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EFFECT OF AMMONIUM METAVANADATE ON THE MOUSE PERITONEAL MACROPHAGE LYSOSOMAL ENZYMES

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Female $B_6C_3F_1$ mice were injected intraperitoneally with ammonium metavanadate (2.5 or 10 mg V/kg), ammonium chloride, or sodium phosphate buffer (0.1 M, pH 7.2) every 3 d for 6 wk. Resident peritoneal macrophage (PEM) cytolysates were prepared and assayed for intracellular enzyme activities of β-glucuronidase, N-acetyl-β-D-glucosaminidase, acid phosphatase, and lysozyme, to investigate possible reasons for the depressive effect of ammonium metavanadate on the intracellular killing of Listeria monocytogenes *by murine PEM. Acid phosphatase activity per 10^6 cells for the 2.5 and 10 mg V/kg groups was depressed by 22.8 and 44.7%, respectively, when compared to phosphate buffer controls. No significant effect by vanadium treatment was observed with regard to the other three enzymes. Kinetic studies (in vitro) on the effect of ammonium metavanadate (5, 10, 15, and 20 mM) on the above enzymes showed similar patterns of effect by vanadium. Lineweaver-Burk analysis of acid phosphatase indicated linear noncompetitive inhibition by vanadium with a K_i of 14.8 mM. NH_4Cl and 10 mg V/kg treatments also enhanced extracellular secretion of β-glucuronidase and lysozyme from PEM, which could be attributed to the presence of ammonium ion. The decrease in acid phosphatase activity might contribute, in part through its interference in the phosphorylation/dephosphorylation, to the diminished intracellular killing ability of PEM.*

INTRODUCTION

The immunomodulating effects of vanadium have been recently disclosed (Ramanatham and Kern, 1983). Studies in this laboratory revealed that these effects were targeted primarily toward cell-mediated immunity (Cohen et al., 1986). Dose-dependent decreases in phagocytosis and intracellular killing of challenging *Listeria monocytogenes* by peritoneal macrophages were demonstrated in mice treated intraperitoneally with ammonium metavanadate (Cohen et al., 1989). Production of superoxide anion necessary for intracellular killing of *Listeria* was significantly reduced, and the enzymes glucose-6-phosphate dehydrogenase and gluta-

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thione reductase, both involved in respiratory burst activity, were also inhibited (Cohen and Wei, 1988).

Apart from highly toxic oxygen radicals, such as O_2^- and OH , lysosomal enzymes were shown to be important contributors to the antimicrobial function of macrophages (Gordon, 1980). In the case of *Listeria,* it was shown that following internalization into the phagosome and phagolysosome fusion, the microorganisms were subjected to the action of lysosomal enzymes (North and Mackaness, 1963a, 1963b). Goldstein et al. (1978) demonstrated the coincidence of reduction in lysosomal enzyme activities with the impairment of bactericidal activity of alveolar macrophages. The ability of lysosomal enzymes to degrade membrane glycoproteins, glycolipids, and glycosaminoglycans is thought to play an important role in (1) intracellular oxygen-independent killing of invading microbial pathogens and (2) expression of processed antigen on the surface of antigen-presenting cells, such as macrophages (Roitt et al., 1989). Mouse peritoneal macrophages were shown to contain intracellular lysosomal acid hydrolases, which can be released in substantial amounts into culture medium in the absence of cell death (Schorlemmer et al., 1977).

Several environmental chemicals and metals such as magnesium, mercury, and cadmium were shown to inhibit lysosomal acid hydrolases from *Diplococcus pneumoniae* at concentrations of 2 m*M* (Hughes and Jeanloz, 1968). This effect was attributed to the reversible formation of mercaptides between sulfhydryl groups of the enzyme (required for catalytic activity) and heavy metal ions.

The present study was carried out to investigate the effect of vanadate on activities and kinetics of select lysosomal enzymes as a possible contributing mechanism to the depression of the intracellular killing ability in peritoneal macrophages from vanadate-treated mice. The inhibitory effect of ammonium metavanadate on in vitro enzyme systems was also studied.

MATERIALS AND METHODS

Chemicals

All chemicals used were of reagent grade. Ammonium metavanadate (NH_4VO_3) was purchased from J. T. Baker and Co. (Philipsburg, N.J.), and sodium phosphate and ammonium chloride from Mallinckrodt Inc. (Paris, Ky.). Phenolphthalein-β-glucuronide, 2-nitrophenyl-*N*-acetyl-β-D-glucosaminide, *p*-nitrophenyl phosphate, lyophilized *Micrococcus lysodeikticus,* β-glucuronidase, *N*-acetyl-β-D-glucosaminidase, lysozyme, and acid phosphatase were obtained from Sigma Chemical Company (St. Louis, Mo.). Medium 199 was purchased from Gibco Laboratories (Grand Island, N.Y.).

