Int. J. Immunopharmac., Vol. 13, No. 8, pp. 1167–1176, 1991.
Printed in Great Britain.

0192–0561/91 $3.00 + .00
Pergamon Press plc.
International Society for Immunopharmacology.

MODULATION OF Fc_γ RECEPTOR EXPRESSION AND FUNCTION IN MOUSE PERITONEAL MACROPHAGES BY AMMONIUM METAVANADATE*

KRISHNA VADDI and CHENG-I WEI†

Food Science and Human Nutrition Department, University of Florida, Gainesville, FL 32611-0163, U.S.A.

(*Received* 13 *March* 1991 *and in final form* 1 *May* 1991)

Abstract — Resident peritoneal macrophages (PEM) harvested from female $B_6C_3F_1$ mice given an intraperitoneal injection of ammonium metavanadate (2.5 or 10 mg V/kg), an equivalent amount of ammonium in the form of ammonium chloride, or sodium phosphate buffer (0.1 M, pH 7.2) every third day for 6 weeks, were subjected to flow cytometric analysis of $Fc_\gamma 2a$ and $Fc_\gamma 2b$ receptor expression, and photometric microassay to measure receptor mediated binding and phagocytosis of sheep red blood cells (SRBC). The NH_4Cl and 10V groups showed 21.7 and 17.2% lower mean fluorescence channel (MFC) values and 7.1 and 5.9% lower values in percentage fluorescence-positive cells than the phosphate buffer control with respect to $Fc_\gamma 2a$ expression. For $Fc_\gamma 2b$ expression, the 10V group showed significantly ($P<0.05$) lower MFC (31.2%) and percentage fluorescence-positive cells (15.7%) than the phosphate buffer control. Though the four groups did not show a significant difference in $Fc_\gamma 2a$ mediated binding and phagocytosis of SRBC, the 10V group showed a significantly lower $Fc_\gamma 2b$ mediated binding and phagocytosis. The results indicate that the reduction in $Fc_\gamma 2b$ expression and function could contribute toward the previously observed depression in phagocytosis, NADPH-oxidase and superoxide generation in peritoneal macrophages obtained from vanadate-treated animals.

Cell surface receptors for the Fc portion of IgG ($Fc_\gamma R$) are membrane phospholipoproteins; their expression on the macrophage surface is integral to the function of the immune system. Interaction of opsonized pathogens with macrophage Fc receptors initiates cellular defense mechanisms including phagocytosis, microbicidal activity, antibody-dependent cell-mediated cytotoxicity, and production of reactive intermediate metabolites (Pontzer & Russell, 1990).

Studies on the expression and function of these receptors are of clinical significance, particularly in patients with leukemia and immunodeficiency. These patients are shown to have major aberrations in the number and distribution of these receptors on cells of the immune system (Pepys & Pepys, 1983). No attempt was made earlier to study the effect of immunosuppressive drugs on the function of $Fc_\gamma R$ as a possible mechanism of their action.

Studies on the structural heterogeneity of $Fc_\gamma R$ lead to the identification of two subclasses, $Fc_\gamma 2a$ and $Fc_\gamma 2b$ receptors for IgG2a and IgG2b, respectively (Anderson & Grey, 1977). $Fc_\gamma 2aR$ is shown to function by activating either protein kinase or membrane adenylate cyclase through mechanisms different from the hormone sensitive system (Fernandez-Botran & Suzuki, 1986). $Fc_\gamma 2bR$ acts by triggering the arachidonic acid metabolic cascade through the cyclo-oxygenase pathway (Hirata & Suzuki, 1987) which in turn leads to the activation of membrane adenylate cyclase via a prostaglandin receptor (Nitta & Suzuki, 1982a). There are accumulating reports on the pathway of signal transduction for O_2^- generation (Fugita, Irita, Takashige & Minakami, 1984; Maridonneau-Parini, Tringale & Tauber, 1986; Bromberg & Pick, 1983). Arachidonic acid released by receptor stimulation was shown to be important for activation of

*Florida Agricultural Experiment Station Journal Series No. R-01158.
†Author to whom correspondence should be addressed.

NADPH-oxidase (Sakata, Ida, Tominga & Onoue, 1987), a complex enzyme required for the production and release of reactive oxygen species such as superoxide anions (Babior & Peters, 1981).

Several factors could account for the selective modulation of surface receptors. Translocation of receptor molecules by membrane interiorization and reinsertion (Michl, Unkeless & Silverstein, 1980), and recycling back to the surface of the macrophage plasma membrane following endocytosis (Muller, Steinman & Cohn, 1980) have important roles in determining the receptor density. The lateral movement in the plane of diffusion, and the cytoskeleton-regulated motion of the receptors are also shown to be the other controlling mechanisms (Michl *et al.*, 1980). Metal ions can significantly modulate the receptor expression either by inducing alterations in the cell membrane/cytoplasm complex (Zucker, Elstein, Easterling & Massaro, 1988) or by inhibiting microtubule and microfilament assembly and disassembly (Chou, 1986).

Depression in the phagocytic and intracellular killing abilities of mouse peritoneal macrophages (PEM) following intraperitoneal treatment with ammonium metavanadate was observed in our laboratory (Cohen, Wei, Kao & Tan 1986). The decreased intracellular killing of PEM was later shown to be related to the reduced production of superoxide and NADPH-oxidase (Cohen & Wei, 1988). In an attempt to better understand the basis of these vanadate-mediated effects, we studied PEM for modulation of $Fc_\gamma R$ expression and function. The results suggest that the down-regulation of $Fc_\gamma 2bR$ expression following vanadate exposure could contribute, in part, to the earlier observed effects.

