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Effects of Ammonium Metavanadate Treatment Upon Macrophage Glutathione Redox Cycle Activity, Superoxide Production, and Intracellular Glutathione Status

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Female $B_6C_3F_1$ mice were given intraperitoneal injections of ammonium metavanadate (2.5 or 10 mg V/Kg), ammonium chloride, or sodium phosphate buffer every 3 days for 6 weeks. Resident peritoneal macrophages were harvested, lysed by freeze-thawing, and the resulting cytolysate was assayed for total protein content and enzyme activities of glutathione reductase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase. In addition, peritoneal macrophages were assayed for superoxide production using nitroblue tetrazolium reduction, as well as for intracellular levels of oxidized and reduced glutathione. Exposure of mice to vanadium resulted in a dose-trend depression in the three macrophage enzyme activities as compared with the controls. Vanadium treatment resulted in a similar decrease in the production of superoxide anion, and an increase in levels of oxidized glutathione; however, the total glutathione pool (reduced plus oxidized forms) was not affected.

Key words: mice, protein, enzyme activities

INTRODUCTION

The enzymatic events in both phagocytosis and killing of ingested microorganisms by various leukocytes have been well studied [19,48,49], but less so in the murine peritoneal macrophage (PEM) [4,27]. During the respiratory burst, there is an increase in cellular respiration, glycolysis, and oxidation of glucose through the hexose monophosphate shunt (HMS) [6,26,40] to provide the energy required for the phagocytic process as well as the oxygen used for intracellular killing. Thus, enzymes of the HMS and the glutathione redox cycle—glucose-6-phosphate dehydrogenase (G6PDH), glutathione reductase (GSHRX), and glutathione peroxidase (GSHPX)—play a role in macrophage phagocytosis and killing of microorganisms.

Exposure to vanadium-containing dusts in the workplace has been correlated with an increased incidence of pulmonary tuberculosis [18] as well as enhanced susceptibility to pneumonia and other respiratory diseases [46,50,53,57]. Although industrial settings are the primary sites for exposure to these dusts, ambient air levels of vanadium—the byproduct of oil and coal combustion for heat and energy production, are known to be high in metropolitan areas. The latter were shown in an epidemiological investigation to have a positive correlation with an increased incidence of deaths due to cancer, pneumonia, or bronchitis [55].

In vitro studies with macrophages exposed to vanadium described alterations in phagocytic capacities and lysosomal enzyme activity as well as high cell mortality [62]. Our earlier in vivo studies showed that mice treated intraperitoneally (IP) with sublethal doses of ammonium metavanadate (NH_4VO_3; 2.5, 5.0, or 10 mg V/Kg) displayed dose-dependent alterations in their immune responses [10]. Resistance to *Escherichia coli* endotoxin (LPS) was increased while resistance to viable *Listeria monocytogenes* was depressed; the IP LD_{20} value decreased 250-fold following a 3-wk exposure to 2.5 mg V/Kg (1/8 IP LD_{50}). LPS lethality resulted, in part, from the phagocytosis and processing by macrophages and subsequent release of glucocorticoid-antagonizing factor (GAF) [7] which inactivated several enzymes required for body energy maintenance such that energy depletion and endotoxemic shock led to host death. Removal of *Listeria* is a three-phase process that encompasses direct engulfment by resident macrophages, followed by the recruitment and accumulation of activated macrophages [36]. The decreases in phagocytic activity of peritoneal macrophages obtained from vanadium-treated mice [10] would result in less GAF production and increased survival time for *Listeria* in the peritoneal cavity.

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Many metals have been studied for their effects on murine PEM viability, antimicrobial/phagocytic activity [32,58], the oxidative burst [1,37], and macrophage oxygen uptake [15,31]. Other studies have focused on the role of metal interactions with structural components such as microtubules [2], membrane receptors [14], or ionophores [54] in the modulation of macrophage activity. Our in vitro studies using purified G6PDH and GSHRX indicated that NH_4VO_3 was a potent inhibitor [11,12]. GSHRX contributes to enhanced NADPH oxidase and HMS activity early in the respiratory burst [56,64] and vanadium inhibition may impact upon the production of superoxide anion. This study determined the effects of NH_4VO_3 on three enzymes involved in energy production for phagocytotis and for the removal of peroxides, as well as the production of superoxide anion.