In Vivo Studies

Dosing Regimen Ammonium metavanadate was dissolved in 0.1 *M* sodium phosphate buffer (pH 7.2) to yield solutions of 0.25 and 1.0 mg V/ml (V equivalent to metal, vanadium) for delivery of 2.5 and 10.0 mg V/kg body weight (one-eighth and one-half the ip LD50, respectively). These concentrations were chosen to bracket the doses used in earlier studies (Cohen et al., 1986) such that dose-dependent effects on the parameters studied could be inferred. Sodium phosphate buffer was used as the primary control. Ammonium chloride at 1.05 mg/ml was used as a second control so that mice received the equivalent amount of ammonium ion comparable to the high vanadium dosage. These solutions were filter sterilized, stored at 4°C, and brought to room temperature before injection.

Experimental Animals Six-week-old female $B_6C_3F_1$ mice (18–20 g, Harlan Sprague-Dawley Co., Indianapolis, Ind.) were fed Purina lab chow 5001 and water ad libitum. The body weights were recorded weekly to allow for appropriate doses of vanadium or control solutions. The mice were separated into 4 groups: 10 mg V/kg (10V), 2.5 mg V/kg (2.5V), phosphate buffer (PO_4), and ammonium chloride (NH_4Cl). Each group consisted of a minimum of 12 mice for cytolysate enzyme analysis, and 6 mice for intra- and extracellular enzyme activity studies. Intraperitoneal (ip) injections of vanadium or control solutions were given over a period of 6 wk, every 3 d, to allow for maximal excretion of the previous dose prior to injection (Cohen et al., 1986). Two days following final injection, mice were sacrificed and peritoneal macrophages harvested.

Cytolysate Preparation Peritoneal macrophages (PEM) were harvested separately from 3 mice/group/d in cold Hanks balanced salt solution (HBSS, pH 7.5) using the procedure of Cohen et al. (1986). After resuspending the PEM pellets in 3 ml of fresh HBSS, cells from 3 mice were combined to yield 1 cell population/treatment group/assay/d. The pooled suspension was repelleted and brought up in 3 ml of HBSS. Cell viability, as determined by trypan blue exclusion, and cell concentrations were determined routinely with yields of 80–85% macrophages and viabilities of more than 90%. The cell concentration was adjusted to 10^7 macrophages/ml, and cells were lysed by repeated (3 times) freeze-thawing in a dry ice : acetone system followed by centrifugation to pellet the sheared membranes. The supernatant was transferred to a test tube held on ice and immediately used for enzyme assays. The cytolysate of each treatment group was analyzed four times for each of the four test enzymes: β-glucuronidase, *N*-acetyl-β-D-glucosaminidase, acid phosphatase, and lysozyme. The process of PEM cytolysate preparation for lysosomal enzyme activity determination was repeated 3 more times until all 12 mice/group were killed.

Cell Preparation for Assay of Intra- and Extracellular Enzyme Activities PEM were harvested on the same day from each of 6 mice/group in medium 199 containing 5% bovine serum albumin and 1% glutamine. After pelleting in separate test tubes, PEM were washed and resuspended in 3 ml of fresh culture medium; cells from 2 mice were pooled. Following determination of cell concentration and viability, the volume was brought to 12 ml with the same medium. The cell suspension was dispensed into four 60 × 15 mm culture dishes (Corning Lab., Houston, Tex.), each with 4–6 × 10^6 cells. A total of 12 dishes was prepared for each treatment group.

The cultures were kept at 37°C in a 5% CO_2, humidified atmosphere for 9 d. Three days after seeding, the medium from each of the 12 dishes/group was collected and assayed separately for enzyme activities. Three milliliters of fresh medium was added to each dish and incubation was continued. On d 6 after seeding, culture medium was collected again for enzyme assay. Out of the 12 dishes/group, 3 were used for protein estimation (Lowry et al., 1951), following cellular hydrolysis by adding 1 ml of 0.2 *N* NaOH solution. Fresh medium was added to the remaining dishes and cultivation was continued for an additional 3 d. At the end of cultivation, medium from all nine dishes was analyzed for extracellular enzyme activities. Cells from three dishes were used for protein determination. Those from the remaining six dishes were used for analysis of intracellular enzyme activity. Medium 199 (1 ml) was added; the cells were scraped off the dish using a rubber policeman and then lysed by freeze-thawing as previously described. Each sample was analyzed in duplicate for activities of β-glucuronidase, *N*-acetyl-β-D-glucosaminidase, and lysozyme.

