INFECTION AND IMMUNITY, Apr. 1971, p. 632-633

Vol. 5, No. 4
Printed in U.S.A.

Amino Acid Oxidase in Leukocytes: Evidence Against a Major Role in Phagocytosis

LAWRENCE R. DECHATELET, CHARLES E. MCCALL, AND M. ROBERT COOPER

Departments of Biochemistry and Medicine, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27103

Received for publication 13 December 1971

The addition of either D- or L-amino acids fails to increase hexose monophosphate shunt activity of resting or phagocytizing neutrophils. This is presumptive evidence against a major role for amino acid oxidase in the bactericidal activity of the cell.

During the phagocytic and bactericidal activities of neutrophils, there is a significant increase in the production of H_2O_2 by the cell (7), which may play a role in the destruction of bacteria by (i) iodination of the bacterial cell wall (10), (ii) generation of bactericidal aldehydes from amino acids (16), and (iii) reaction with ascorbic acid to yield a bactericidal-free radical intermediate (12). Concomitant with the increase in H_2O_2 production are increases in hexose monophosphate shunt (HMS) activity and in oxygen consumption (19).

The exact mechanism by which the H_2O_2 is generated is a matter of considerable interest and much debate. Although some workers feel the critical enzyme is reduced nicotinamide adenine dinucleotide oxidase (1), others favor reduced nicotinamide adenine dinucleotide phosphate oxidase (8). We have suggested that the oxidation of ascorbic acid might be involved in H_2O_2 generation (L. R. DeChatelet et al., Fed. Proc., p. 1275, 1971). Lehrer and Cline (2) recently identified a D-amino acid oxidase in leukocytes and proposed that this enzyme might be responsible for the metabolic changes seen with phagocytosis. This proposal is especially attractive since it contains a built-in mechanism for the recognition of the "foreignness" of phagocytized microorganisms. However, the fact that the ingestion of inert latex particles will stimulate H_2O_2 production is evidence that this cannot be the sole means of H_2O_2 production. Eckstein et al. (5) investigated the amino acid oxidase of guinea pig and human neutrophils. They confirmed the presence of D-amino acid oxidase but found an equal activity toward L-amino acids. Furthermore, they found normal levels of both activities in cells of patients with chronic granulomatous disease of childhood and concluded that it was unlikely that either enzyme was the primary source of H_2O_2 during phagocytosis.

Because the stimulation of the HMS is intimately associated with H_2O_2 production, we decided to employ this sensitive measurement to assess the role of the amino acid oxidases in the normal physiology of the neutrophil.

Glucose utilization via the HMS was estimated by a modification of the method of Holmes et al. (6), employing glucose differentially labeled in the C-1 or C-6 position. All solutions were prepared in Dulbecco's phosphate-buffered saline (PBS) (11). Each incubation flask contained 0.16 mg of D-glucose-*1*-^{14}C (0.20 μCi of radioactivity). Phagocytosis was initiated in appropriate flasks by the addition of 0.15 ml of polystyrene particles (Difco; 0.8 μm diameter) at a ratio of approximately 12 particles per phagocyte. Amino acid solutions were neutralized to *p*H 7.0, and sufficient PBS was added to give a final volume (after the addition of cells) of 2.50 ml. The reaction was initiated by the addition of 1.0 ml of cell suspension, containing 5×10^6 neutrophils. The cells were isolated from the blood of healthy volunteer subjects by the method previously described (4). Better than 90% of the cells isolated were polymorphonuclear leukocytes as determined by chamber differential. The $^{14}CO_2$ liberated in the course of a 1-hr incubation period at 37 C was trapped in a center well containing 0.50 ml of hyamine hydroxide and was quantitated in a Packard Tri-Carb liquid scintillation spectrometer.

The results in Table 1 demonstrate that the addition of amino acids to either resting or phagocytizing neutrophils results in no significant stimulation of HMS activity. Cells observed by phase microscopy were seen to contain large numbers of polystyrene particles, indicating that active phagocytosis was occurring. Alanine and threonine were chosen as representative amino acids because they have been demonstrated to be the best substrates for the leukocyte D-amino acid oxidase (2). Similarly, the addition of these amino

TABLE 1. *Effect of amino acids on $^{14}CO_2$ production from glucose-1-^{14}C in resting and phagocytizing human neutrophils*

Addition	Counts/min in $^{14}CO_2$[a]	
	Resting	Phagocytizing
None	2,760	18,119
D-Alanine (4 mM)	2,678	16,545
D-Alanine (40 mM)	2,450	19,821
L-Alanine (4 mM)	2,981	17,400
L-Alanine (40 mM)	2,020	16,540
D-Threonine (4 mM)	2,040	15,200
D-Threonine (40 mM)	2,240	19,500
L-Threonine (4 mM)	2,260	17,950
L-Threonine (40 mM)	2,950	19,250

[a] Each value represents the mean of three determinations.

acids in concentrations as high as 40 mM had no significant effect on the oxygen consumption of either resting or phagocytizing leukocytes (*data not shown*).