EXPERIMENTAL PROCEDURES

Materials

All chemicals used were of reagent grade. Ammonium metavanadate (NH_4VO_3) was purchased from J. T. Baker and Co. (Philipsburg, NJ), and sodium phosphate and ammonium chloride from Mellinckrodt Inc. (Paris, KY). Bovine serum albumin (BSA), fetal bovine serum (FBS), Hank's balanced salt solution (HBSS), and RPMI 1640 were purchased from Gibco Laboratories (Grand Island, NY). Affinity purified mouse myeloma proteins (IgG2a and IgG2b), the $F(ab')_2$ fraction of mouse IgG, and sheep erythrocytes (SRBC) were from Sigma Chemicals (St. Louis, MO). Fluorescein isothiocyanate (FITC) conjugated monoclonal rat anti-mouse IgG2a and IgG2b ($F(ab')_2$ fractions) were from Zymed Laboratories Inc. (San Francisco, CA). Monoclonal rat anti-mouse macrophage antibody was from Accurate Chemical & Scientific Corp. (Westbury, NY). Anti-SRBC antibodies (IgG2a and IgG2b) were generous gift of Dr T. Suzuki of the University of Kansas Medical Center (Kansas City, KS).

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 atom) for delivery of 2.5 and 10.0 mg V/kg body weight (1/8 and 1/2 i.p. LD_{50}, 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 solution at 1.05 mg/ml was used as the second control so that mice received the equivalent amount of ammonium ion given to mice receiving the high vanadium dosage. These solutions were filter sterilized, stored at refrigerated temperatures 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, 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 (10V), 2.5 mg V/kg (2.5V), phosphate buffer (PO_4), and ammonium chloride (NH_4Cl); each group consisted of a minimum of four mice for flow cytometric analysis of IgG2a and IgG2b receptor expression, and six mice for photometric microassay of receptor-mediated binding and phagocytosis. Intraperitoneal (i.p.) injections of vanadium or control solutions were given over a period of 6 weeks, once every 3 days to allow for maximal excretion of the previous dose prior to injection (Cohen *et al.*, 1986). Two days following the final injection, the mice were sacrificed and peritoneal macrophages harvested.

Flow cytometry

Flow cytometric analysis of Fc_γ receptors was performed by the method of Titus, Sharrow & Segal (1983) with some modifications. Peritoneal macrophages (PEM) were harvested separately from

each of the four mice per group and day, in cold RPMI 1640 using the procedure of Cohen *et al.* (1986). Peritoneal macrophage pellets were resuspended in cold phosphate buffered saline (PBS) containing 0.2% BSA and 0.1% sodium azide (PBS/BSA/Azide) at a concentration of 10^6 cells/ml. Equal volumes (0.5 ml) of the above suspension and a solution containing heat aggregated mouse IgG2a or IgG2b (250 μg/ml) were mixed and incubated with continuous shaking at 4°C for 30 min to allow for attachment of immunoglobulins to Fc receptors. Following incubation, the cells were pelleted and washed twice with fresh PBS/BSA/Azide to remove excess IgG. The pellets were resuspended in 250 μl PBS. After equal volumes of FITC-F(ab')$_2$ rat anti-mouse IgG solutions (40 μg/ml) were added, the mixtures were incubated for another 30 min at 4°C. The cells were washed three times with cold PBS/BSA/Azide solution at 4°C to remove excess antibody and then resuspended in the same buffer for analysis. Peritoneal macrophage populations were identified in the scatter plots by labelling the cells with rat anti-mouse macrophage monoclonal antibody and then with FITC-F(ab')$_2$ rabbit anti-rat IgG.

Fluorescence was quantified using a FACStar flow cytometer (Becton-Dickinson, San Jose, CA) equipped with an organ laser. The laser power was 250 mW and the excitation wavelength was set at 488 nm. A 530/30 nm bandpass filter was used to reflect the green channel. Fluorescent monodispersed carboxylated microspheres (Polysciences, Warrington, PA) were used to standardize fluorescence and scatter signals before each run, on a daily basis. For all experiments, cell size and scatter thresholds were established to include only the macrophage population in the analysis. For each treatment, 10,000 events were counted. Log amplification was used for fluorescence analysis and light scatter signals were collected with linear amplification. Data were acquired in list mode and processed with a Consort 30 Data Management System (Becton-Dickinson, San Jose, CA). Results were displayed as single parameter correlated histograms.

Isotype controls without the primary antibody for nonspecific labelling as well as the inclusion of naive cells served to eliminate auto-fluorescence interference.

Photometric microassay of Fc receptor function

This assay was conducted according to the modified method of Rummage & Leu (1985). Mouse PEM were harvested using RPMI 1640 supplemented with 200 mM L-glutamine, 25 mM HEPES, and 50 μg/ml gentamycin. After washing twice with the same medium, the cells were resuspended at a concentration of 5×10^6 cells/ml in RPMI 1640 supplemented with 10% FBS. Macrophage monolayers were prepared by dispensing 0.1 ml of the above suspension into individual wells of a 96-well microtiter plate (Corning Laboratory). Following incubation for 1 h at 37°C in a 5% CO_2 humidified atmosphere, nonadherent cells were removed by two washes with the 0.9% saline. Incubation was continued either at 37°C for phagocytic index determination or at 4°C for binding index determination.