In Vitro Studies on the Effect of Ammonium Metavanadate on Enzyme Kinetics

NH_4VO_3 was dissolved in 0.1 *M* sodium phosphate buffer (pH 7.2) as various stock solutions to be used for studying the effect of vanadate on the kinetics of the 4 lysosomal enzymes. Equal aliquots of freshly prepared NH_4VO_3 stock solutions were added immediately before the addition of substrate to the individual reaction mixtures to yield final NH_4VO_3 concentrations of 5, 10, 15, and 20 m*M*. These concentrations corresponded to the doses used in previous in vivo studies at which significant depression in intracellular killing of mouse peritoneal macrophages was reported (Cohen et al., 1989). Controls for these in vitro studies included NH_4Cl and sodium phosphate.

Lineweaver-Burk analysis was used to determine inhibition kinetics of NH_4VO_3 on the four pure enzyme systems. The assay methods are described below.

Enzyme Assays The activity of β-glucuronidase in the cytolysates, culture medium, and enzyme preparations was assayed by the modified

method of Hashimoto et al. (1986). Sample (0.1 ml) was added to a mixture containing 0.1 ml acetic acid buffer (1 *M*, pH 4.5) and 0.1 ml phenolphthalein β-glucuronide (315 μ*M*) solution in H_2O. Following incubation for 30 min at 37°C with shaking in a water bath, 1.2 ml of glycine/sodium dodecyl sulfate (SDS) buffer (glycine 0.2 *M*, SDS 0.2%, pH 11.7) and 1 ml of distilled water were added to terminate the reaction. Absorbance of the mixture was measured at 550 nm against a sample blank containing all the components except substrate. Enzyme activity values for cytolysates and culture media were expressed in mU/10^6 cells, and for enzyme solutions in mU. One unit is defined as the amount of enzyme releasing 1 μmol of product in 1 min under the specified experimental conditions.

The method of Dean et al. (1979) was followed to measure the activity of *N*-acetyl-β-D-glucosaminidase. Sample (0.1 ml) was added to 0.1 ml of buffered substrate solution containing 16 m*M* 2-nitrophenyl *N*-acetyl-β-D-glucosaminide in 0.1 *M* pyridine HCl (pH 4.5) and 0.2% Triton X-100. The mixture was incubated for 10 min at 37°C with shaking in a water bath. After incubation, 1.2 ml of glycine/SDS buffer and 1 ml of water were added to the incubation mixture. Absorbance was measured at 420 nm against a sample blank containing all components but the substrate. Enzyme activity values were expressed and units defined as those for β-glucuronidase.

Acid phosphatase activity was measured by the method of Schnyder and Baggiolini (1978). Sample (0.1 ml cytolysate, medium, or enzyme preparation) was incubated for 10 min at room temperature with 1 ml of 4.5 m*M* *p*-nitrophenyl phosphate in 0.1 *M* acetic acid-sodium acetate buffer (pH 4.5) containing 0.05% Triton X-100. The reaction was stopped by adding 1 ml of 2 *N* NaOH, and absorbance was measured spectrophotometrically at 405 nm. Enzyme activity values were expressed and units defined as those of β-glucuronidase and *N*-acetyl-β-D-glucosaminidase.

A modified method of Schnyder and Baggiolini (1978) was used to measure lysozyme activity. Sample (0.1 ml) was added to 2.5 ml of buffered substrate solution consisting of *Micrococcus lysodeikticus* (100 μg/ml) in 0.067 *M* sodium phosphate buffer (pH 6.25). After mixing, the change in absorbance at 450 nm was monitored for a period of 3 min. In contrast to the other 3 enzymes, 1 unit of lysozyme activity is defined as the amount of enzyme that causes a ΔA_{450} of 0.001/min in a standard *Micrococcus lysodeikticus* suspension in a 2.6-ml reaction mixture at 37°C and pH 6.25.

Statistical Analysis

The statistical significance of the differences observed in the enzyme activities and cytolysate protein levels among the four treatment

groups was determined using the analysis of variance combined with a Duncan's analysis of the means test at p level of .05.

