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Increased leucoattractant binding and reversible inhibition of neutrophil motility mediated by the peroxidase/H_2O_2/halide system: effects of ascorbate, cysteine, dithiothreitol, levamisole and thiamine

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SUMMARY

Human blood neutrophils manifested markedly decreased motility following exposure to the horseradish peroxidase (HRP)/H_2O_2/halide system *in vitro*. These cells were protected from this inhibitory effect (of the HRP/H_2O_2/halide system) by inclusion of concentrations in the reaction system of ascorbate, cysteine, levamisole and thiamine which stimulate neutrophil migration and inhibit activity of the HRP/H_2O_2/halide system. The reversible nature of the oxidative inhibition of migration was demonstrated by exposing neutrophils to the HRP/H_2O_2/halide system for 15 min followed by washing to remove the components of the peroxidative system, and subsequent addition of ascorbate, cysteine, levamisole, thiamine and the reducing agent, dithiothreitol. Neutrophils so treated completely recovered normal or increased motility induced by the leucoattractants endotoxin-activated serum or synthetic chemotactic tripeptide f-met-leu-phe. This reversible loss of migratory responsiveness following exposure of neutrophils to the HRP/H_2O_2/halide system was not associated with decreased cell viability or adherence. However, membrane oxidation was accompanied by increased uptake of radiolabelled f-met-leu-phe and degranulation. The increased leucoattractant uptake was decreased by ascorbate, levamisole and thiamine. These agents also prevented oxidation of the neutrophil membrane by the HRP/H_2O_2/halide system as measured indirectly by inhibition of iodination.

INTRODUCTION

We have previously reported that the increased neutrophil motility mediated by ascorbate, levamisole and thiamine appears to be related entirely to the antioxidant properties of these molecules by inhibiting activity of the peroxidase/H_2O_2/halide system *in vitro* and *in vivo* (Anderson, 1981a; Anderson, 1981b; Theron *et al.*, 1981). Although the myeloperoxidase (MPO)/H_2O_2/halide system is an important component of neutrophil antimicrobial defences (Klebanoff & Clark, 1978) it may also regulate immune reactivity by inhibition of cellular immune functions such as neutrophil motility and lymphocyte transformation to mitogens (Theron *et al.*, 1981) as well as the activity of humoral factors such as leucoattractants (Clark & Klebanoff, 1979) and antiproteases such as α-l-antitrypsin (Carp & Janoff, 1980). Antioxidants may, therefore, have an important rôle in immunology, probably as a result of their ability to maintain essential molecules on leucocyte membranes in a reduced state and thereby sustain or enhance immune reactivity.

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In this study we have investigated the reversibility of the oxidative inhibition of neutrophil motility mediated by the horseradish peroxidase (HRP)/H_2O_2/halide system in the presence and absence of the antioxidants, as well as their effects on certain activities essential for normal leucocyte locomotion *viz* the uptake of radiolabelled leucoattractants, adherence and glycolysis. The possible importance of antioxidants in immunology is discussed and a model of leucoattractant-induced chemotaxis is suggested on the basis of the results obtained.

MATERIALS AND METHODS

Chemicals and drugs. Ascorbate and thiamine were obtained from the Sigma Chemical Co. (St Louis, Missouri, USA), cysteine and dithiothreitol were obtained from Merck Chemicals (Darmstadt, West Germany) and levamisole was kindly gifted by Janssen Pharmaceutica (Beerse, Belgium).

Neutrophil motility. Neutrophils were obtained from heparinized venous blood (5 units of heparin ml^{-1}) from normal adult volunteers and were resuspended to a final concentration of 6×10^6 ml^{-1} in HEPES (*N*-2-hydroxyethyl-ethylpiperazine-*N*′-2-ethanesulphonic acid obtained from the Sigma Chemical Co.) buffered Hanks' balanced salt solution (HBSS: GIBCO Laboratories, Grand Island, New York, USA) supplemented with 0·05% bovine serum albumin (BSA) following hypotonic lysis of residual erythrocytes with 0·84% ammonium chloride as described previously (Anderson & Van Rensburg, 1979).