MATERIALS AND METHODS[1]

Dosing Regimen

Ammonium metavanadate (NH_4VO_3, J.T. Baker Co., Philipsburg, NJ) was dissolved in 0.1 M sodium phosphate buffer (pH 7.2) to yield solutions of 0.25 and 1.0 mg V/ml buffer ("V" equivalent to metal atom and not the compound or vanadate ion) for delivery of 2.5 and 10.0 mg V/Kg body weight (1/8 and 1/2 IP LD_{50}, respectively). These concentrations were chosen to bracket the doses used in earlier studies [10] such that dose-dependent effects on the parameters studied could be inferred. Sodium phosphate buffer was the primary control; ammonium chloride (1.05 mg NH_4Cl/ml) was used as a second control so that mice received the equivalent amount of ammonium ion given to mice receiving the high vanadium dosage. Solutions were filtered and stored in sterile tubes, refrigerated, and brought to room temperature before injection.

Six-week-old female $B_6C_3F_1$ mice (18–20 g, Harlan Sprague-Dawley Co., Indianapolis, IN) 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 four groups: 10 mg V/Kg, 2.5 mg V/Kg, phosphate buffer, or ammonium chloride; each group consisted of a minimum of 12 mice. IP injections were given every 3 days for a period of 6 wk to allow for a maximal excretion of the previous dose prior to injection [10]. Two days following the final injection, the mice were sacrificed and peritoneal macrophages harvested.

Cytolysate Preparation

PEM were harvested in Hanks' balanced salt solution (HBSS, pH 7.5) using the procedure of Cohen et al. [10]. After resuspending the macrophage pellets in 3 ml fresh HBSS, the cells from 3 mice were combined to yield one cell population per treatment group per assay per day. The pooled suspension was pelleted and brought up in 3 ml of H/T solution (2:1 (v/v) solution of HBSS:TE [triethanolamine buffer, 0.05 M, pH 7.5]). Cell viability (using trypan blue exclusion) and cell concentration were measured with yields routinely containing 80–85% macrophages and viabilities >90%.

The cell concentration was adjusted to 10^7 cells/ml H/T solution. After freeze-thawing three times in a dry ice:acetone system, the suspension was centrifuged to pellet the sheared membranes. The supernatant was transferred to a test tube held on ice and immediately used for the enzyme assays. Protein levels were determined using the Bio-Rad system (Bio-Rad Laboratories, Richmond, VA); each treatment cytolysate was analyzed five times for each enzyme preparation.

Assay of Glucose-6-Phosphate Dehydrogenase Activity

The method of Lohr and Waller [30] as modified by Vogt et al. [61] was used to analyze the activity of the G6PDH in the cytolysate. Aqueous maleimide (0.1 ml, 4 mM), $MgCl_2$ (0.05 ml, 3 mM), $NADP^+$ (0.1 ml, 1.2 mM in 1% $NaHCO_3$), and 0.1 ml of the lysate were added to TE buffer (0.55 ml, 27.5 mM). After 5 min incubation at 25°C, glucose-6-phosphate (0.1 ml, 4 mM) was added; the clear solution was mixed and transferred to a cuvette; and the absorbance at 340 nm measured every 30 sec for 5 min. The reference cell contained all components excluding the $NADP^+$ and the cytolysate. The total moles of NADPH formed per minute was calculated and the specific activity was defined as 0.1 mole of NADPH formed/min per mg protein. In this study, as well as with the other enzymes, the assay was repeated five times for each treatment cytolysate and was performed with freshly harvested PEM in six separate experiments.