RESULTS

Kinetic analysis of the lysosomal enzymes revealed K_m values of 271.1 μM for β-glucuronidase, and 1.70 and 2.41 mM for *N*-acetyl-β-D-glucosaminidase and acid phosphatase, respectively. The K_m value for lysozyme could not be determined because of the lack of a molecular weight value for the substrate (*Micrococcus lysodeikticus*) used. Addition of ammonium chloride or ammonium metavanadate at the highest concentration of 20 mM had no significant ($p > .05$) effect on K_m values of β-glucuronidase, *N*-acetyl-β-D-glucosaminidase, and acid phosphatase. However ammonium metavanadate resulted in a dose-dependent linear noncompetitive inhibition of acid phosphatase (Fig. 1), with a K_i value of 14.8 mM (Fig. 2). Acetate buffer controls showed 21.4, 39.3, 48.9, and 57.2% inhibition in acid phosphatase activity with 5, 10, 15, and 20 mM NH_4VO_3, respectively. NH_4Cl showed no inhibitory effect on acid phosphatase activity. NH_4VO_3 and NH_4Cl had no effect on the activities of β-glucuronidase, *N*-acetyl-β-D-glucosaminidase, or lysozyme at any biologically relevant concentration (a maximum final concentration of 20 mM). Addition of sodium phosphate at 0.1 M did not affect β-glucuronidase, *N*-acetyl-β-D-glucosaminidase, or lysozyme activity, but inhibited acid phosphatase activity by 16.3%. When compared with so-

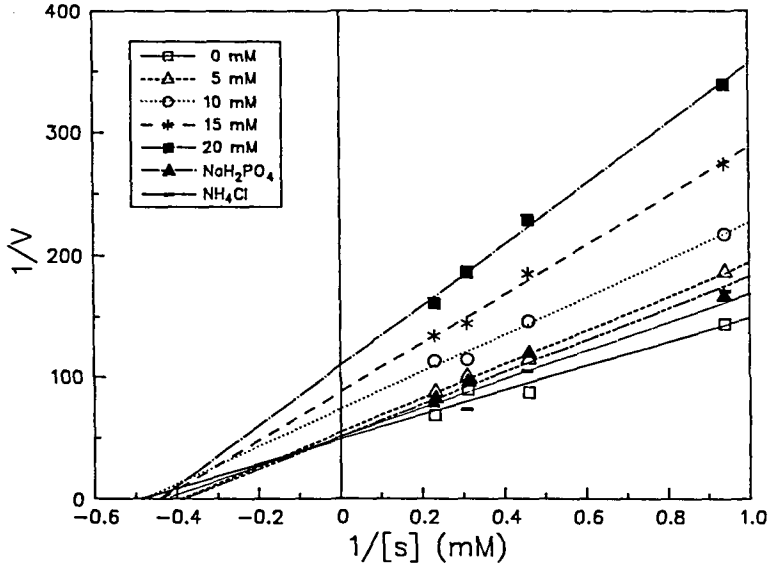

FIGURE 1. Inhibition of acid phosphatase activity by ammonium metavanadate at concentrations of 5, 10, 15, and 20 mM in the pure enzyme system. Enzyme level was held constant and vanadate levels were varied from 0 to 20 mM.