Two leucoattractants were used: (i) EAS, *viz* fresh autologous serum activated with 100 μg bacterial endotoxin (*Escherichia coli* 0127: B8, Difco Laboratories, Detroit, Michigan, USA) ml^{-1} which was diluted eight-fold with HBSS before use; and (ii) the synthetic chemotactic tripeptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (f-met-leu-phe; Miles Laboratories Inc., Elkhart, Indiana, USA) which was used at a final concentration of 5×10^{-7} M (previously found to be the optimal leucotactic concentration). To assess the effects of cysteine on neutrophil motility varying concentrations of the amino acid were added to neutrophils prior to assessment of motility and remained in the cell compartment throughout the incubation period. Ascorbate, levamisole and thiamine were used in this study at concentrations which had previously been shown to stimulate neutrophil motility and cause inhibition of the peroxidase/H_2O_2/halide system. These concentrations are 5×10^{-2} M for ascorbate (Anderson & Theron, 1979), 5×10^{-3} M for levamisole (Anderson, 1981b) and 5×10^{-2} M for thiamine (Theron *et al.*, 1981). The final neutrophil concentration in all studies of migration was 3×10^6 ml^{-1}. The assays of motility were performed in modified Boyden chambers (Wilkinson, 1971) with 5-μm pore size membrane filters (Sartorius-membranfilter, Göttingen, West Germany) and a 2 hr incubation period. The results are expressed as the number of cells which have completely traversed the filter per microscope high-power field as an average of triplicate filters.

Kinetics of chemoattractant binding of cells exposed to the HRP/H_2O_2/halide system. Neutrophils at a final concentration of 1×10^7 ml^{-1} were exposed to 0·25 units ml^{-1} of HRP (Type VI, Sigma Chemical Co.) 0·1 μM H_2O_2 (Merck Chemical Co.) and 10 mM sodium iodide (Merck Chemical Co.) for 15 min at 37°C in 0·15 M phosphate-buffered saline (PBS). The cells were then washed once and resuspended to 2×10^7 ml^{-1} in BSA-supplemented HBSS. To 100-μl aliquots of cells contained in the 6-mm diameter wells of Linbro tissue-culture plates (Flow laboratories Inc., Rockville Maryland, USA) was added 0·2 μCi of f-met-leu-phe, *N*-phenylalanine-ring-2,6-^{3}H (N), i.e. (^{3}H) f-met-leu-phe, (New England Nuclear Corp., Boston, Massachusetts, USA). Incubation was carried out at 37°C and the cells harvested at varying time intervals using a multiple automated sample harvester. Uptake of the radiolabelled leucoattractant was assessed using a liquid scintillation spectrophotometer. Control systems with untreated neutrophils were included.

The effects on binding of the radiolabelled leucoattractant of migration stimulatory concentrations of ascorbate, levamisole and thiamine were also assessed. Ascorbate and thiamine were used at final concentrations in the assay system of 1×10^{-1} M, 5×10^{-2} M and $2{\cdot}5 \times 10^{-2}$ M; levamisole concentrations investigated were $7{\cdot}5 \times 10^{-3}$ M, 5×10^{-3} M and $2{\cdot}5 \times 10^{-2}$ M. A fixed incubation

period of 2 hr was used in these studies. Pure neutrophil suspensions of > 90% purity and viability were used in these studies (Anderson & Van Rensburg, 1979).

Exposure of neutrophils to the peroxidase/H_2O_2/iodide system and the effects of the antioxidants. In this series of experiments the effects of the peroxidase/H_2O_2/iodide system on neutrophil motility to both EAS and f-met-leu-phe in the presence and absence of migration stimulatory concentrations of ascorbate, cysteine, levamisole and thiamine were investigated. Neutrophils (5×10^6) in 0·5 ml were pre-incubated with 0·25 units of HRP, 10 mM sodium iodide and H_2O_2 at three different concentrations *viz* 0·1, 1 and 10 μM. The reaction systems were incubated at 37°C for 30 min in a final volume of 1 ml of HBSS without BSA after which the supernatants containing the reactants were removed following centrifugation. The cells were resuspended in BSA-supplemented HBSS and tested for migratory responsiveness.