Assay of Glutathione Reductase Activity

The method of Racker [43] was used to assay GSHRX activity in the cytolysates. Potassium phosphate buffer (KPO_4, 0.05 ml, 1 M, pH 7.6), bovine serum albumin (0.1 ml, 0.1% in 0.1 M KPO_4 buffer), NADPH (0.1 ml, 0.1 mM), and 0.1 ml of lysate were added to 0.55 ml water. Oxidized glutathione (GSSG, 0.1 ml, 3.3 mM) was added, the solution was mixed, and the decrease in absorbance at 340 nm was monitored every 30 sec for 5 min against a reference without NADPH. Specific activity was defined as 1.0 nmole NADPH oxidized/min per mg protein present.

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[1]Unless otherwise indicated, all chemical reagents are products of Sigma Chemical Company, St. Louis, MO.

Assay of Glutathione Peroxidase Activity

The method of Paglia and Valentine [42] was used to assay GSHPX activity in the cytolysates. Bovine mucosa GSHRX (0.05 ml, 1 unit), NADPH (0.1 ml, 0.2 mM), and 0.1 ml of lysate were added to 0.71 ml of a solution containing NaN_3, reduced glutathione (GSH), and Na_2EDTA (each 1 mM in 50 mM sodium phosphate buffer, pH 7). After 5 min incubation at 25°C, aqueous cumene hydroperoxide (0.04 ml, 1.5 mM) was added and the absorbance at 340 nm was monitored every 30 sec for 5 min. The data was treated as above in the GSHRX experiment. A solution containing HBSS in place of the cytolysate was also assayed to determine the contribution from spontaneous oxidation of NADPH by the peroxide. This background rate was subtracted from the observed rate in order to obtain the true activity in the cytolysate. The reference blank contained all reagents but the lysate and NADPH.

Assay of Superoxide Anion Production by Means of Nitroblue Tetrazolium Reduction

The method of Godfrey and Wilder [21] was used to measure superoxide production in PEM fed opsonized zymosan. The macrophage suspension was adjusted to 10^6 cells/ml in complete medium (C-MEM) containing 20% heat-inactivated fetal calf serum (v/v). Aliquots containing 10^5 cells were transferred to a glass coverslip held in a Leighton tube, 0.9 ml of 37°C C-MEM was added, and the tubes stoppered with foam plugs. The tubes were incubated at 37°C (with 5% CO_2) for 1 hr to allow for cell attachment prior to the addition of fresh medium containing opsonized zymosan (1 mg/ml) [25] and nitroblue tetrazolium chloride (NBT, 0.5 mg/ml in C-MEM). After allowing 1 hr for phagocytosis, the coverslips were removed and rinsed with warmed PBS, the cells fixed in methanol and then stained with 0.2% (w/v) safranin O (in 1% acetic acid) for 5 min before mounting onto slides for counting. The relative number of NBT-positive cells was determined from a population of 200 cells per slide. Only cells that contained nondiffuse regions of deep blue stain were scored as positive. Ten slides per treatment were prepared for each of three separate experiments.

Assay of Macrophage Reduced and Oxidized Glutathione Content

The method of Tietze [60] was employed to measure the levels of GSSG and total glutathione (GSH + GSSG) in PEM. The pooled cell suspension of four mice was adjusted to 10^7 cells/ml H/T solution; 2.0 ml was put aside and the remaining solution frozen for later use in assaying total glutathione. The 2 ml suspension was immediately analyzed for GSSG since previous work indicated that prolonged storage of GSH resulted in the increased formation of GSSG. After the cytolysate was prepared, N-ethylmaleimide (NEM, 1.5 ml, 50 mM) was added to 1.5 ml of sample and the mixture placed on ice for 1 hr. In a 20-ml separatory funnel, the solution was extracted five times with 3 volumes of ethyl ether. The recovered aqueous layer was flushed with nitrogen to remove any remaining ether, the volume recorded, and the sample assayed for GSSG.