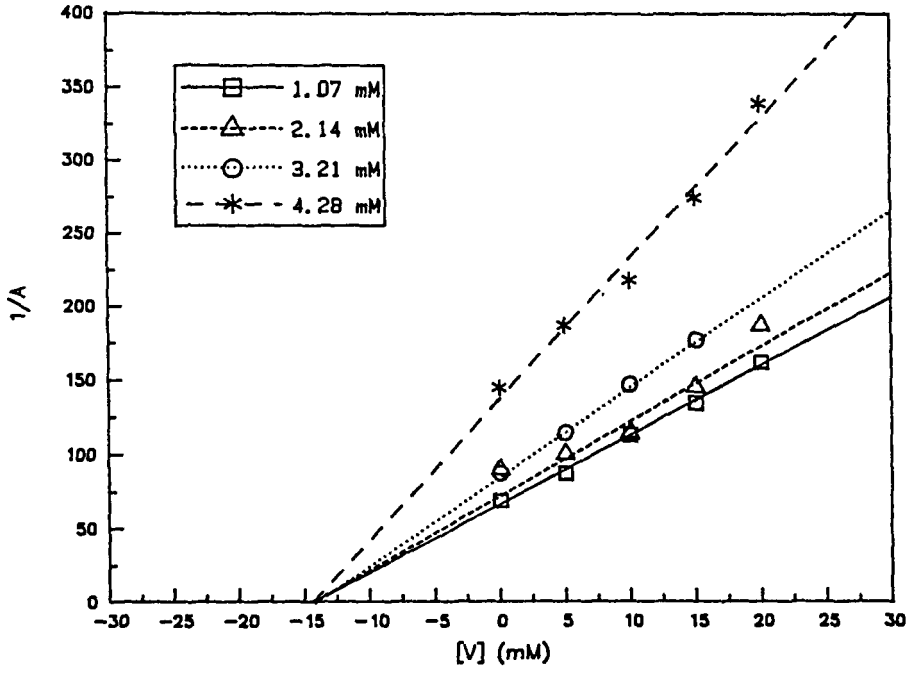

FIGURE 2. Dixon plot of concentration of vanadate versus 1/*A* for the determination of K_i for acid phosphatase; *A* is the absorbance change per min at 405 nm.

dium phosphate control, the NH_4VO_3 inhibition of acid phosphatase activity was found to be 5.1, 23.0, 32.6, and 40.9% at 5, 10, 15, and 20 m*M* vanadate, respectively.

A comparison of PEM intracellular enzyme activities per 10^6 cells among the four animal groups revealed that *N*-acetyl-β-D-glucosaminidase activity in the NH_4Cl group was significantly ($p < .05$) lower than the phosphate buffer control group (Table 1). β-Glucuronidase, acid phosphatase, and lysozyme activities were also lower in the NH_4Cl group than the phosphate buffer group, but the differences were not significant ($p > .05$).

Except for acid phosphatase and *N*-acetyl-β-D-glucosaminidase, the β-glucuronidase and lysozyme activities in the 2.5V group were similar to those of the phosphate buffer control (Table 1). Acid phosphatase activity in the 2.5V was significantly lower than in the phosphate buffer or NH_4Cl group. *N*-Acetyl-β-D-glucosaminidase activity in the 2.5V group was significantly ($p < .05$) lower than the phosphate buffer group (Table 1).

β-Glucuronidase, *N*-acetyl-β-D-glucosaminidase, and lysozyme in the 10V group were similar to those of the NH_4Cl group. Acid phosphatase activity in this group compared to the phosphate buffer and NH_4Cl groups was 44.7 and 42.2% less ($p < .05$), respectively (Table 1). The 10V

TABLE 1. Effect of Vanadate on the Activities of Different Lysosomal Enzymes in Mouse Peritoneal Macrophages

Enzyme	Phosphate buffer	NH_4Cl	2.5V	10V
β-Glucuronidase (mU)	0.84 ± 0.27	0.72 ± 0.08	0.81 ± 0.12	0.74 ± 0.21
N-acetyl-β-D-glucosaminidase (mU)	14.35 ± 0.07[a]	13.89 ± 0.11[b,c]	14.07 ± 0.28[c]	13.78 ± 0.20[b]
Acid phosphatase (mU)	1.14 ± 0.04[a]	1.09 ± 0.14[a]	0.88 ± 0.05[b]	0.63 ± 0.06[c]
Lysozyme (U)	5.87 ± 0.92	5.13 ± 0.99	5.38 ± 0.71	5.25 ± 0.64

Note. Mean values ± SD from eight determinations. Values bearing different superscripts are significantly different ($p < .05$). Enzyme activities are expressed per 10^6 cells.

group also had significantly less *N*-acetyl-β-D-glucosaminidase and acid phosphatase activities than the 2.5V group.

PEM intracellular enzymes from the four animal groups showed similar kinetic behavior patterns as observed in the in vitro studies. The percent reduction observed in acid phosphatase activity was approximately 25.6 and 45.7% for 2.5V and 10V treatments, respectively (Fig. 3).