Reversibility of the inhibition of motility mediated by the HRP/H_2O_2/halide system. In these experiments neutrophils were exposed as described earlier to the HRP/H_2O_2/iodide system using the same HRP and NaI concentrations and 10 μM H_2O_2 in the *absence of the antioxidants*. Following a 15 min exposure to this system the treated oxidized cells were centrifuged to remove the components of the HRP/H_2O_2/halide system and resuspended in BSA-supplemented HBSS containing migration stimulatory concentrations of ascorbate, cysteine, levamisole and thiamine as well as the known reducing agent, dithiothreitol, which was used at concentrations of 10^{-2} M and 10^{-3} M. The cells were then tested for migratory responsiveness to EAS and the results obtained with the oxidized cells in the control systems were compared with those of the oxidized cells to which the various antioxidants and the reducing agent had been added.

Effects of the HRP/H_2O_2/halide system on neutrophil adherence. Neutrophils were exposed to 0·25 units HRP, 10 μM H_2O_2 and 10 mM NaI for 15 min, centrifuged to remove the peroxidative system and resuspended to 1×10^6 ml^{-1} in BSA-supplemented HBSS. Aliquots of 1 ml were introduced into 35-mm diameter tissue-culture dishes (Falcon) and incubated for 60 min at 37°C. Adherence was measured directly by microscopic enumeration of neutrophils adherent to the surface of the dish and indirectly by counting the non-adherent cells in the supernatant fluid.

MPO-mediated iodination of ingested protein. This was determined by the method of Root and Stossel (1974) with minor modifications. To 0·1 ml of the pure neutrophil suspension (1×10^7 ml^{-1}) was added 0·1 ml of *Candida albicans* (1×10^8 ml^{-1}), 0·1 ml of fresh autologous serum, 0·1 ml of the various cysteine concentrations, 0·1 ml of an ^{125}I solution (0·6 μCi ml^{-1}) obtained from New England Nuclear (Boston, Massachusetts, USA) as sodium iodate and 0·5 ml PBS. Incubation was for 60 min at 37°C on a turntable after which the extent of incorporation of ^{125}I into acid-precipitable protein was determined by solid scintillation counting. Results are expressed as nmol ^{125}I in the protein precipitate.

Effects of cysteine on the HRP/H_2O_2/iodide system. To determine the effects of cysteine on the iodination of BSA mediated by the HRP/H_2O_2/iodide system the various cysteine concentrations were incubated with 0·25 units HRP, 10 μM H_2O_2, 0·06 μCi of ^{125}I and 5 mg BSA in a total reaction volume of 1 ml. The tubes were incubated and processed as described for the MPO system. Only cysteine was investigated for its effects on the MPO or HRP/H_2O_2/iodide system as the inhibitory activities of ascorbate, levamisole and thiamine have been previously documented (Anderson and Theron, 1979; Anderson, 1981; Theron *et al.*, 1981).

Effects of ascorbate, cysteine, levamisole and thiamine on the oxidation of the neutrophil membrane mediated by the HRP/H_2O_2/iodide system. To 0·1-ml aliquots containing 5×10^6 of a suspension of pure neutrophils in Linbro tissue-culture plates was added 0·25 units HRP, 10 μM H_2O_2 and 0·06 μCi ^{125}I and varying concentrations of ascorbate, cysteine, levamisole and thiamine in a final reaction volume of 0·2 ml HBSS. Control systems contained no antioxidants. Incubation was for 60 min at 37°C after which the cells were harvested on an automated sample harvester and the extent of iodination of the neutrophils was assessed.