Sheep red blood cells were washed three times with and then resuspended in HBSS at a concentration of 2×10^8 cells/ml. Following dilution at 1:20 with PBS, IgG2a or IgG2b fraction of the rabbit anti-SRBC antibodies was each incubated with an equal volume of SRBC suspension in a 37°C water bath for 30 min. The SRBC – IgG conjugates were washed twice with and then resuspended in cold HBSS at the same concentration as above and stored at 4°C until needed.

Opsonized SRBC suspension in HBSS (0.1 ml) as added to each macrophage monolayer kept either at 37 or 4°C, for phagocytosis and binding studies, respectively. After incubating for 1 h, monolayers were washed three times with 0.9% saline. The microtiter plates designated for phagocytic index determinations were treated with 0.2 ml of 0.09% hypotonic saline to lyse extracellular bound SRBCs and then washed with normal saline. Plates designated for binding index were gently washed with normal saline to remove unbound SRBC. A 0.05 ml aliquot of 50% FBS in distilled water is added to each well for 3 min to cover the macrophage unoccupied area, and removed by inversion of the plate. The monolayers were dried at 50°C for 20 min and fixed with 0.1 ml of absolute methanol for 3 min. The plates were then washed with distilled water, dried at 50°C for 20 min, and then scanned at 405 nm, to monitor the RBCs still bound to or phagocytozed by macrophages, using a microtiter plate reader (BioRad, Model 2550). Peritoneal macrophage samples from each animal/group were run in quadruplicate. Results were expressed as the mean and standard deviation of the mean of 16 samples (i.e. four animals/group). Controls included macrophages alone and macrophage monolayers added with nonopsonized SRBC to correct for nonspecific attachment. Binding and phagocytic indices were calculated using the following formula: Index (%) = $[1 - EA_{405}/EA(\text{IgG})A_{405}] \times 100$, where EA_{405} = absorbance of PEM with nonopsonized

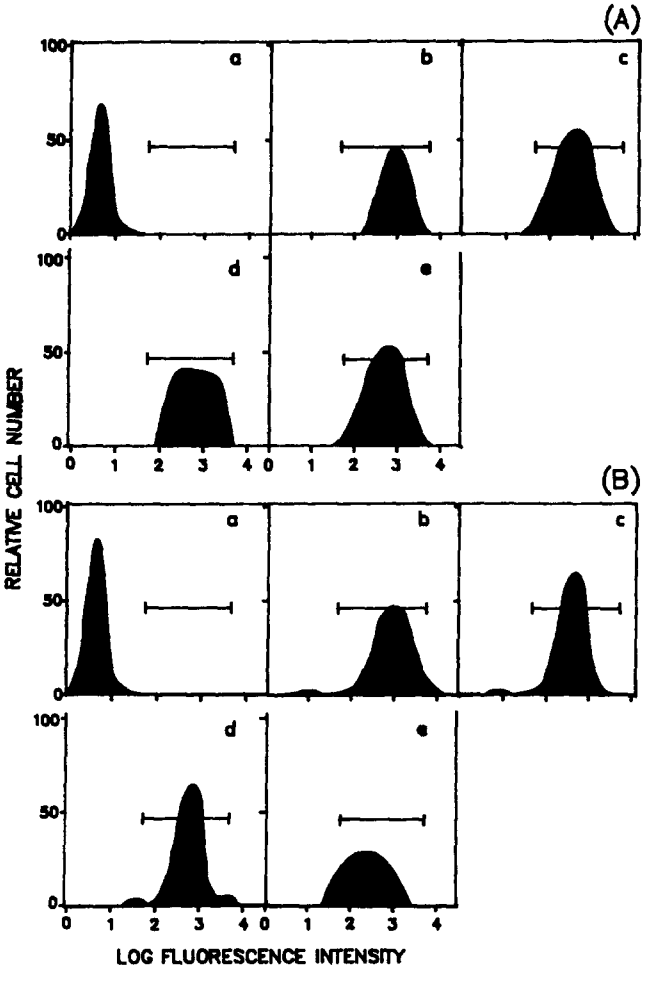

Fig. 1. Effect of ammonium metavanadate on $Fc_{\gamma}2aR$ (A) and $Fc_{\gamma}2bR$ (B) expression in PEM as expressed by single parameter histograms of green fluorescence. Receptors were stained respectively with heat aggregated IgG2a or IgG2b, and then $F(ab')_2$ fraction of FITC-α mouse IgG. Representative data from one of four replicate experiments are presented. (a) Isotype control, (b) phosphate buffer control, (c) NH_4Cl, (d) 2.5V and (e) 10V.

SRBC, and $EA(IgG)A_{405}$ = absorbance of PEM with SRBC opsonized either with IgG2a or IgG2b.

Statistical analysis

Mean fluorescence, percentage fluorescence-positive cells, phagocytic and binding indices were analysed using analysis of variance (ANOVA) treatment. Duncan's analysis of means was used to determine the significance of difference among the treatments at P level of 0.05.