It seems unlikely that the lack of effect could be explained on the basis of exclusion of the amino acids from the cell. Ascorbic acid in considerably lower concentrations (0.10 to 1.0 mM) will more than double the HMS activity of resting neutrophils (3). Epinephrine, norepinephrine, dihydroxyphenylalanine, and adrenochrome will cause a 5- 10-fold stimulation of the HMS of resting neutrophils at a concentration of 1 mM or less (Qualliotine et al., J. Reticuloendothel. Soc., *in press*). Rosenberg and Downing (15) have demostrated that leukocyte suspensions in vitro actively transport neutral amino acids against a concentration gradient, so that the intracellular concentration becomes many times higher than the concentration in the medium. Finally, in the case of the phagocytizing neutrophils, some of the amino acids are certain to be taken up in the formation of the phagocytic vesicle [as has been demonstrated for the uptake of nitroblue tetrazolium dye (14)], and yet there is no increment in HMS activity attributable to the amino acids under these conditions.

Previous work has conclusively demonstrated that hydrogen peroxide will stimulate the HMS in normal human neutrophils (14). The present results demonstrate that high concentrations of either D- or L-amino acids have no effect on the HMS activity. These data support the contention of Eckstein et al. that amino acid oxidases are not the primary source of H_2O_2 generation during phagocytosis (5). Although the amino acid oxidases are certainly present within the leukocyte, their physiologic role remains obscure.

The excellent technical assistance of Pamela Shirley is gratefully acknowledged.

This research was supported by grants from the Brown-Hazen Fund, the Forsyth Cancer Service, and by Public Health Service grant AI-10732 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Cagen, R. H., and M. L. Karnovsky. 1964. Enzymatic basis of the respiratory stimulation during phagocytosis. Nature (London) **204**:255.
2. Cline, M. J., and R. I. Lehrer. 1969. D-amino acid oxidase in leukocytes: a possible D-amino-acid-linked antimicrobial system. Proc. Nat. Acad. Sci. U.S.A. **62**:756–763.
3. Cooper, M. R., C. E. McCall, and L. R. DeChatelet. 1971. Stimulation of leukocyte hexose monophosphate shunt activity by ascorbic acid. Infect. Immunity **3**:851–853.
4. DeChatelet, L. R., and M. R. Cooper. 1970. A modified procedure for the determination of leukocyte alkaline phosphatase. Biochem. Med. **4**:61–68.
5. Eckstein, M. R., R. L. Baehner, and D. G. Nathan. 1971. Amino acid oxidase of leukocytes in relation to H_2O_2-mediated bacterial killing. J. Clin. Invest. **50**:1985–1991.
6. Holmes, B., A. R. Page, and R. A. Good. 1967. Studies of the metabolic activity of leukocytes from patients with a genetic abnormality of phagocytic function. J. Clin. Invest. **46**: 1422–1432.
7. Iyer, G. Y. N., D. F. M. Islam, and J. H. Quastel. 1961. Biochemical aspects of phagocytosis. Nature (London) **192**: 535–541.
8. Iyer, G. Y. N., and J. H. Quastel. 1963. NADPH and NADH oxidation by guinea pig polymorphonuclear leukocytes. Can. J. Biochem. Physiol. **41**:427–434.
9. Karnovsky, M. L. 1962. Metabolic basis of phagocytic activity. Physiol. Rev. **42**:143–168.
10. Klebanoff, S. J. 1967. Iodination of bacteria: a bactericidal mechanism. J. Exp. Med. **126**:1063–1078.
11. Merchant, D. J., R. H. Kahn, and W. H. Murphy, Jr. 1960. Handbook of cell and organ culture. Burgess Publishing Co., Minneapolis.
12. Miller, T. E. 1969. Killing and lysis of gram-negative bacteria through the synergistic effect of hydrogen peroxide, ascorbic acid, and lysozyme. J. Bacteriol. **98**:949–955.
13. Nathan, D. G., R. L. Baehner, and D. K. Weaver. 1969. Failure of nitro blue tetrazolium reduction in the phagocytic vacuoles of leukocytes in chronic granulomatous disease. J. Clin. Invest. **48**:1895–1904.
14. Reed, P. W. 1969. Glutathione and the hexose monophosphate shunt in phagocytizing and hydrogen peroxide-treated rat leukocytes. J. Biol. Chem. **244**:2459–2464.
15. Rosenberg, L. E., and S. Downing. 1965. Transport of neutral and dibasic amino acids by human leukocytes: absence of defect in cystinuria. J. Clin. Invest. **44**:1382–1393.
16. Strauss, R. R., B. B. Paul, A. A. Jacobs, and A. J. Sbarra. 1970. Role of the phagocyte in host-parasite interactions XXII. H_2O_2-dependent decarboxylation and deamination by myeloperoxidase and its relationship to antimicrobial activity. J. Reticuloendothel. Soc. **7**:754–761.