Effects of exposure of neutrophils to the HRP/H_2O_2/halide system on degranulation. Pure neutrophil suspensions containing 5×10^6 cells were exposed to 0·25 units HRP, 10 μM H_2O_2 and 10 mM NaI for 15 min at 37°C. Following incubation the peroxidative system was removed by centrifugation and the cells washed once in PBS and resuspended to 5×10^6 ml^{-1}. The cells were then tested for spontaneous degranulation by measurement of release of alkaline phosphatase at

varying time intervals from 5 to 180 min. Post-phagocytic degranulation was assessed by inclusion of opsonised *C. albicans* in the incubation mixture to give a neutrophil:microorganism ratio of 1:20. Enzyme release was measured at varying time intervals from 1 to 40 min. Alkaline phosphatase was measured using a colorimetric assay based on using *p*-nitrophenyl phosphate as the enzyme substrate. Results are expressed as enzyme units per 5×10^6 neutrophils.

Glycolysis. This was measured by the amount of lactate production using recognized procedures (Hohorst, 1962). Pure neutrophil suspensions were resuspended to a final concentration of 2×10^7 ml^{-1} in PBS containing 10 mM glucose. Each assay tube contained 6×10^6 control neutrophils or neutrophils which had been exposed to the HRP/H_2O_2/halide system for 15 min, 0·1 ml of EAS or f-met-leu-phe at final concentrations of 5% or 5×10^{-6} M, respectively. Tubes were incubated at 37°C for 5, 10, 15 and 30 min and the reactions terminated and the systems deproteinized by the addition of 1 ml of cold 0·6 M perchloric acid. After centrifugation 0·2-ml aliquots of the supernatant were assayed for lactate in the presence of NAD and lactate dehydrogenase at 25°C for 60 min. The change in optical density at 340 nm was measured spectrophotometrically and results expressed as μg lactate per 6×10^6 neutrophils.

Cell viability. Viability testing of neutrophils following exposure to 0·25 units of HRP, 10 μM H_2O_2 and 10 mM NaI for 15 min was done by trypan blue (0·1%) dye exclusion.

RESULTS

Results are expressed as the mean value with standard error for each set of experiments. Statistical analyses of data were performed by the Student's *t*-test (*t*-statistic for two means).

Studies of motility

The effects of ascorbate, levamisole and thiamine on the inhibition of neutrophil motility to EAS mediated by the HRP/H_2O_2/halide system are shown in Table 1. These three agents at the

Table 1. Effects of ascorbate, levamisole and thiamine on the inhibition of neutrophil migration to EAS and f-met-leu-phe mediated by the HRP/H_2O_2/halide system *in vitro*

Reaction system	Neutrophil migration to: 10% EAS	Neutrophil migration to: 5×10^{-7} M f-met-leu-phe
Neutrophils + HBSS	162 ± 27†	92 ± 15
Neutrophils + HRP/H_2O_2/NaI* only	42 ± 6 ($P < 0·005$)	14 ± 5 ($P < 0·005$)
Neutrophils + HRP/H_2O_2/NaI + ascorbate at:		
$2·5 \times 10^{-2}$ M	112 ± 13	88 ± 10
5×10^{-2} M	131 ± 15	108 ± 4
1×10^{-1} M	173 ± 18	131 ± 4
Levamisole at:		
$2·5 \times 10^{-3}$ M	135 ± 16	85 ± 7
5×10^{-3} M	166 ± 20	98 ± 12
$7·5 \times 10^{-3}$ M	177 ± 34	105 ± 16
Thiamine at:		
$2·5 \times 10^{-2}$ M	94 ± 3	78 ± 21
5×10^{-2} M	110 ± 10	99 ± 27
1×10^{-1} M	118 ± 29	90 ± 15

* Neutrophils were incubated with 0·25 units of HRP, 10 μM H_2O_2 and 10 mM NaI in the presence and absence of ascorbate, levamisole and thiamine at the concentrations shown, washed and tested for migratory responsiveness to EAS and f-met-leu-phe.