RESULTS

Flow cytometry

Results of flow cytometric analyses of $Fc_{\gamma}2aR$ and $Fc_{\gamma}2bR$ expressions are shown in Fig. 1 and Table 1. Mean fluorescence channel (MFC) indicates the relative amounts of fluorescent antibody bound to the PEM, which in turn reflects the density of the respective receptors. The expression, "percentage fluorescence-positive cells", reflects the fraction of total cells showing fluorescence greater than the background of isotype controls. Similar $Fc_{\gamma}2aR$ expression (as MFC) was observed with the phosphate buffer control and the 2.5V group. However, the ammonium chloride and 10V groups exhibited the respective 21.7 and 17.2% lower $Fc_{\gamma}2aR$ expression than the phosphate buffer control, though the differences were not significant ($P>0.05$). As to the percentage fluorescence-positive cells, a 7.1 and 5.9% lower value was respectively observed in the NH_4Cl and 10V groups when compared with the phosphate buffer group.

Table 1. Flow cytometry of mouse peritoneal macrophages for Fc,2a and Fc,2b receptors*

Treatment group	Mean fluorescence channel		% Fluorescence-positive cells	
	IgG2a	IgG2b	IgG2a	IgG2b
Isotype control	120.28 ± 15.52	109.45 ± 9.95	0.07 ± 0.02	0.10 ± 0.04
Phosphate buffer control	1194.08 ± 117.19	997.65 ± 90.36	95.47 ± 1.56	97.73 ± 1.62
NH_4Cl	934.96 ± 171.74	1157.48 ± 207.41	88.66 ± 10.80	96.97 ± 1.55
2.5V	1065.25 ± 133.99	1047.64 ± 202.92	97.75 ± 1.65	97.43 ± 1.08
10V	988.54 ± 95.57	686.38 ± 138.09†	89.96 ± 8.79	82.37 ± 1.90†

*Mean ± S.D., $n = 4$.
†Values are significantly different ($P<0.05$) from all others in the same column.

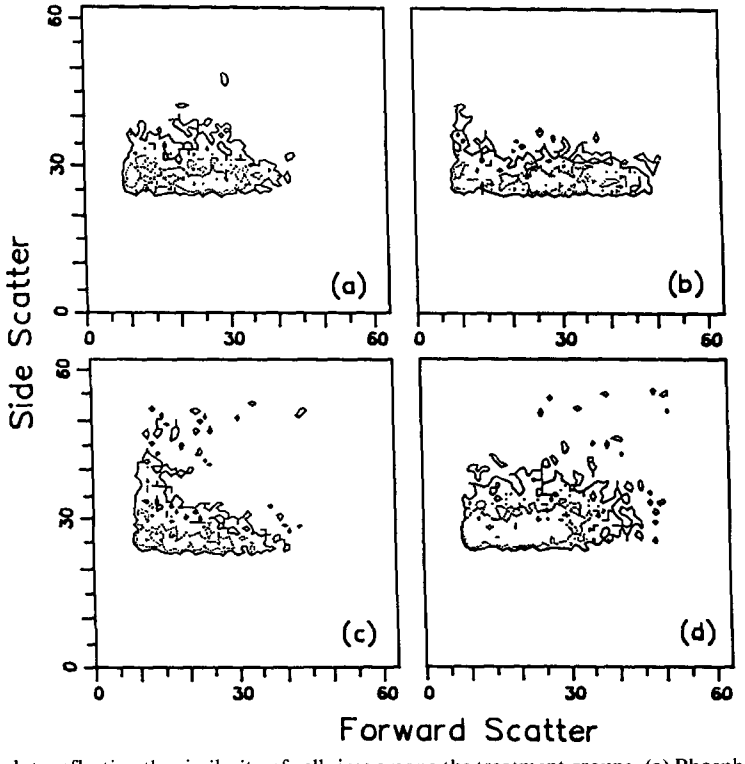

Fig. 2. FSC vs SSC plots reflecting the similarity of cell sizes among the treatment groups. (a) Phosphate buffer control, (b) NH_4Cl, (c) 2.5V and (d) 10V.

For Fc,2bR expression, similar values for MFC and percentage fluorescence-positive cells were obtained for the phosphate buffer, ammonium chloride and 2.5V groups. Only the 10V group showed a significant ($P<0.05$) reduction in values of MFC (31.2 to 40.7%) and percentage fluorescence-positive cells (15.1 to 15.7%) when compared with the other three groups.

In order to exclude the possibility that the variation in cell size might contribute to differences in receptor densities, the four treatment groups were compared with respect to their relative cell sizes as expressed by the plots of forward scatter (FSC) vs side scatter (SSC). All treatments exhibited similar plot values (Fig. 2). The mean X and Y axis positions for the phosphate buffer, NH_4Cl, 2.5V and 10V groups were 25.4 and 33.9, 28.9 and 31.7, 23.2 and 35.3, 25.9 and 33.6, respectively.