Activities for the secreted lysosomal enzymes (β-glucuronidase, *N*-acetyl-β-D-glucosaminidase and lysozyme) from cultured PEM over a 9-d period are presented in Figure 4. Acid phosphatase was not found in

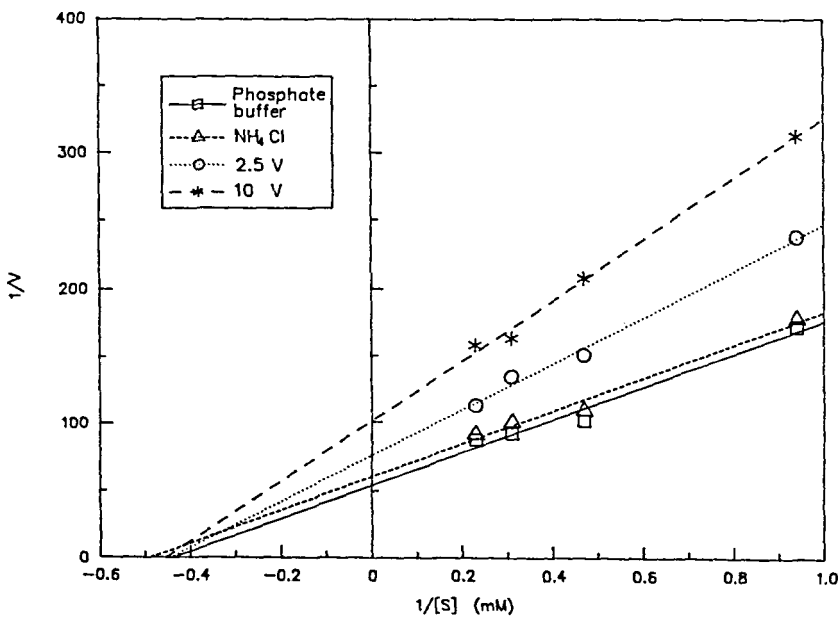

FIGURE 3. Ammonium metavanadate inhibition plot of intracellular acid phosphatase activity of murine peritoneal macrophages with *p*-nitrophenyl phosphate as substrate in 1 ml volume at room temperature.