† Results as mean value with standard error of three different experiments.

Table 2a. Effects of cysteine on neutrophil motility to EAS and on the MPO-mediated iodination of *C. albicans* and the HRP-mediated iodination of BSA

Cysteine concentration	Neutrophil motility to EAS (as cells per HPF)	MPO-mediated iodination of *C. albicans* (as nmol ^{125}I deposited)	HRP-mediated iodination of BSA (as nmol ^{125}I deposited)
Control (no cysteine)	171±20*	0·63±0·02*	0·28±0·02*
5×10^{-3} M cysteine	250±24	0·10±0·01†	0·03±0·01†
$1{\cdot}0 \times 10^{-2}$ M cysteine	290±10†	0·9±0†	0·03±0·0†
$2{\cdot}5 \times 10^{-2}$ M cysteine	340±12†	0±0†	0±0†

* Results as the mean value with standard error for three different experiments.
† $P < 0{\cdot}05$.

Table 2b. The effects of cysteine on the inhibition of motility to EAS mediated by the HRP/H_2O_2/halide system

Reaction system	Migratory responsiveness to EAS
Neutrophils exposed to:	
1. HBSS only (control)	172±5†
2. HBSS+HRP+H_2O_2+NaI*	69±9 ($P < 0{\cdot}005$)
3. HBSS+HRP+H_2O_2+NaI+5×10^{-3} M cysteine	129±28
4. HBSS+HRP+H_2O_2+NaI+1×10^{-2} M cysteine	200±24
5. HBSS+HRP+H_2O_2+NaI+5×10^{-2} M cysteine	317±26

* Neutrophils were incubated with 0·25 units of HRP, 10 μM H_2O_2, and 10 mM NaI in the presence and absence of the various cysteine concentrations. Following removal of the peroxidative system by centrifugation, after 15 min incubation, the cells were tested for migratory responsiveness to EAS.
† Results as the mean value with standard error for three separate experiments.

concentrations tested protected and even enhanced the neutrophil migratory response to EAS. Similar results were obtained when f-met-leu-phe was used as the leucoattractant. Cysteine at concentrations of 5×10^{-3} M caused stimulation of motility to EAS. Results for 5×10^{-3} M, 1×10^{-2} M, and $2{\cdot}5 \times 10^{-2}$ M cysteine are shown in Table 2a. These concentrations of cysteine also protected neutrophils from the inhibition of motility mediated by the HRP/H_2O_2/halide system (Table 2b). The effects of cysteine on the MPO-mediated iodination of *C. albicans* and the HRP-mediated iodination of BSA are shown in Table 2a. Cysteine at concentrations which mediated stimulation of motility and protection of the neutrophil migratory response from the inhibitory activity of the HRP/H_2O_2/halide system caused considerable inhibition of MPO- and HRP-mediated iodination reactions.

The reversibility of the oxidative inhibition of neutrophil motility to EAS by the HRP/H_2O_2/halide system

This was observed by exposing oxidized cells to the reducing agent, dithiothreitol, as well as the various antioxidants. Oxidized cells completely recovered normal and even enhanced migratory responsiveness to EAS, thus demonstrating that the oxidative inhibition of motility mediated by the HRP/H_2O_2/iodide system is completely reversible. These results are shown in Table 3.

The effects of exposure of neutrophils to the HRP/H_2O_2/halide system on binding of the radiolabelled leucoattractant, ^{3}H f-met-leu-phe

These are shown in Fig. 1. Oxidation of neutrophils by the HRP/H_2O_2/halide system caused a

Table 3. Assessment of the reversibility of the inhibition of neutrophil migration mediated by the HRP/H_2O_2/halide system by incubation of oxidized cells with ascorbate, cysteine, dithiothreitol, levamisole and thiamine

