic and microbicidal studies. The technique to assess the phagocytic capacity and microbicidal activity of murine phagocytic cells is an adaptation of a light microscopic procedure initially described by Schmid and Brune (10) using human peripheral blood PMNs. Briefly, a phagocyte:yeast (*Saccharomyces cerevisiae*) ratio of 1:3 was utilized. Sterile capped plastic test tubes (12 by 75 mm) to which 0.20 ml of methylene blue (25.6×10^{-3} g/100 ml in Hanks balanced salt solution), 0.25 ml of the cell suspension, 0.25 ml of yeast in fetal calf serum, and 0.10 ml of cadmium acetate or cadmium chloride in saline were added simultaneously and then incubated at 37°C in a Dubnoff shaking water bath for 30 min. Preliminary studies conducted to adapt the method of Schmid and Brune (10), which used human cells, to a murine system revealed optimal activity for the three cell types at 30 min. This time was then chosen as the time for measurement. The tubes were centrifuged at 312 $\times$ *g* for 6 min at 4°C after removal from the water bath. The supernatant was discarded, and the cell pellet was resuspended in 20 μl of Hanks balanced salt solution. A wet mount was then prepared for microscopic examination, and a minimum of 200 phagocytic cells were counted per preparation. Dead yeast cells stained blue with the methylene blue while live yeast remained unstained. The total number of yeast, live and dead, ingested per 100 phagocytic cells was tabulated and expressed as the phagocytic capacity. The total number of dead yeast, i.e., the percentage of the ingested yeast which stained blue, within the macrophages was counted and expressed as a function of the cellular microbicidal activity. All phagocytic and microbicidal activity studies were conducted in duplicate.

Statistical evaluation. Data are presented as the mean ± standard error. Significance was determined by Student's *t* test and is noted where $P < 0.05$ in which experimental values were compared to control values. Comparisons within experimental groups were not conducted.

RESULTS

The phagocytic capacity (i.e., the total number of ingested yeast per 100 cells) was significantly reduced, in a dose-associated manner, in peritoneal macrophages, alveolar macrophages, and PMNs incubated in medium containing either cadmium chloride, or, with the exception of PMNs, in cadmium acetate (Tables 1 to 3). In the present study the most avid phagocytic cell type was the PMN (Table 3) and the least

TABLE 1. *Influence of cadmium salts on the phagocytic capacity and microbicidal activity of peritoneal macrophages*[a]

Salt (meq/liter)	Peritoneal macrophages	
	Phagocytic capacity (no. of injested yeast/100 cells)	Microbicidal activity ([no. dead/(no. dead + no. alive)]/100 cells)
Cadmium chloride		
0	85 ± 8.8	39 ± 3.8
8.0×10^{-3}	61 ± 3.5[b]	39 ± 3.5
8.0×10^{-2}	43 ± 3.2[b]	35 ± 3.1
8.0×10^{-1}	15 ± 2.9[b]	30 ± 15.1
Cadmium acetate		
0	81 ± 7.9	44 ± 5.7
8.0×10^{-3}	51 ± 8.1[b]	41 ± 6.0
8.0×10^{-2}	41 ± 10.5[b]	40 ± 6.8
8.0×10^{-1}	33 ± 3.8[b]	40 ± 6.7

[a] Data are presented as mean ± standard error; $n = 8$ in all groups.
[b] $P < 0.05$.

TABLE 2. *Influence of cadmium salts on the phagocytic capacity and microbicidal activity of pulmonary alveolar macrophages*[a]

Salt (meq/liter)	Pulmonary alveolar macrophages	
	Phagocytic capacity (no. of injested yeast/100 cells)	Microbicidal activity ([no. dead/(no. dead + no. alive)]/100 cells)
Cadmium chloride		
0	36 ± 1.8	30 ± 2.0
8.0×10^{-3}	30 ± 0.5[b]	14 ± 6.0[b]
8.0×10^{-2}	9 ± 2.7[b]	10 ± 5.1[b]
8.0×10^{-1}	3 ± 0.6[b]	2 ± 0.47[b]
Cadmium acetate		
0	38 ± 1.7	32 ± 0.9
8.0×10^{-3}	28 ± 2.0[b]	30 ± 1.9
8.0×10^{-2}	30 ± 6.3[b]	20 ± 0.5[b]
8.0×10^{-1}	18 ± 5.0[b]	8 ± 4.4[b]

[a] Data are presented as mean ± standard error; $n = 8$ in all groups.
[b] $P < 0.05$.