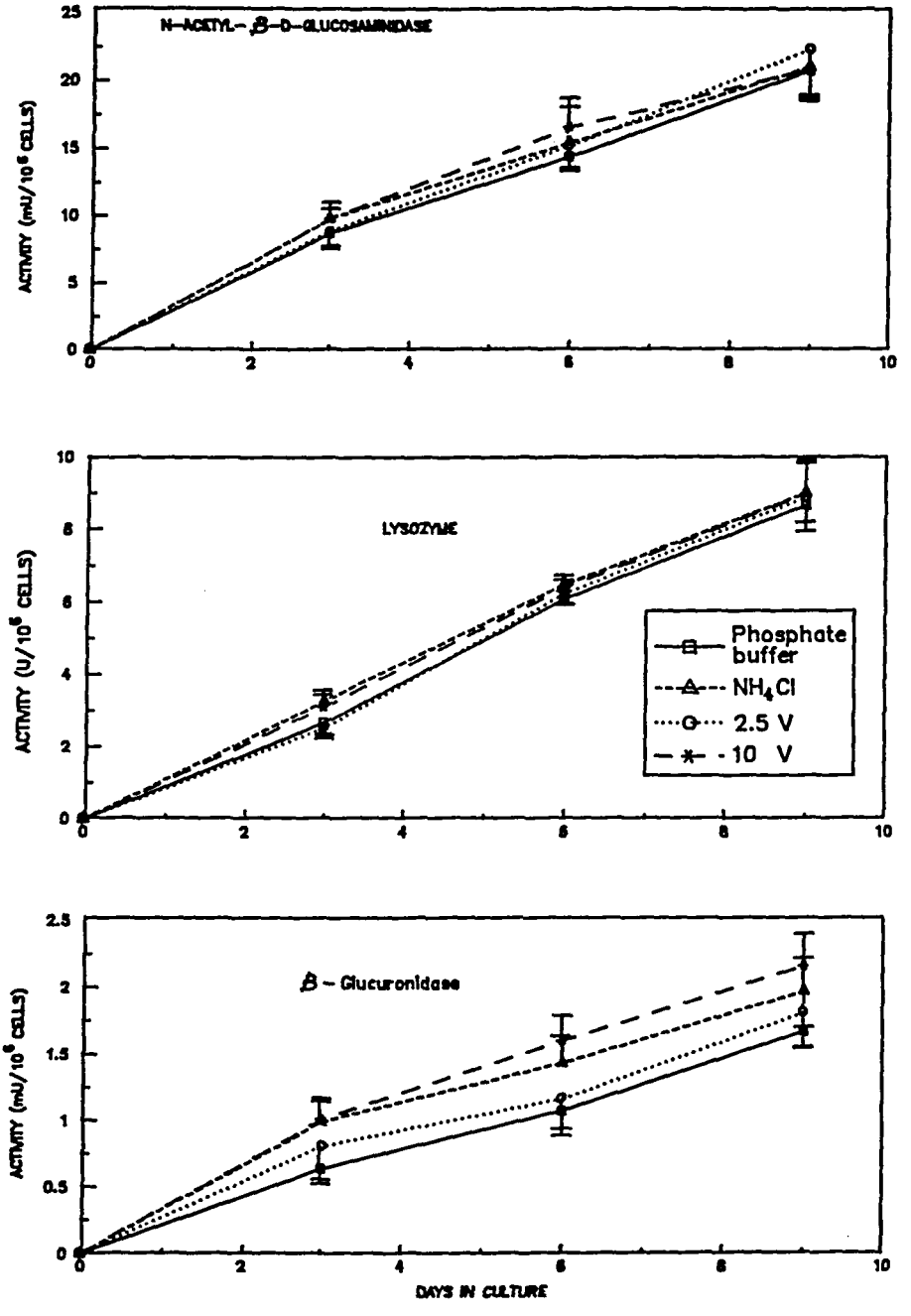

FIGURE 4. Effect of ammonium metavanadate on the extracellular secretion of β-glucuronidase, *N*-acetyl-β-D-glucosaminidase, and lysozyme in the murine peritoneal macrophages. Mean ± SD (n = 12) for each time point studied.

measurable amounts in the culture medium, while the other 3 enzymes were secreted at fairly constant amounts over the 9-d duration. In the first 3-d period, β-glucuronidase and lysozyme secretion were significantly ($p < .05$) higher in NH_4Cl and 10V groups than the other two groups. The secretion of *N*-acetyl-β-D-glucosaminidase was also higher in NH_4Cl and 10V groups but not significant ($p > .05$). Over the remaining period, the activities of the enzymes were very similar among all the treatment groups. The activities measured on the 9th day of incubation were 0.76, 0.72, 0.78, and 0.70 mU for β-glucuronidase, 12.95, 13.15, 12.58, and 12.67 mU for *N*-acetyl-β-D-glucosaminidase, and 4.87, 4.62, 5.04, and 5.23 U for lysozyme when expressed per 10^6 cells for the control, NH_4Cl, 2.5V, and 10V groups, respectively; the differences among the four treatments were not significant ($p > .05$).

The mean protein contents on d 6 of incubation were determined to be 71.2, 67.7, 70.8, and 73.3 $\mu g/10^6$ cells for phosphate buffer, NH_4Cl, 2.5V, and 10V groups, respectively; on d 9, the protein contents were determined to be 67.8, 61.3, 66.2, and 62.4 $\mu g/10^6$ cells for the same respective treatment groups.

DISCUSSION

The depression of phagocytic activity and intracellular killing of PEM are among the major immunotoxic effects of vanadium in mice treated with NH_4VO_3 (Cohen et al., 1989). Lysosomal enzymes are involved not only in the killing of ingested microorganisms, but also with the surface expression of microbial epitopes on macrophages (Roitt et al., 1989). Any agent interfering with lysosomal hydrolases either by inhibiting their activity or disrupting their proper export to lysosomes can have adverse consequences on cell-mediated immune response. This study was conducted to determine if vanadium treatment could inhibit the activities of lysosomal enzymes (including lysozyme and other acid hydrolases) in PEM, which in turn would depress intracellular killing of *Listeria*.

The activities of the intracellular lysosomal enzymes in phosphate buffer control mice (Table 1) were in accordance with those reported earlier by Schnyder and Baggiolini (1978). Except for *N*-acetyl-β-D-glucosaminidase and acid phosphatase, NH_4VO_3 treatment of mice at levels proven to inhibit PEM intracellular killing ability (Cohen et al., 1989) had no effect on β-glucuronidase and lysozyme activities (Table 1). Similar findings were reported by Waters et al. (1975), who showed specific activities of lysozyme and β-glucuronidase in alveolar macrophages were only slightly depressed by V_2O_5 at 50 μg V/ml; acid phosphatase was, however, inhibited by about 70% at this dose.

The data showing lower enzyme activities in ammonium chloride and 10V treated mice than in the phosphate buffer controls are in con-

sistence with the observations of Jessup et al. (1982). They inferred that ammonium ions raised the intralysosomal pH after entering the lysosomes by being protonated and trapped. As a consequence water enters and causes the organelles to swell, and results in loss of lysosomal enzymes.