Reaction system	Neutrophil migration to EAS
1. Neutrophils + HBSS	199 ± 31†
2. Neutrophils + HRP + H_2O_2 + NaI	75 ± 11 ($P < 0{\cdot}005$)
3. Neutrophils + HRP + H_2O_2 + NaI followed by exposure to:*	
a. 5×10^{-2} M ascorbate	253 ± 24
b. 5×10^{-2} M cysteine	286 ± 38
c. 1×10^{-3} M dithiothreitol	317 ± 49
d. 5×10^{-3} M levamisole	166 ± 20
e. 5×10^{-2} M thiamine	166 ± 14

* Neutrophils were exposed to 0·25 units HRP, 10 μM H_2O_2 and 10 mM NaI for 15 min after which the peroxidative system was removed by centrifugation and the cells then incubated with ascorbate, cysteine, dithiothreitol, levamisole and thiamine and tested for chemotactic responsiveness.

† Results as the mean value with standard error for three different experiments.

significant increase in the rate and extent of uptake of ^{3}H f-met-leu-phe (P value $< 0{\cdot}005$ for the 3 hr incubation period). Co-incubation of neutrophils with migration stimulatory concentrations of ascorbate, levamisole and thiamine during exposure to the HRP/H_2O_2/iodide system prevented the increased uptake of ^{3}H f-met-leu-phe. The mean percentage inhibition of increased leucoattractant uptake mediated by the HRP/H_2O_2/halide system was 75 ± 25, 97 ± 3 and 61 ± 24 for 5×10^{-3} M ascorbate, 5×10^{-2} M thiamine and $7{\cdot}5 \times 10^{-3}$ M levamisole, respectively.

Oxidation of the neutrophil membrane by the HRP/H_2O_2/I^{125} system

This was shown indirectly by demonstrating iodination of neutrophils. These results are shown in Table 4 and demonstrate that inhibition of neutrophil motility mediated by the HRP/H_2O_2/halide system is associated with oxidation of the neutrophil. Co-incubation of neutrophils with migration stimulatory concentrations of ascorbate, cysteine, levamisole and thiamine during exposure to the HRP/H_2O_2/^{125}I system prevented iodination of the neutrophil membrane.

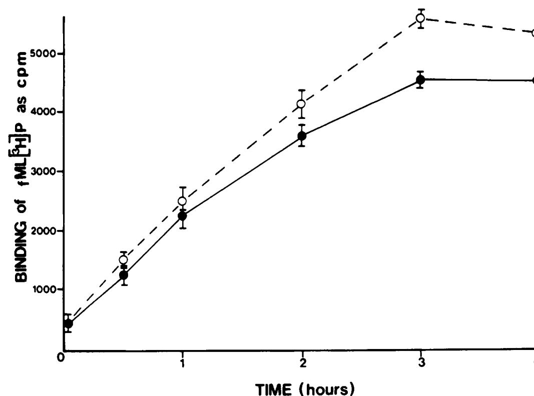

Fig. 1. The effects of exposure of neutrophils to the HRP/H_2O_2/NaI system on uptake of tritiated f-met-leu-phe. These are the normalized results of ten separate experiments. (o) Cells exposed to the HRP/H_2O_2/NaI system (●) Control cells.

Table 4. The effects of ascorbate, cysteine, levamisole and thiamine on iodination of neutrophils mediated by the HRP/H_2O_2/halide system

Reaction system	Iodination of neutrophils (as nmol ^{125}I deposited)
5×10^6 neutrophils + HRP + H_2O_2 + NaI only*	0·29 ± 0·04
5×10^6 neutrophils + HRP + H_2O_2 + NaI +	
1×10^{-3} M ascorbate	0·03 ± 0·01†
5×10^{-3} M ascorbate	0·02 ± 0·01†
1×10^{-3} M cysteine	0·10 ± 0·5†
5×10^{-3} M cysteine	0·02 ± 0·01†
1×10^{-3} M levamisole	0·07 ± 0·04†
5×10^{-3} M levamisole	0·04 ± 0·01†
1×10^{-2} M thiamine	0·24 ± 0·09
5×10^{-2} M thiamine	0·17 ± 0·03†

* Neutrophils in HBSS were exposed to 0·25 units HRP, 10 μM H_2O_2 and Na ^{125}I for 60 min in the presence and absence of ascorbate, cysteine, levamisole and thiamine and the extent of iodination assessed.