Photometric microassay

No significant differences ($P>0.05$) were observed among the four groups with respect to Fc,2aR-mediated binding and phagocytosis of IgG-

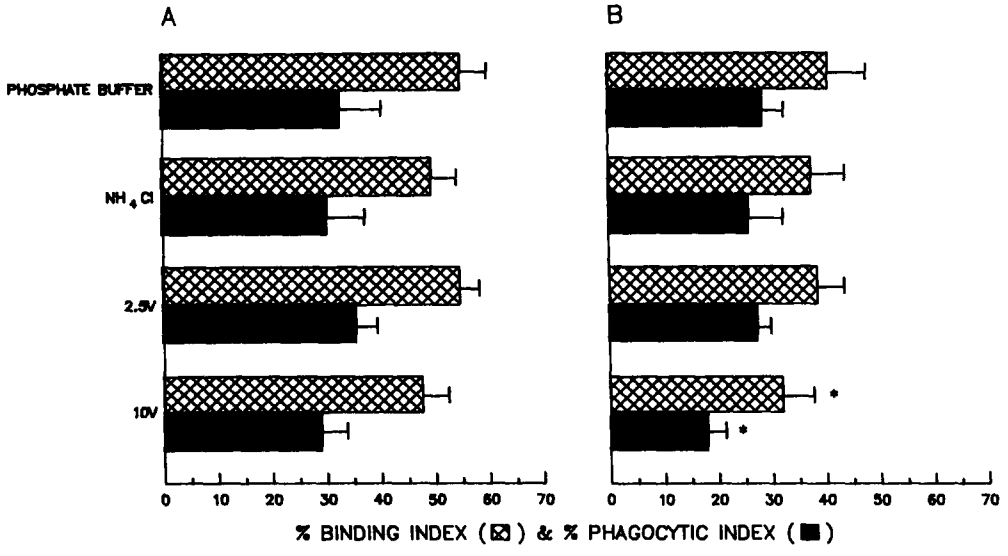

Fig. 3. Effect of vanadium treatment on percentage phagocytic and binding indices of (A) IgG2a and (B) IgG2b opsonized SRBC. Values (mean ± S.D.) significantly different ($P<0.05$) from others in the same group are indicated by *.

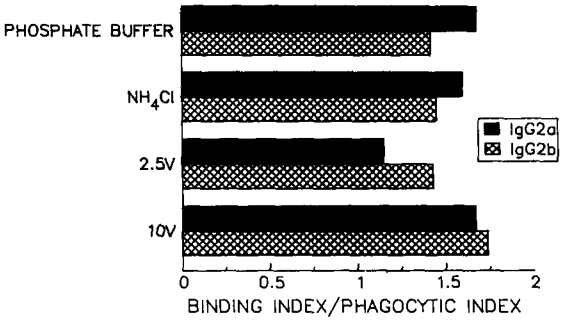

Fig. 4. Comparison of the ratios of phagocytic/binding indices among vanadium-treated and control groups.

opsonized SRBC (Fig. 3). However, significant ($P<0.05$) decreases in $Fc_{\gamma}2b$ mediated binding and phagocytosis were noticed for the 10V group when compared with the other three groups. The 10V group had a 20.1 and 35.7% lower binding and phagocytosis when compared with the phosphate buffer control, and 13.5 and 28.3% lower than the NH_4Cl group. There was no significant difference in $Fc_{\gamma}2b$-mediated binding and phagocytosis in the 2.5V, the phosphate buffer and the NH_4Cl groups.

A comparison of the ratios of binding index/phagocytosis index among the four groups revealed that the 2.5V group had the lowest value (1.15) when IgG2a-opsonized SRBC were used, while the 10V group had the highest (1.74) when IgG2b-opsonized SRBC were used. In both cases, the difference was not significant (Fig. 4).

DISCUSSION

The rationale behind the present study was to explain some of the mechanisms involved in the observed depression of mouse PEM function following intraperitoneal treatment with vanadate for 6 weeks at levels that are not toxic to the cells (Cohen & Wei, 1988). The data shown in this paper indicate that the previously observed depression of phagocytic activity, superoxide generation and NADPH-oxidase activity could, in part, be due to the down-regulation of the $Fc_{\gamma}R$ in PEM following vanadate treatment.

Though the $Fc_{\gamma}2aR$ expression in the phosphate buffer control and 2.5V groups was very similar, its expression in the NH_4Cl and 10V groups was depressed, though not statistically significant (Fig. 1 and Table 1). Amines such as methylamine and hydroxylamine have been shown to inhibit the formation of IgG-FcR (Sander, Erdei, Fesus & Gergely, 1983). Since NH_4Cl also has similar biological effects as these compounds (Jessup, Leone, Bodmer & Dean, 1982), and the 10V and NH_4Cl groups have equal concentrations of ammonium ion, it is possible that changes in intracellular pH due to ammonium ions might play an important role in exerting the observed effect.

The 10V group also showed significant ($P<0.05$) differences in $Fc_{\gamma}2bR$ expression, in terms of the percentage of receptor positive cells and mean fluorescence, compared with the other three groups

(Fig. 1 and Table 1). Phosphorylation of receptors was suggested to lead to trapping of recycling receptors within the cell, either through accelerated internalization or by a retardation of movement to the cell surface (Klausner, Harford & Van Renswoude, 1984; Beguinot, Hanover, Ito, Richert, Willigham & Pastan, 1985; Lin, Chen, Lazar, Carpenter, Gill, Evans & Rosenfield, 1986). Earlier observed effects of vanadate as a stimulator of protein phosphorylation (Catalan, Martinez, Aragones & Diaz, 1989; Yang, Brown & Chan, 1989) and inhibitor of phosphatases (Tessier, Chapdelaine & Chevalier, 1989; Vaddi & Wei, 1991) support this possible mechanism for the down-regulation of Fc$_r$R expression.