NADPH (0.05 ml, 0.2 mM) and 0.1 ml of cytolysate were added to 0.8 ml of a 0.75 mM solution of DTNB (5,5′-dithiobis-(2-nitrobenzoic acid), and the mixture incubated at 25°C for 5 min. Bovine GSHRX (0.05 ml, 1 unit) was added and the absorbance at 412 nm recorded every 15 sec for 3 min. The reference blank contained DTNB and NADPH but no enxyme since this led to reduction of DTNB. Each cytolysate solution was assayed 10 times; each treatment was analyzed using freshly harvested cells in 3 separate experiments. The total GSSG present was calculated from a standard curve prepared from the analysis of commercial GSSG mixtures.

To determine the total glutathione content, the frozen cell suspension was processed to yield the cytolysate, and a 0.1 ml aliquot assayed directly in the system described above. From the values for total glutathione and GSSG in the cytolysate, the amount of free GSH was calculated.

Statistical Analysis

Enzyme activities, glutathione levels, cytolysate protein levels, and the NBT assay results were analyzed using an analysis of variance (ANOVA) treatment. Duncan's analysis of the means was applied at P levels of 0.05 and 0.01 to determine significance among the treatments.

RESULTS

Based upon the known inhibitory effects of vanadate (V) on cellular protein degradation [38], the cytolysates were assayed for protein content in order to account for any possible contributions to enzyme activity from a buildup of nondegraded enzyme (Table 1). The protein level was significantly increased by the 10V (10 mg V/Kg BW) treatment with intracellular protein levels 2.2–2.5 times greater than in the controls, and 1.9 times that from the cells of the 2.5V mice. The 2.5V regimen increased the level of protein in macrophages by 18–30% above the controls, but this increase was not significant ($P < 0.05$).

The effect of mouse pretreatment with 2.5 or 10 mg V/Kg body weight resulted in dose-trend depressions of macrophage enzyme activity and was more severe regarding the G6PDH and GSHRX than with the GSHPX. The G6PDH activity (Table 1) from the 2.5V mice was 59% of the controls, while that from the 10V was only

TABLE 1. Mouse Peritoneal Macrophage Enzyme Activities and Cytolysate Protein Levels After 6 Weeks of Intraperitoneal Exposure to Ammonium Metavanadate

Treatment[a]	Activity (units/mg protein)[b]			N	Cytolysate protein levels[c]
	G6PDH	GSHRX	GSHPX		
2.5V	1087.2 ± 610.8	129.8 ± 65.5**	924.6 ± 141.1	11	14.1 ± 4.6
10V	542.8 ± 289.8***	77.1 ± 40.9***	542.9 ± 241.6***	10	26.6 ± 12.1***
N+	1977.3 ± 797.4	235.3 ± 76.1	1072.8 ± 170.0	11	10.7 ± 3.2
P	1735.1 ± 464.7	221.9 ± 37.8	982.6 ± 302.8	11	12.0 ± 4.1

[a]Treatments: 2.5 mg V/kg (2.5V), 10 mg V/kg (10V), NH_4Cl (N^+), sodium phosphate buffer (P).
[b]Units defined as 1.0 nmole NADPH oxidized/min for GSHRX and GSHPX, and 0.1 nmole NADPH formed/min for G6PDH. Each value is the mean ± S.D. of 6 separate experiments with 5 replicates per cytolysate.
[c]microgram protein present in 10^6 Mϕ using N cytolysates.
,*Value only significantly different from controls at $P < 0.05$ and at $P < 0.01$, respectively.

29%. The studies of GSHRX revealed similar results; the 2.5V treatment activities were 56% of the control values while the 10V activities were significantly ($P < .01$) reduced to 34% of the controls. In the GSHPX assay, the 10V treatment resulted in the lowest peroxidase activity. This value was only 53% of the controls and was significantly lower than the 2.5V treatment and the controls ($P < 0.01$). Among the three enzymes studied, the 2.5V treatment exhibited the least inhibitory effect upon the GSHPX. Within each of these studies, the ammonium chloride and phosphate buffer control animals did not show significant differences between their enzyme activities ($P < 0.05$).

