Assessment of neutrophil function – I

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Introduction

Neutrophils with monocytes form the primary defence of the body against infection. Thus they will exhibit chemotaxis to a site of bacterial invasion, and there they will phagocytose the invaders, aided by serum components, opsonins.

Opsonization and neutrophil granules

There are 3 mechanisms for opsonization. Firstly, specific antibody that reacts with the bacterial cell walls will then attach to the surface of neutrophils via their Fc receptors, which are for the heavy chains of antibody molecules. Secondly, the classical pathway of complement activation yields opsonic C3b, which attaches to a complement receptor (CR) on the neutrophil surface; thirdly, the alternative pathway properdin system also generates C3b. In fact each neutrophil has 120,000 receptors for the Fc of IgG and 38,000 surface receptors for C3b (designated CR1 for C3b and CR3 for iC3b). After ingestion most bacteria can be killed in the phagosomes by the action of 'reactive oxygen intermediates' (ROI) or by the action of cationic proteins and lysosomal proteases.

There are two types of granule within the neutrophil. The primary azurophic granules contain acid hydrolases and neutral proteases, and the vital myeloperoxidase (MPO) which generates bactericidal hypochlorite from hydrogen peroxide. The specific granules contain lysozyme, lactoferrin, cathepsin G, a collagenase and a protease that will generate C5a from C5. In the process of phagocytosis each type of granule fuses with the phagosome and releases its contents into it. So the lysozyme can digest the peptidoglycan of gram-positive bacterial cell walls, which it does by digesting the bond between muramic acid and N-acetylgucosamine. Then the generated reactive oxygen intermediates together with cationic proteins aid the killing and disintegration of bacteria.

Adherence to vascular endothelium and chemotaxis

When an acute inflammatory response develops at a local site where there is bacterial invasion, there is increased blood flow through dilated arterioles and through newly opened capillary and venular beds, and concurrently an increase of micro-vascular permeability is established. The result is that there is margination of neutrophils, adhesion to the endothelium of the post-capillary venules and then migration into the tissues. The mature neutrophil has a negative surface charge due to its excess of surface sialic acid. This means that it is repulsed by normal endothelium which bears a like negative surface charge. Reduction of this surface charge by alterations of surface glycoproteins results in adhesion of neutrophils to the endothelium.

In brief the sequence of events is adhesion of neutrophils to the endothelium, migration along a chemotactic gradient into the tissues, ingestion of bacteria, activation of oxidative metabolism by the respiratory burst, secretion of enzymes into the phagosomes and also externally.

The physical properties of the endothelial cells influence adherence. It is known that in response to a chemotactic stimulus Mac-1 and p150,90 adhesive glycoproteins appear on the surface of the neutrophil, arising as the membrane is expanded by the fusion of secondary granules from beneath (Springer & Anderson, 1986). Mac-1 is actually the complement receptor type 3, which mediates binding and phagocytosis of particles opsonized with the C3bi ligand. However, in those rare patients with deficiency of this glycoprotein (Springer et al., 1984), the functional defects are actually much greater than this. The autosomal recessive results in life threatening bacterial infections, a lack of pus formation and persistent granulocytosis. This defect does not account for a difference in surface charge.

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Leukotriene B4 has been shown to stimulate neutrophil adhesion to cultured vascular endothelial cells (Gimbrone et al., 1984). In fact both PAF acether and LTB4 mediate neutrophil chemotaxis through an intact endothelial cell monolayer (Hopkins et al., 1984). These effects are presumably mediated via a change in intracellular calcium distribution because the chemotaxis of neutrophils is stopped by agents that elevate intracellular cyclic AMP. Fibronectin has no influence on the movement of neutrophils through basement membranes. However the glycoprotein laminin, which is an integral part of membranes, has been shown to bind to the neutrophil surface, to help attachment to type IV collagen of the basement membrane and to promote neutrophil migration through the membrane (Terranova et al., 1986).

Chemotactic factors determine the migration of neutrophils. It is estimated that a 1% difference in the strength of a chemotactic agent from the front to the rear of a cell determines cell locomotion along the axis of a concentration gradient (Uden et al., 1986). The numerous chemotactic agents are conveniently subdivided into those that are cell or plasma derived, and those that are exogenous bacterial factors (Table I). The latter can be mimicked by artificial agents (Valerius, 1984).

Bacterial factor may share a cell surface receptor with the tripeptide FMLP. Zymosan works by activation of the complement cascade so producing C5a, which is a major in vivo chemoattractant.

The biochemistry of phagocytosis is described in standard works (Wilkinson, 1982; Dale & Foreman, 1984) and will not be detailed here. The process is mediated by Fc and C3 surface receptors and an invagination occurs on the surface as actin:myosin polymerization affects underlying microfilaments (Valerius, 1984; Hartwig, 1986).

### The respiratory burst
Phagocytosis is accompanied by a sudden burst of oxygen consumption that is known as the ‘respiratory burst’ (Table II).