Previous studies have shown strong evidence for the modulation of FcR expression by agents that cause changes in intracellular cAMP levels (Vogel, Weedon, Joost & Rosenstreich, 1981). Fc$_r$2bR was shown in a series of studies to trigger the arachidonate metabolic cascade through the cyclooxygenase pathway and resulted in an accumulation of intracellular cAMP (Nitta & Suzuki 1982a, b; Fernandez-Botran & Suzuki, 1984). The conversion of arachidonate to prostaglandins (PG), when a certain level is reached, activates the PG receptor coupled adenylate cyclase system (Hirata, Fernandez-Botran & Suzuki, 1987). Down-regulation of cAMP production has been shown to reduce the expression of receptors on the macrophage surface in an autocrine manner (Muschel, Rosen, Rosen & Bloom, 1977).

Signals transmitted by Fc$_r$2bR appear to be important with respect to NADPH-oxidase activation since arachidonate was demonstrated as an important second messenger for superoxide generation (Bromberg & Pick, 1983) through its involvement in the interaction of the component molecules of the NADPH-oxidase complex (Pick & Mizel, 1982). Muschel *et al.* (1977) demonstrated the inhibitory effect of insulin on Fc$_r$R function which acts without affecting the intracellular cAMP levels. These findings are important since vanadate was shown to mimic some of the biological actions of insulin (Tamura, Brown, Dubler & Larner, 1983; Kowalski, Gazzano, Fehlmann & Van-Obberghen, 1983) including the enhancement of the degree of receptor phosphorylation, and activation of glycogen synthase. All the findings discussed above, when taken collectively, indicate that the vanadate-mediated depression of the phagocytic activity could be due, at least in part, to its effect on the Fc$_r$2bR.

Since all vanadium-treated and control groups showed similar contour plots of FSC vs SSC (Fig. 2), the possibility of changes in the surface area or the cell volume causing the observed difference in fluorescence intensity of PEM from 10V treated mice was excluded. Forward scatter was shown to be a reasonably good measure of cell volume in the absence of drastic changes in cell size (Shapiro, 1988).

Results of the photometric microassay to evaluate the effects of vanadate on Fc$_r$R mediated binding and phagocytosis (Fig. 3) are corroborative of the findings of flow cytometric analysis. The phagocytosis and only the 10V group exhibited significantly ($P<0.05$) NH_4Cl and 10V groups exhibited lower IgG2a mediated binding and lower IgG2b mediated binding and phagocytosis. The method used to quantitate FcR mediated phagocytosis and binding was shown to offer distinct advantages over the traditional ^{51}Cr-labelled erythrocyte method in terms of sensitivity and reproducibility (Rummage & Leu, 1985). At the concentrations of IgG2a and IgG2b used in this assay, binding of opsonized erythrocytes at 4°C, which prevents internalization of FcR bound conjugates, was found to be equivalent to the net difference between total (bound and internalized complexes) and phagocytic (internalized) index values derived at 37°C (Rummage & Leu, 1985).

Because the 10V group has a higher ratio of binding index/phagocytic index than the other three groups, though the difference was not significant (Fig. 4), a separate mechanism other than a consequence of reduction in binding may also be involved in the depressive effect of vanadate on phagocytosis. The well-established effects of metal ions, including vanadate, on microtubule and actin filament organization (Wang & Choppin, 1981; Chou, 1986; Yamamoto, Fujioka, Iinuma, Takano, Maeno, Nagai & Ito, 1984), and the role of the cytoskeleton in the process of phagocytosis (Wang, Michl, Pfeffer, Silverstein & Tamm, 1984) indicate that vanadate might be interfering with the formation of the structural framework of the phagocytic cups. However, there is neither enough experimental data nor previous observations to support this hypothesis.

The effect of vanadate on FcR expression also explains the previously observed increase in the resistance to LPS lethality in mice treated with 10 mg V/kg (Cohen *et al.*, 1986). The effect of LPS as an inhibitor of interferon-γ induced FcR-mediated H_2O_2 generation, through the induction of IL-1 production (Arend, Ammons & Kotzen, 1987) in murine peritoneal macrophages, could contribute to its lethality. This effect coupled with the previous

explanation of glucocorticoid inhibition and subsequent caloric shock (Berry, 1978) might synergistically result in LPS lethality. As observed by Berry (1978), the lethality is enhanced by the interaction of macrophages, T-cells and endotoxin, and agents that could down-regulate this interaction might offer some protection against LPS. Vanadate, by altering the macrophage recognition and phagocytosis, may have a negative effect on the antigen presentation which, in turn, reduces the macrophage and T-cell interaction.

We have not studied whether the observed effects of vanadate on Fc,2b receptor are due to down-regulation of receptor recycling or receptor binding affinity, but earlier literature suggests the former to be a more probable mechanism of action (Klausner *et al.*, 1984; Catalan *et al.*, 1989).

In conclusion, the studies presented in this paper explain some of the findings related to the modulation of murine immune response by ammonium metavanadate. Since the phagocytosis process is the result of simultaneous occurrence of a variety of complex biochemical events, factors regulating a specific reaction have every chance of being translated into a cascade of other reactions, ultimately resulting in amplified responses. These effects assume even more importance in determining functional characteristics during inflammation, since they are further intensified by changes in the cell cycle.

Acknowledgements — The technical assistance of Melissa G. Chen at the Flow Cytometry Core Laboratory, Interdisciplinary Center for Biotechnology Research, University of Florida is gratefully acknowledged. We also thank Dr T. Suzuki and Noreen Connoly for their kind donation of anti-SRBC antibodies. This study was supported by a grant from the Centers for Disease Control (RO3-OH 02583).