† $P < 0{\cdot}05$.

Adherence studies

Exposure of neutrophils to the HRP/H_2O_2/iodide system using H_2O_2 concentrations of up to 10 μM had no effects on neutrophil adherence. For a reaction system containing 0·25 units HRP/10 μM H_2O_2/10 mM NaI the adherence value was 331 ± 35 adherent neutrophils per low-power microscope field compared with a control value of 351 ± 35. These results are the mean values with standard errors of three separate experiments.

Degranulation studies

Exposure of neutrophils to the HRP/H_2O_2/halide system caused increased spontaneous and post-phagocytic degranulation. These results are shown in Fig. 2.

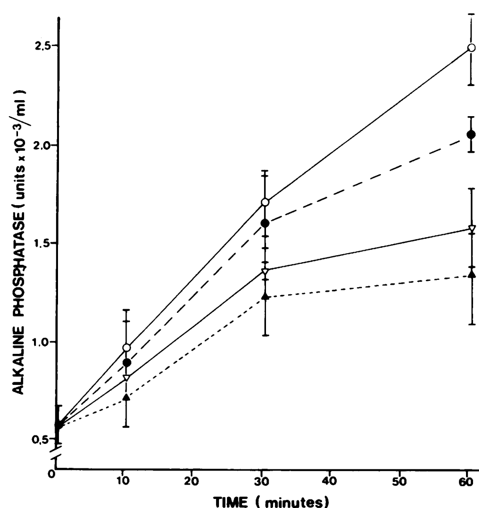

Fig. 2. The effects of exposure of neutrophils to the HRP/H_2O_2/NaI system on spontaneous degranulation in control cells (▲) and treated neutrophils (▽) and post-phagocytic degranulation in control (●) and treated neutrophils (○).

Glycolysis
Exposure of neutrophils to the HRP/H_2O_2/halide system had no effects on the stimulation of glycolysis which accompanies exposure to leucoattractants. The results obtained after exposure to 5% EAS were 37·5 ± 5·5 and 42·4 ± 7·5 μg lactate per 5×10^6 cells for control and oxidized cells, respectively.

Viability studies
There was no loss of viability of neutrophils following a 15 min exposure to the HRP/H_2O_2/halide system. Results were 97 ± 2 and 94 ± 3 percentage viability for control and oxidized cells, respectively (results of three experiments).

DISCUSSION

In this study we have found that ascorbate, cysteine, levamisole and thiamine share a common mechanism of stimulation of neutrophil migration. This is by protection of the neutrophil membrane from oxidative damage by the peroxidase/H_2O_2/halide system. We have previously reported that levamisole and thiamine have no peroxidase inhibitory activity *per se* but function rather as antioxidants or co-oxidants by scavenging toxic oxidative products of the peroxidase/H_2O_2/halide system (Anderson, 1981; Theron *et al.*, 1981). Cysteine also does not possess peroxidase inhibitory activity (unpublished observations) and probably acts as an antioxidant by virtue of its oxidizable sulphydryl group. Ascorbate, on the other hand, does possess peroxidase inhibitory activity and can also act as a potent reducing agent (McCall *et al.*, 1971). These agents as a consequence of their antioxidant activities most probably stimulate neutrophil migration by inhibition of auto-oxidative membrane damage mediated by the MPO/H_2O_2/halide system which is activated during the interaction of leucoattractants and phagocytic stimuli with neutrophils. In support of this theory we have deomonstrated that the various antioxidants prevent iodination of the neutrophil membrane. The oxidative inhibition of neutrophil motility mediated by the HRP/H_2O_2/halide system is completely reversible as shown by exposing oxidized cells to the known reducing agent, dithiothreitol, as well as to ascorbate, cysteine, levamisole and thiamine which caused complete recovery of migratory responsiveness to leucoattractants. Cell viability was unaffected by exposure of neutrophils to the HRP/H_2O_2/halide system which correlates well with the reversibility of the oxidative inhibition of motility. Adherence and glycolytic activity are not affected by oxidation of the neutrophil membrane and cannot therefore be implicated in the inhibition of migratory responsiveness. The most likely candidates for reversible oxidative inactivation could be structural or contractile membrane proteins or glycoproteins which may have to be in a reduced state for normal function. Oliver *et al* (1978) have reported that critical minimal levels of reduced glutathione are necessary for microtubule assembly since they protect tubulin from direct attack by oxidants. It is therefore possible that membrane sulphydryl groups are the target of oxidative products of the MPO/H_2O_2/halide system. Oxidation of the thioether group of methionine to the sulphoxide form is a reversible reaction (Jori *et al.*, 1968) and maintenance of these groups in a reduced form may also be important for motility.

