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

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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 H2O2 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 H2O2 production are increases in hexose monophosphate shunt (HMS) activity and in oxygen consumption (19).

The exact mechanism by which the H2O2 is generated is a matter of considerable interest and much debate. Although some workers feel the chief 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 H2O2 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 H2O2 production is evidence that this cannot be the sole means of H2O2 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 H2O2 during phagocytosis.

Because the stimulation of the HMS is intimately associated with H2O2 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-1-14C (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 pH 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 × 10⁸ 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 14CO2 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 acids...
TABLE 1. Effect of amino acids on \(^{14}\text{CO}_2\) production from glucose-\(^1\text{C}\) in resting and phagocytizing human neutrophils

<table>
<thead>
<tr>
<th>Addition</th>
<th>Counts/min in (^{14}\text{CO}_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resting</td>
</tr>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>D-Alanine (4 mM)</td>
<td>2,760</td>
</tr>
<tr>
<td>D-Alanine (40 mM)</td>
<td>2,450</td>
</tr>
<tr>
<td>L-Alanine (4 mM)</td>
<td>2,981</td>
</tr>
<tr>
<td>L-Alanine (40 mM)</td>
<td>2,020</td>
</tr>
<tr>
<td>L-Threonine (4 mM)</td>
<td>2,040</td>
</tr>
<tr>
<td>D-Threonine (40 mM)</td>
<td>2,240</td>
</tr>
<tr>
<td>L-Threonine (4 mM)</td>
<td>2,260</td>
</tr>
<tr>
<td>L-Threonine (40 mM)</td>
<td>2,950</td>
</tr>
</tbody>
</table>

* 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 demonstrated 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 \(\text{H}_2\text{O}_2\) generation during phagocytosis (5). Although the amino acid oxidases are certainly present within the leukocyte, their physiologic role remains obscure.

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LITERATURE CITED