The production and release of superoxide anion in PEM from vanadium-treated mice was significantly decreased in a dose-dependent manner ($P < 0.01$, Fig. 1). The control resident macrophages yielded populations of 41–45% positive cells while those from the 2.5V mice were only 26% positive. The 10V-treated mice had only 18% NBT-positive macrophages containing the insoluble blue formazan; a value significantly lower than the controls and the 2.5V group.

There were no significant differences among the four groups in the total GSH pool per million cells (Fig. .2) as a result of the 6-wk treatments. When the macrophages were assayed for just the GSSG, only the 10V mice had significantly ($P < 0.05$) higher levels than the controls. Vanadium treatment increased GSSG levels relative to the paired controls by 25% and 63% in the 2.5V and 10V groups, respectively. Consequently, vanadate treatment also resulted in lowered levels of free reduced GSH, with the 10V levels 13% lower than the controls.

The cytolysates were also analyzed to determine if there was any shift in the ratio of levels of GSSG to GSH (Fig. 3). The ratios of GSSG to free GSH and to the total GSH pool were dose-dependently, but not significantly, increased by vanadate treatments. These mice had GSSG levels equivalent to 48–63% of the total GSH pool compared to 42% for the controls. Regarding the free GSH, the GSSG levels in cells from vanadate-treated mice yielded ratios of 0.84:1–1.59:1, while controls averaged 0.74:1–0.80:1.

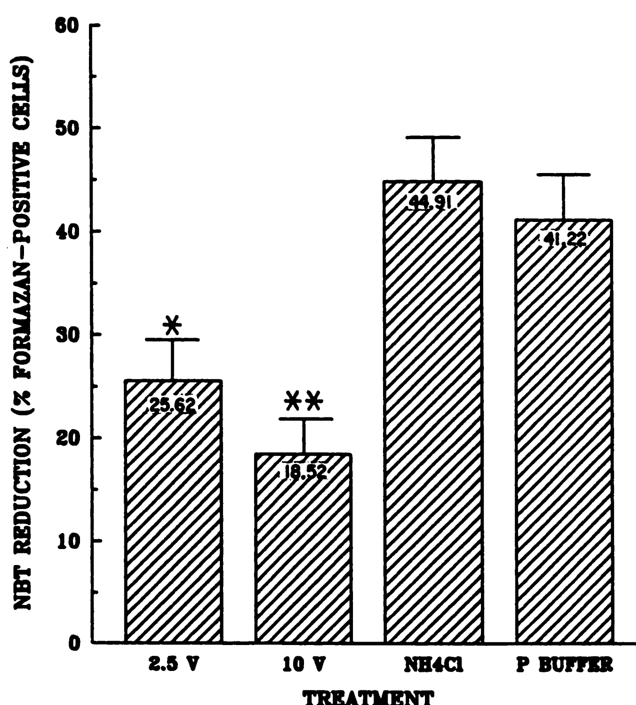

Fig. 1. Percentages of cells that stained deep blue with precipitated formazan within phagocytic vesicles 30 min after ingestion of opsonized zymosan (NBT-positive cells). Mouse dousing regimens (treatments) are as outlined in Materials and Methods. Each bar represents the mean (± SD) of four assays performed in duplicate on separate days. Values that are significantly different from controls (*) and from each other (**) at $P < 0.01$ are noted. The percentage formazan-positive cells were determined from populations of 200 cells per slide.