#### Table II Features of the respiratory burst

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Increased oxygen utilization of neutrophils.</td>
</tr>
<tr>
<td>2.</td>
<td>Increased oxidation of glucose via the HMP shunt.</td>
</tr>
<tr>
<td>3.</td>
<td>Increased formation of hydrogen peroxide.</td>
</tr>
<tr>
<td>4.</td>
<td>Formation of superoxide anion $O_2^-$.</td>
</tr>
</tbody>
</table>

Respiratory burst activity involves the oxidation of increased amounts of glucose via the hexose monophosphate shunt. This can be measured by the oxidation of $1^{-14}C$-glucose to radioactive carbon dioxide, which is adsorbed onto a cotton wool plug that is saturated with hyamine (Root et al., 1972). Concurrently the respiratory burst generates reactive oxygen intermediates such as superoxide via the activation of a membrane NADPH oxidase, that catalyses the one electron reduction of oxygen to superoxide, using the reducing equivalents provided by NADPH formed by the HMP shunt (Babior, 1978; Klebanoff, 1980; Rossi et al., 1986).

$$\text{NADPH} + \text{H}^+ + 2\text{O}_2 \rightarrow \text{NADP}^+ + 2\text{H}^+ + 2\text{O}_2^-$$

This membrane oxidase enzyme complex contains phospholipids and a unique (non-mitochondrial) cytochrome b245 which has a redox potential low enough to reduce molecular oxygen to superoxide. Hence activated neutrophils will secrete superoxide and hydrogen peroxide into their environment. The latter is created by the spontaneous dismutation of superoxide (2$\text{O}_2^- + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O}_2$)

The following sequence indicates how the various reactive oxygen intermediates (ROIs) are formed.

```
<table>
<thead>
<tr>
<th>Molecular</th>
<th>Superoxide</th>
<th>Hydrogen Peroxide</th>
<th>Hydroxyl Radical</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>O_2</td>
<td>+e</td>
<td>+e</td>
<td>+e</td>
<td>OH_2</td>
</tr>
<tr>
<td></td>
<td>2H</td>
<td>HzO</td>
<td>+OH^-</td>
<td>+2H^+</td>
</tr>
</tbody>
</table>
```

The various forms of reactive oxygen intermediates formed by neutrophils are shown in Table III.
The amount of superoxide that is formed can be quantitated in various ways:

1. Ferricytochrome c is reduced by superoxide to ferrocytochrome c

\[ \text{O}_2^- + \text{Fe}^{3+} : \text{cyt c} \rightarrow \text{O}_2 + \text{Fe}^{2+} : \text{cyt c} \]

(Curnutte & Babiör, 1974).

2. By chemiluminescence that largely measures single oxygen formation (Trush et al., 1978).

3. Nitro-blue tetrazolium reduction (Baehner & Nathan, 1968; McCall et al., 1974; Levinsky et al., 1983).


The hydrogen peroxide formation by activated neutrophils can be measured by the oxidation of \(^{14}\text{C}-\text{formate}\) (Iyer et al., 1961).

### References


SPRINGER, T.A. & ANDERSON, D.C. (1986). The importance

### Table III

<table>
<thead>
<tr>
<th>Types of oxygen metabolite formed by activated neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary metabolites</strong></td>
</tr>
<tr>
<td>superoxide O(_2^-)</td>
</tr>
<tr>
<td>hydrogen peroxide H(_2)O(_2)</td>
</tr>
<tr>
<td><strong>Secondary metabolites</strong></td>
</tr>
<tr>
<td>hydroxyl radical OH</td>
</tr>
<tr>
<td>singlet oxygen O(_2)</td>
</tr>
<tr>
<td>hypochlorous acid HOCl</td>
</tr>
<tr>
<td>chloramines HR-NH-Cl and HR-NCl</td>
</tr>
</tbody>
</table>

**Bacterial killing**

Hydrogen peroxide that is formed by activated neutrophils is used by the enzyme myeloperoxidase (MPO), that imparts the yellow colour to neutrophils, to form hypochlorous acid which kills bacteria in the phagosomes.

\[ \text{H}_2\text{O}_2 + \text{Cl}^- \rightarrow \text{OCl}^- + \text{H}_2\text{O} \]

MPO also deaminates and decarboxylates aminoacids in the presence of hydrogen peroxide and chloride. That reaction yields chloramines and aldehydes which cause oxidative damage to proteins.

The importance of the oxidative burst is stressed by reports of impaired bacterial killing in those rare patients with 'chronic granulomatous disease of childhood' (Holmes *et al.*, 1966) or persons with myeloperoxidase deficiency (Rosen & Klebanoff, 1976). Males with the X-linked chronic granulomatous disease (CGD) are defective in cytochrome b 245 (Segal *et al.*, 1983), whilst the female carriers have only half the required amount. In CGD patients the hexose monophosphate shunt activity of leucocytes and also myeloperoxidase iodination of proteins is poor. The patients are usually identified by the inability of their neutrophils to reduce nitroblue-tetrazolium on a slide test (Ward, 1974; Baehner & Nathan, 1968), and there are even ways of screening antenatal samples of blood (Levinsky *et al.*, 1983).
of the Mac-1,LFA-1 glycoprotein family in monocyte and granulocyte adherence. *Ciba Foundation Symposium, 118*, 102.


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