Exposure of neutrophils to the HRP/H_2O_2/halide system was associated with a significant increase in the uptake of radiolabelled f-met-leu-phe and a consistent, slight, spontaneous degranulation. The stimulatory effect on degranulation was more evident when a phagocytic stimulus was used. These results suggest a positive correlation between degranulation, uptake of leucoattractants and oxidation of the neutrophil membrane. It is possible that interaction of leucoattractants with the membrane of a phagocytic cell during exposure to a concentration gradient of a leucoattractant may cause a highly localized stimulation of oxidative metabolism. Activation of HMS activity and superoxide production by leucoattractants is well-established (Bass, De Chatelet & McCall, 1978; Becker, Sigman & Oliver, 1979). Localized membrane oxidation may trigger degranulation if the cytoplasmic granules could recognize and fuse with the region of oxidized cell membrane. The granules may thus provide extra receptors for leucoattractants as suggested by Wilkinson (Wilkinson, P.C., personal communication, 1978) and thereby

sustain the localized response at the site of exposure to the leucoattractant and confer orientation upon the cell. Indirect evidence in support of this theory is that some children with chronic granulomatous disease have primary abnormalities of neutrophil motility (Clark & Klebanoff, 1978; Anderson, 1981c). Furthermore, markedly abnormal neutrophil chemotaxis associated with impaired degranulation is found in individuals with Chediak–Higashi syndrome (Clark & Kimball, 1971). This oxidation/reduction model of the interaction of regions of oxidized cell membrane and cytoplasmic granules in neutrophil chemotaxis is hypothetical and considerable further investigation is required to establish or disprove its existence. Incubation of ascorbate, levamisole and thiamine with neutrophils during exposure to the HRP/H_2O_2/halide system prevented the increased uptake of radiolabelled f-met-leu-phe. The increased uptake of f-met-leu-phe accompanying membrane oxidation probably is a consequence of a general membrane oxidation. Inclusion of the antioxidants prevents this and may be important in sustaining the gradient-detecting mechanism of the cell.

Antioxidants have been reported to inhibit the oxidative conversion of certain precarcinogens to carcinogens *in vitro* (Zenser *et al.*, 1980). Recent studies indicate that they may also be important immunopharmacological agents of potential value in the immunotherapy or prophylaxis of conditions associated with decreased immune responsiveness. It is of interest that in the present study three of four antioxidants described, *viz* ascorbate, cysteine and thiamine, are important dietary constituents. Furthermore, there has been considerable interest recently in the rôle of retinoic acid and its derivatives as immunostimulatory agents in cancer (Medawar & Hunt, 1981) and this property may also be related to the antioxidant activity of these compounds (Kensler & Trush, 1981). These observations suggest that although immunopharmacotherapy with large doses of a single antioxidant may be useful in immunocompromised individuals that adequate dietary intake of various essential antioxidants may confer on the normal individual good immunological responsiveness linked to correct nutrition.

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