DISCUSSION

Exposure of mice to ammonium metavanadate has previously been shown to alter their resistance to *L. mono-*

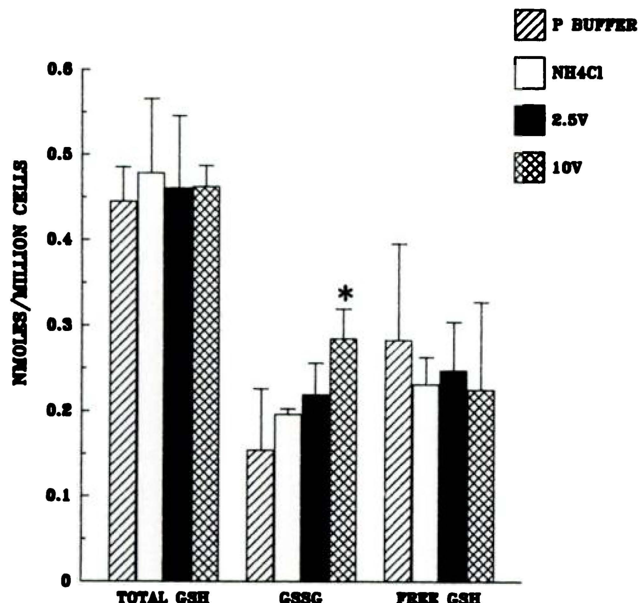

Fig. 2. Murine peritoneal macrophage total (combined forms), oxidized, and reduced glutathione content after 6-week exposure of mice to 2.5 mg V/kg, 10 mg V/Kg, ammonium chloride, or sodium phosphate buffer. The bars represent the mean (± SD) from 3 experiments per treatment performed on separate days. Ten replicates were assayed from freshly harvested cells on each day. Values significantly different from the controls (*) at $P < 0.05$ are noted.

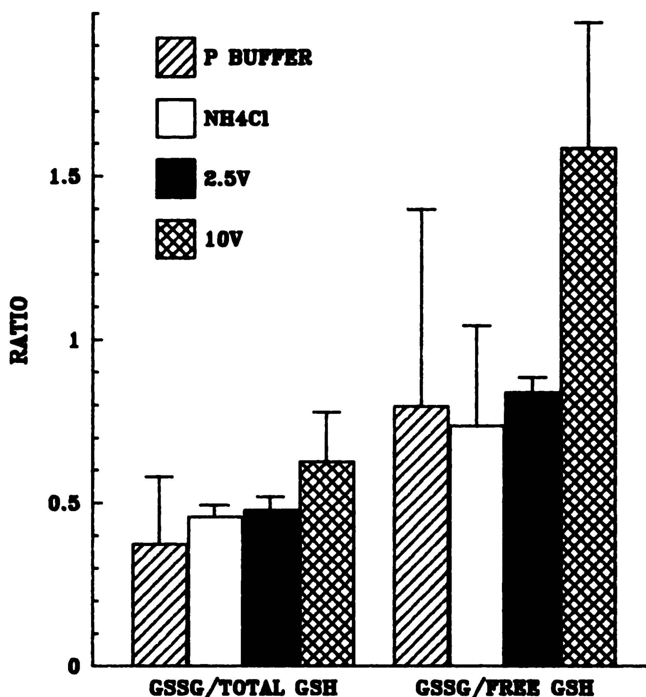

Fig. 3. The ratio of the total oxidized glutathione to (right) reduced glutathione and to (left) the total glutathione pool (oxidized plus reduced forms) in macrophages harvested after 6-wk pretreatment of mice with vanadium or control solutions. Details are as indicated in Figure 2.

cytogenes and to enhance resistance to *E. coli* endotoxin [10]. The depressed phagocytic capacity of the resident PEM from vanadate-treated mice appears to be the immunologic event which links these observations since reduced phagocytic and antigen-processing activities would protect the host from endotoxin lethality by reducing the release of GAF but would allow for increased bacterial survival. Resistance to *Listeria* requires recruitment of macrophages to the site of infection, a process mediated by monokine release from stimulated T-cells. The effects of vanadium exposure in vivo on T- and B-cells are not clear; but in vivo [34,59] and in vitro [44] studies have shown that vanadium inhibited mitogenic responses of T-cells and thymocytes during differentiation and proliferation stages.