TABLE 3. *Influence of cadmium salts on the phagocytic capacity and microbicidal activity of PMNs*[a]

Salt (meq/liter)	PMNs	
	Phagocytic capacity (no. of injested yeast/100 cells)	Microbicidal activity ([no. dead/(no. dead + no. alive)]/100 cells)
Cadmium chloride		
0	136 ± 19.1	34 ± 6.0
8.0×10^{-3}	91 ± 11.3[b]	29 ± 4.5
8.0×10^{-2}	67 ± 6.1[b]	36 ± 4.7
8.0×10^{-1}	47 ± 5.5[b]	33 ± 6.0
Cadmium acetate		
0	145 ± 27.4	32 ± 4.0
8.0×10^{-3}	137 ± 11.6	32 ± 0.2
8.0×10^{-2}	108 ± 18.1	46 ± 12.7
8.0×10^{-1}	58 ± 10.3[b]	25 ± 2.4

[a] Data are presented as mean ± standard error; $n = 8$ in all groups.
[b] $P < 0.05$.

phagocytically active cell was the pulmonary alveolar macrophage (Table 2). However, there did not appear to be any selective alteration in the phagocytic capacity of one cell type by either of the cadmium salts.

Although a somewhat uniform suppression in the phagocytic capacity of the cells incubated in medium containing cadmium chloride or cadmium acetate (with the exception of PMNs) was observed, a concomitant alteration in their intracellular microbicidal activity was not observed (Tables 1 to 3). Peritoneal macrophages and PMNs incubated in media containing either cadmium chloride or cadmium acetate manifested no alteration in their ability to kill the intracellular yeast (Tables 1 and 3). However, pulmonary alveolar macrophages had a significantly impaired microbicidal activity when incubated in cadmium chloride, and, to a lesser extent, cadmium acetate (Table 2). No alteration in the microbicidal activity of the alveolar macrophages was observed when the cells were incubated in media containing 8.0×10^{-3} meq of cadmium acetate per liter; however, a similar concentration of cadmium chloride elicited a 53% decrease in the microbicidal activity of the cells. An impaired killing ability of the alveolar macrophages incubated in medium containing cadmium acetate was initially observed at a concentration of 8.0×10^{-2} meq of cadmium acetate per liter. At this concentration of cadmium acetate, a 37% decrease in the microbicidal activity of the alveolar macrophage was observed, whereas a 67% reduction was noted in cells exposed to $CdCl_2$.

DISCUSSION

The present study has demonstrated a significant impairment in the phagocytic capacity of alveolar macrophages, peritoneal macrophages, and PMNs incubated in medium containing either cadmium chloride or (with the exception of PMNs) cadmium acetate. The impaired phagocytic capacity was associated with the cadmium concentration in the medium. Cells exposed to $CdCl_2$ exhibited a depression in their phagocytic capacity similar to those incubated in medium containing cadmium acetate. The exception to this observation occurred with PMNs which were effected more by the chloride than the acetate salt of the cadmium. Ward et al. (14) also observed a difference between the complex of a metal and its effect in that the chloride of gold was more effective in inhibiting [^{14}C]proline incorporation into fibroblasts than was gold thioglucose.

Although a suppressed phagocytic capacity of cells incubated in cadmium was demonstrated, a concomitant impairment in the intracellular microbicidal activity of either peritoneal macrophages or PMNs was not manifested. However, pulmonary alveolar macrophages did elicit a marked depression in the microbicidal activity when incubated in medium containing cadmium acetate or cadmium chloride. Previous studies (7) similarly demonstrated that the alveolar macrophage was the most sensitive phagocytic cell type to a cadmium-induced inhibition of the respiratory burst. Because Cd^{2+} has been demonstrated to significantly impair alveolar macrophage metabolic activities and because Cd^{2+} is a common air pollutant and a component of cigarette smoke, a compromised pulmonary host defense may be manifested after exposure to cadmium (12). The cadmium-induced alteration in alveolar macrophage microbicidal activity may be related to certain unique metabolic and biochemical characteristics of these cells (1).

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