Another effect of ammonium ion-mediated increase in intralysosomal pH is depletion of free mannose 6-phosphate receptors in cells (Sly et al., 1981). The newly synthesized lysosomal enzymes are diverted into secretory vesicles to be exported out rather than to lysosomes, because their normal segregation into lysosomes depends on their binding to the unoccupied mannose 6-phosphate receptors in Golgi bodies (Gonzalez-Noriega et al., 1981). However, acid phosphatase is not likely to be affected by the above mentioned mechanisms since it is exported to lysosome as a transmembrane protein and later cleaved into a soluble form (Waheed et al., 1988).

Vanadium treatment caused a dose-dependent decrease in intracellular acid phosphatase activity. Acid phosphatase levels in macrophages were used in earlier studies to reflect their intracellular killing ability. Goldstein et al. (1978) noticed the absence of acid phosphatase activity in macrophages with impaired microbiocidal activity. The inhibition of acid phosphatase might interfere with phosphorylation/dephosphorylation, the major regulatory events for activating NADH-dependent oxidase (Hayakawa et al., 1986), and for eliciting appropriate cell response to stimulation by extracellular ligands (Schneider et al., 1981).

In vitro studies on the effect of NH_4VO_3 on the activities of pure lysosomal enzymes were conducted to substantiate findings of the in vivo studies. The K_m values observed for acid phosphatase, β-glucuronidase, and *N*-acetyl-β-D-glucosaminidase are similar to those reported for the substrates used (Wang and Touster, 1972; Ayoub and McCarty, 1968). However, β-glucuronidase was not inhibited by vanadate. The metal did not interact with either substrate or the aglycone product.

Earlier observations on the effects of metal ions on the *N*-acetyl-β-D-glucosaminidase activity are varied. Hughes and Jeanloz (1968) reported that *N*-acetyl-β-D-glucosaminidase was inhibited by metals such as magnesium, mercury and cadmium at 2 m*M*. They attributed this to the presence of sensitive sulfhydryl groups, whereas Ayoub and McCarty (1968) found no effect by calcium and magnesium ions at 1 m*M*. In the present study, *N*-acetyl-β-D-glucosaminidase and lysozyme displayed similar behaviors irrespective of the presence of vanadate in the reaction mixture. No literature reports are available describing the effects of vanadate on lysozyme activity.

Studies on the inhibitory effect of vanadate on acid phosphatase revealed a dose-dependent linear non-competitive inhibition with a K_i value of 14.8 m*M*. Moran et al. (1989) also reported a noncompetitive inhibition by sodium vanadate on acid phosphatase activity from *Yarro-*

wia lipolytica. Tessier et al. (1989) demonstrated in several epithelial cell types the inhibitory effect of sodium orthovanadate on acid phosphatases including prostatic acid phosphatase, which affects dephosphorylation. Acid phosphatase from Madin Darby bovine kidney cells was depressed about 43% when cells were exposed to 1 m*M* sodium vanadate (Bracken and Sharma, 1989).

Acid phosphatase was not tested for extracellular secretion because it was reported not to be secreted extracellularly (Warren, 1989). A higher secretion of the other three enzymes was observed during the first 3-d period in the 10V and NH_4Cl groups (Fig. 4). These two groups also had consistently lower intracellular enzyme levels (Table 1). Based on the insignificant differences in extracellular enzyme activities among the four treatment groups after the 3-d period, the ammonium ion was believed not to cause any permanent damage to the enzyme transportation mechanism. The restoration of normal enzyme secretions on d 6 and 9 can be explained by the discontinuance of an external supply of vanadate or ammonium ions. Currently, no literature can be found on the interference of the synthesis of lysosomal enzymes by vanadate or ammonium ion. The fact that enzyme secretion was similar among all treatment groups over the experimental period also indicates that enzyme synthesis remains unaffected irrespective of vanadate treatment.

The results thus suggest that the decreased bactericidal activity in the PEM of vanadate treated mice is not due to direct inhibition of either activity or synthesis of β-glucuronidase, *N*-acetyl-β-D-glucosaminidase, or lysozyme. The inhibition of acid phosphatase activity by vanadate might be important in relation to the diminished intracellular killing ability of PEM through the interference in phosphorylation-/dephosphorylation involved in respiratory burst function, coupled with the already established theory of insufficient $NADP^+$ formation to drive the glucose-6-phosphate dehydrogenase and glutathione reductase cycle for cellular energy. Studies on the effect of vanadium on signal transduction pathways and antibody-dependent cell-mediated cytotoxicity and receptor damage might provide further insight into the molecular mechanisms of vanadium mediated immunotoxic effects.

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