The study described here sought to determine if vanadate pretreatment could contribute to the earlier observed depressed phagocytic responses and *Listeria* killing by inhibiting enzymes related to the respiratory burst in PEM (including G6PDH, GSHRX, and GSHPX). The burst produces the substrates necessary for energy-producing pathways for phagocytosis as well as for the uptake of oxygen for the formation of toxic oxygen metabolites to be used for killing the ingested pathogen.

The value for the G6PDH activity of the nonelicited, nonactivated murine PEM from control mice fell into the predicted range for this type of cell [29]. The treatment of mice for 6 wk with 2.5 or 10 mg V/Kg doses led to a 41% and 71% decrease in the G6PDH activity, respectively. In vitro studies with cadmium indicated that macrophage flavin- and pyridine-linked dehydrogenases could be inhibited by the metal acting as a dithiol binding agent [39]. In cell-free systems, vanadate has been shown to bind thiol groups to yield thioesters of vanadium [47]. In vitro inhibition of the G6PDH by vanadate was shown to be the result of inhibition of the binding of the pyridine cofactor to the active site [11]; a similar mechanism in the intact cell is possible. Vanadium exposure that inhibited glycolysis enzymes such as hexokinase [9] might also affect the activity of the HMS by decreasing production of the G6PDH substrate.

Cellular GSHRX was believed to act only as the substrate regeneration enzyme for GSHPX-mediated detoxification of cellular peroxides. Strauss et al. [56] showed that GSHRX participated in the initiation of the energy producing steps needed for phagocytosis as well as for the enhanced conversion of oxygen to superoxide. Comparative values for GSHRX activity in resident PEM were not available although the values obtained in this study were similar to levels within alveolar macrophages [20]. Several inorganic anions, including vanadium, have been shown to inhibit the GSHRX when it was studied as

a purified protein [12,38], but no studies in PEM have been reported. The exposure of mice to 2.5 and 10 mg V/Kg for 6 wk resulted in significant decreases (43% and 66%, respectively) in GSHRX activity. The depressed levels of activity of the macrophage GSHRX relative to the controls paralleled that observed with the G6PDH; this demonstrated the interdependence of these two enzymes in the cell. In studies with commercially prepared enzymes, the G6PDH was more sensitive than the GSHRX to the inhibitory effects of vanadate [11,12].

In vitro studies have shown that vanadate increased the activity of NAD(P)H oxidase as well as oxygen uptake in microsomal and membrane preparations of mouse hepatocytes [35,45] in the presence of exogenous NADPH. Based on the GSHRX work by Strauss et al. [56], with decreases in activities of both the G6PDH and GSHRX, the decreased availability of reducing equivalents might negate any enhanced oxidase activity and so the production of superoxide anion following phagocytosis was expected to decrease. The production of superoxide anion measured indirectly by NBT reduction was decreased in a dose-dependent manner following vanadate treatment. The qualitative NBT-reduction study yielded a normal population of 41–45% positive cells from control mice [21], while 2.5V- and 10V-mice yields were only 26% and 18% positive, respectively. Although quantitative studies of NADPH oxidase activity or assays of ferricytochrome c reduction would have better described the effect of vanadam treatment on superoxide production, the use of qualitative studies has been shown to accurately reflect the status of macrophages exposed to exogenous agents or during diseased states of the host [5,25].

The possible contribution from the effects of vanadium on macrophage phagocytosis were considered since in an earlier study, treatment of mice with 2.5V or 10V decreased phagocytosis in cultured macrophages by 13 and 21%, respectively [10]. With levels of NBT-positive cells 40 (2.5V) and 58% (10V) lower than the controls, the effects from decreased NADPH oxidase or GSHRX activity likely accounted for the remainder. Using the observed relative activities of GSHRX, expected positive populations would be 24% for the 2.5V mice and 15% for the 10V mice (using 43% as the control level). This decrease in superoxide production was in keeping with the previously observed increased lethality due to *Listeria* infection in vanadate-treated mice since these anions play a role in the listericidal activity of PEM [22].