REFERENCES

ANDERSON, C. L. & GREY, H. M. (1977). Solubilization and characterization of cell membrane Fc receptors. *J. Immun.*, **118**, 819–825.

AREND, W. P., AMMONS, J. T. & KOTZEN, B. L. (1987). Lipopolysaccharide and interleukin-1 inhibit interferon-γ-induced Fc receptor expression on human monocytes. *J. Immun.*, **139**, 1873–1879.

BABIOR, B. M. & PETERS, W. A. (1981). The O_2^- producing enzyme of human neutrophils. *J. biol. Chem.*, **256**, 2321–2323.

N. D., WILLIGHAM, M. C. & PASTAN, I. (1985). Phorbol esters BEGUINOT, L., HANOVER, J. A., ITO, S., RICHERT, induce transient internalization without degradation of unoccupied epidermal growth factor receptors. *Proc. natn. Acad. Sci. U.S.A.*, **82**, 2774–2778.

BERRY, L. J. (1978). Mediation of endotoxemic effects. In *Toxins: Animal, Plant and Microbial* (ed. Rosenberg, P.), pp. 869–887. Pergamon, New York.

BROMBERG, Y. & PICK, E. (1983). Unsaturated fatty acids as second messengers of superoxide generation by macrophages. *Cell. Immun.*, **79**, 240–252.

CATALAN, R. E., MARTINEZ, A. M., ARAGONES, M. D. & DIAZ, G. (1989). Evidence for a regulatory action of vanadate on protein phosphorylation in brain microvessels. *Biochem. Biophys. Res. Commun.*, **163**, 771–779.

CHOU, I. N. (1986). Dramatic cytoskeletal perturbation induced by epigenetic carcinogenic metals. *Toxicologist*, **6**, 105–105.

COHEN, M. D. & WEI, C. I. (1988). Effects of ammonium metavanadate treatment upon macrophage glutathione redox cycle activity, superoxide production, and intracellular glutathione status. *J. Leukocyte Biol.*, **44**, 122–129.

COHEN, M. D., WEI, C. I., KAO, K. J. & TAN, H. K. (1986).Effect of ammonium metavanadate on the murine response. *J. Tox. envir. Hlth*, **19**, 279–298.

FERNANDEZ-BOTRAN, R. & SUZUKI, T. (1984). Prostaglandin-sensitive adenylate cyclase of a murine macrophage-like cell line ($P388D_1$). II. Isolation and partial characterization of PGE_2-binding proteins. *J. Immun.*, **133**, 2662–2667.

FERNANDEZ-BOTRAN, R. & SUZUKI, T. (1986). Biochemical signal transmitted by Fc receptor for immunoglobulin G2a of murine macrophage-like cell line $P388D_1$: mode of action of adenylate cyclase mediated by immunoglobulin G2a binding proteins. *Biochemistry*, **25**, 4388–4400.

FUGITA, I., IRITA, K., TAKESHIGE, K. & MINAKAMI, S. (1984). Diacylglycerol, 1-oleol-2-acetyl glycerol, stimulates superoxide generation from human neutrophils. *Biochem. Biophys. Res. Commun.*, **120**, 318–324.

HIRATA, Y., FERNANDEZ-BORTRAN, R. & SUZUKI, T. (1987). Relationship between $Fc_{,2b}$ receptor and adenylate cyclase of a murine macrophage like cell line, $P338D_1$. *Biochemistry*, **26**, 4183–4192.

HIRATA, Y. & SUZUKI, T. (1987). Protein kinase activity associated with Fc,2a receptor of a murine macrophage like cell line $P388D_1$. *Biochemistry*, **26**, 8189–8195.

JESSUP, W., LEONE, P., BODMER, J. L. & DEAN, R. T. (1982). The effect of weak bases on lysosomal enzyme secretion by mononuclear phagocytes. *Biochem. Pharmac.*, **31**, 2657 – 2662.

KLAUSNER, R. D., HARFORD, J. & VAN RENSWOUDE, J. (1984). Rapid internalization of the transferrin receptor in K562 cells is triggered by ligand binding or treatment with a phorbol ester. *Proc. natn. Acad. Sci. U.S.A.*, **81**, 3005 – 3009.

KOWALSKI, A., GAZZANO, H., FEHLMANN, M. & VAN-OBBERGHEN, E. (1983). Dephosphorylation of the hepatic insulin receptor: absence of intrinsic phosphatase activity in purified receptors. *Biochem. Biophys. Res. Commun.*, **117**, 885 – 893.

LIN, C. R., CHEN, W. S., LAZAR, C. S., CARPENTER, C. D., GILL, G. N., EVANS, R. M. & ROSENFIELD, M. G. (1986). Protein kinase C phosphorylation at THR 654 of the unoccupied EGF receptor and EGF binding regulate functional receptor loss by independent mechanisms. *Cell*, **44**, 839 – 848.

MARIDONNEAU-PARINI, I., TRINGALE, S. M. & TAUBER, A. I. (1986). Identification of distinct activation pathways of the human neutrophil NADPH-oxidase. *J. Immun.*, **137**, 2925 – 2929.

MICHL, J., UNKELESS, J. C. & SILVERSTEIN, S. C. (1980). Modulation of macrophage plasma membrane receptors for IgG and complement. In *Mononuclear Phagocytes, Functional Aspects* (ed. Van Furth, R.), pp. 351 – 369. Blackwell Scientific, Oxford.