GSHPX serves in the macrophage to destroy cellular peroxides which can result from leaching from phagolysosomes. Unlike the GSHRX, there was a significant decrease in the peroxidase activity only after treatment with the higher vanadium dose. The decrease in GSHPX activity relative to the paired controls was only 10% for the 2.5V mice and 47% for the 10V group. Decreases in peroxidase activity help explain the earlier observations in in vivo and in vitro studies where levels of peroxides were increased in several tissues after exposure of mice to pentavalent vanadium [16,17].

Because GSHPX was dependent upon GSHRX for substrate, the resulting changes in activity of the peroxidase were expected to parallel those in the latter. However, activities 20–30% greater (relative to the controls) than the reductase activities at corresponding vanadium doses were common. This suggested that peroxidase activity was maintained in the macrophage at the cost of cellular GSH in order to maintain cell viability against peroxide buildup; viability was unaffected by vanadate treatments.

Exposure to vanadate resulted in alterations in the ratio of oxidized and reduced glutathione in the cell. Studies of the effects of altered GSSG:GSH ratios upon glucose utilization and HMS activity [24], and phagocytosis and NBT reduction [23] have been performed. The levels of total GSH (GSH + GSSG) in the control macrophages were not significantly different from those in the cells form vanadate-treated mice. Vanadate treatment resulted in increases in the ratio of GSSG to both the free GSH and the total glutathione pool. The levels of GSSG were 25% and 63% greater for the 2.5V and 10V mice, respectively, compared with the paired controls. Although cellular GSSG levels were increased by vanadate treatment, a toxic level of the disulfide was evidently not reached; toxicity from a buildup of intracellular GSSG could be prevented by its transport out of the macrophage [61].

This study has clearly shown that intraperitoneal exposure of mice to vanadium for 6 wk could affect three cytoplasmic enzymes crucial to PEM function, as well as the level of substrate (GSH or GSSG) required for their proper activity. The use of the IP route of exposure in our model would seem inappropriate since exposure to vanadium is primarily through inhalation or ingestion. However, a comparison of studies using intratracheally or IP-administered pentavalent vanadium showed similarly rapid patterns of metal clearance into the systemic blood [13,52]. The transient clinical symptoms such as dyspnea, reduced locomotion, depressed appetite, and diarrhea following IP exposure were similar to those observed using other exposure routes. Based upon government-established threshold limit values for ambient vanadium in the work environment [3] and in a high-risk setting [28], our mouse model has been estimated to receive with each dose (2.5V) the equivalent of 110 days and 25 days of exposure, respectively, for a normal adult steel, chemical, or mine worker. Thus, our 6-wk exposure period may provide a model for the analysis of the effects of vanadium on macrophage function after long-term exposure.

After entering cells via the anion exchange pathway for phosphate uptake, vanadium alternates between the +4 and +5 valence states, and binds several intracellular ligands [8,33,41]. No studies of the levels of intracellular vanadium in PEM following IP exposure have been performed, but our studies of macrophages viability suggest that toxic levels of the metal were not attained. A more quantitative analysis is certainly recommended for our future studies.

The results of these experiments explain, in part, the earlier observed dose-dependent depression in macrophage phagocytic capacities after vanadate exposure [10]. Unlike pulmonary macrophages, PEM in the oxygen-poorer environment relied primarily upon glucose metabolism rather than oxidative phosphorylation as the primary source of cellular energy. The absence of endogenous cellular myeloperoxidase in these cells enhanced the importance of the enzymes studied here for the production and detoxification of activated oxygen metabolites used for intracellular killing. The decrease in the assayed enzyme activities as well as the drop in production of superoxide anion likely served as the underlying basis for the enhanced susceptibility of these mice to viable *Listeria monocytogenes*; failures within the respiratory burst have been implicated in the virulence of other intracellular parasites [63]. Studies of *Listeria* ingestion and killing by PEM after vanadate treatment are currently in progress.

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