MULLER, W. A., STEINMAN, R. M. & COHN, Z. A. (1980). Membrane flow during endocytosis. In *Mononuclear Phagocytes, Functional Aspects* (ed. Van Furth, R.), pp. 318 – 330. Blackwell Scientific, Oxford.

MUSCHEL, R. J., ROSEN, N., ROSEN, O. M. & BLOOM, B. R. (1977). Modulation of Fc-mediated phagocytosis by cyclic AMP and insulin in a macrophage like cell line. *J. Immun.*, **119**, 1813 – 1820.

NITTA, T. & SUZUKI, T. (1982a). Fc_{r2b} receptor-mediated prostaglandin synthesis by a murine macrophage cell line ($P388D_1$). *J. Immun.*, **128**, 2527 – 2532.

NITTA, T. & SUZUKI, T. (1982b). Biochemical signals transmitted by Fc_r receptors: triggering mechanisms of the increased synthesis of adenosine-3,5′-cyclic monophosphate mediated by Fc_{r2b}-receptors of a murine macrophage-like cell line ($P388D_1$). *J. Immun.*, **129**, 2708 – 2714.

PEPYS, E. O. & PEPYS, M. B. (1983). Demonstration of lymphocyte surface markers using alkaline phosphatase-labeled reagents. Application to the enumeration of lymphocyte populations in whole peripheral blood. *Meth. Enzymol.*, **93**, 164 – 177.

PICK, E. & MIZEL, D. (1982). Role of transmethylation in the elicitation of an oxidative burst in macrophages. *Cell. Immun.*, **72**, 277 – 302.

PONTZER, C. H. & RUSSELL, S. W. (1990). Interferons exhibit temporally distinct regulation of two bovine macrophage receptors. *J. Leukocyte Biol.*, **47**, 258 – 264.

RUMMAGE, J. A. & LEU, R. W. (1985). Photometric microassay of macrophage Fc and C3b receptor function. *J. Immun. Meth.*, **77**, 155 – 163.

(1987). Arachidonic acid acts as an intracellular activator of NADPH-SAKATA, A., IDA, E., TOMINAGA, M. & ONOUE, K. oxidase in Fc, receptor-mediated superoxide generation in macrophages. *J. Immun.*, **138**, 4353 – 4359.

SANDER, M., ERDEI, A., FESUS, L. & GERGELY, J. (1983). IgG-Fc receptors differ in sensitivity to pimary amines. *Immun. Lett.*, **6**, 265 – 269.

SHAPIRO, H. M. (1988). *Practical Flow Cytometry*, pp. 116 – 118. Alan R. Liss, New York.

TAMURA, S., BROWN, T. A., DUBLER, R. E. & LARNER, J. (1983). Insulin-like effect of vanadate on adipocyte glycogen synthase and on phosphorylation of 95,000 dalton subunit of insulin receptor. *Biochem. Biophys. Res. Commun.*, **113**, 80 – 86.

TESSIER, S., CHAPDELAINE, A. & CHEVALIER, S. (1989). Effect of vanadate on protein phosphorylation and acid phosphatase activity in the canine prostate. *Molec. Cell. Endocrin.*, **64**, 87 – 94.

TITUS, J. A., SHARROW, S. O. & SEGAL, D. M. (1983). Analysis of Fc (IgG) receptors on human peripheral blood leukocytes by dual fluorescence flow microfluorometry. II. Quantitation of receptors on cells that express the OKM1, OKT3, OKT4, and OKT8 antigens. *J. Immun.*, **130**, 1152 – 1158.

VADDI, K. & WEI, C. I. (1991). Effect of ammonium metavanadate on murine peritoneal macrophage lysosomal enzymes. *J. Tox. envir. Hlth*, in press.

VOGEL, S. N., WEEDON, L. L., JOOST, J. O. & ROSENSTREICH, D. L. (1981). Defective Fc-mediated phagocytosis in C3H/HeJ macrophages. II. Correction by cAMP agonists. *J. Immun.*, **126**, 441 – 445.

WANG, E. & CHOPPIN, P. W. (1981). Effect of vanadate on intracellular distribution and function of 10 nm-filaments. *Proc. natn. Acad. Sci. U.S.A.*, **78**, 2363 – 2367.

WANG, E., MICHL, J., PFEFFER, L. M., SILVERSTEIN, S. C. & TAMM, I. (1984). Interferon suppresses pinocytosis but stimulates phagocytosis in mouse peritoneal macrophages: related changes in cytoskeletal organization. *J. Cell Biol.*, **98**, 1328 – 1341.

YAMAMOTO, F., FUJIOKA, H., IINUMA, M., TAKANO, M., MAENO, K., NAGAI, Y. & ITO, Y. (1984). Enhancement of new castle disease virus-induced fusion of mouse L cells by sodium vanadate. *Microbiol. Immun.*, **28**, 75 – 83.

YANG, D. C., BROWN, A. B. & CHAN, T. M. (1989). Stimulation of tyrosine-specific protein phosphorylation and phosphotidylinositol phosphorylation by orthovanadate in rat liver plasma membrane. *Arch. Biochem. Biophys.*, **274**, 659–662.

ZUCKER, R. M., ELSTEIN, K. H., EASTERLING, R. E. & MASSARO, E. (1988). Metal-induced alteration of the cell membrane/cytoplasm complex studied by flow cytometry and detergent lysis. *Toxicology*, **53**, 69–78.