Review Article

Assessment of neutrophil function – II. Laboratory tests of neutrophil function*

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Laboratory tests of neutrophil function

Adherence of neutrophils to nylon fibres

Since adherence is a local phenomenon at sites of inflammation, one can only expect to detect gross abnormalities. Adherence is increased in inflammation (Lentek et al., 1976). It is defective in those rare cases of Mac-1 deficiency (Anderton & Airlaut, 1984). It can also be defective in diabetes (Bagdade et al., 1978).

Fc and C3 surface receptors

For Fc receptors one might study the adherence of neutrophils to ox erythrocytes that are coated with rabbit IgG antibody to those cells. Likewise for C3 receptors one can use ox erythrocytes coated with rabbit IgM antibody to those cells, and the system will contain added mouse complement (Breedveld et al., 1984).

Chemotaxis

This is measured by a Boyden diffusion chamber. A filter separates cells, that can be labelled with radioactive chromium, and the attractant. Hence the number of neutrophils that move through the filter can be quantitated (Boyden, 1962; Ternowitz, 1985).

One can also use a test in which there is migration of neutrophils under an agarose gel. Three wells are cut in agarose gel in a plastic tissue culture dish. The neutrophils are placed between buffer on one side and chemoattractant on the other side. Obviously they migrate preferentially towards the attractant.

This can be zymosan activated serum (i.e. C5a) or a filtrate of a bacterial culture (Nelson et al., 1975).

Phagocytosis

This will involve counts of remaining bacteria after 2 hours of incubation with known numbers of neutrophils (Sieger & Waldman, 1977). Alternatively it can be performed by using bacteria that are pre-labelled with isotopes (Miller & Beck, 1975; Peterson et al., 1977).

Intracellular killing

This is the most vital function to study when a patient’s clinical course suggests susceptibility to infection. The method ascertains the decrease in number of viable bacteria when neutrophils containing Staphylococcus aureus are incubated (Leigh et al., 1980). It is of note that killing capacity is similar under aerobic and anaerobic conditions (Vel et al., 1984), even though there is little or no chemiluminescence and thus no formation of reactive oxygen species in the anaerobic situation. This indicates bacterial killing by means of cationic proteins within the phagosomes (Elbsbach & Weiss, 1983; Houde & Gray, 1986).

Only extracellular bacteria or Candida take up uridine (Yamamura et al., 1977). Hence it has been possible to devise useful rapid micro-assays of phagocytosis using this principle (Rajkovic & Williams, 1985).

Assay of specific neutrophil enzymes

(i) A primary defect of neutrophil myeloperoxidase (Kitahar et al., 1979) can be detected by a colour reaction. The various anti-arthritis drugs that contain thiol groups (d-penicillamine, sodium aurothiomalate, aurothiogluco and tiopronin) have recently been shown to act by scavenging hypochlorite so preventing its action (Cuperus et al., 1985). Indeed many of the anti-arthritis drugs act by inhibiting neutrophil functions.

(ii) Lysozyme extracted from neutrophils can be

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assayed by a turbidometric assay that quantitates the fall in optical density of a standard solution of Micrococcus lysodeikticus in comparison with a standard solution of human lysozyme (Hanse & Anderson, 1973).

(iii) Esterases can be assayed using an artificial substrate such as benzoyl-L-tyrosine ethylester (Odeberg et al., 1975).

(iv) Elastase that is released from neutrophils is a good indicator of changes during septicaemia (Duswald et al., 1985), and of neutrophil activation by means of dialysis membranes (Hörl et al., 1985).

**Iodination by leucocytes**

The basis of this test (Klebanoff & Clark, 1977) is that myeloperoxidase forms hypohalite (e.g. hypochlorous acid) by the combination of chlorine or iodine with oxygen that is derived from hydrogen peroxide.

\[
\begin{align*}
    H_2O_2 + Cl^- & \rightarrow ClO^- + H_2O \\
    H_2O_2 + I^- & \rightarrow IO^- + H_2O
\end{align*}
\]

Neutrophils that lack myeloperoxidase (MPO), that cannot produce hydrogen peroxide or those that have a defect in hexose monophosphate (HMP) shunt activity show defective iodination and bactericidal capacity. The hypohalite that is formed will kill by iodinating the tyrosine residues of bacterial proteins.

In surgical infections there is often failure of opsonization of bacteria and thus defective chemotaxis (Cohen et al., 1984). The iodination technique is sensitive enough to be used to detect such opsonization defects, or subtle depression of neutrophil function that occurs with *E. coli* or *K. pneumoniae* infections (Kellerman et al., 1985). Thus the test is particularly useful for studies of susceptibility to infection at the time of surgery or trauma.

**Chemiluminescence**

The formation of ROIs in neutrophils causes the cells to emit very weak light radiation. Emission in the red bands (\( \lambda_{\text{max}} 634 \) and 703 nm) is thought to represent the formation of singlet oxygen

\[ H_2O_2 + OCl \rightarrow H_2O + Cl^- + ^1O_2 \]

whilst blue wavelength emissions represents excited carbonyls that are produced by oxidation (Trush et al., 1978; Campbell et al., 1984). It is also known now that the interaction between hydrogen peroxide and chloramine produces light (Zgliczynski et al., 1985).

Fortunately the compound luminol readily diffuses into neutrophils and reacts with oxidizing ions that have been produced during phagocytosis. The reaction leads to the formation of an electronically excited aminophthalate ion, which releases light on returning to its ground state. The chemiluminescence is measured using photomultiplier tubes adjusted so as to convert light to electrical energy.

**Applications of chemiluminescence (CL)**

CL is closely related to bacterial killing under aerobic conditions. Patients with chronic granulomatous disease (CGD) produce no CL during phagocytosis and patients with MPO deficiency have a delayed lower CL response. In fact a mixture of MPO, hydrogen peroxide and halides in a cell-free system produces CL and it is bactericidal (Allen et al., 1972).

CL assays are useful for the detection of those rare conditions in which there is an intrinsic defect of neutrophils, or the more common situation in which a patient with a serious bacterial infection turns out to have an opsonic defect (Wilson et al., 1978; Schopf & Mattar, 1984; Stevens & Young, 1977; Easmon et al., 1980; Barbour & Allred, 1980). If there is a serum defect it can be clearly identified by using the following type of protocol (Leigh et al., 1981; Johnson et al., 1984) (Table 1).

CL can be used to quantitate the activity of membrane NADPH oxidase (Minkenberg & Ferber, 1985) and luminol binding microspheres to perform direct measurements of phagosomal superoxide formation (Uchida et al., 1985).

CL can be used to define any form of neutrophil

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**Table 1** Neutrophil or opsonic defects and CL

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Function being tested</th>
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</thead>
<tbody>
<tr>
<td>FMLP</td>
<td>respiratory burst enzymes of neutrophils</td>
</tr>
<tr>
<td>opsonized zymosan</td>
<td>oxidative burst after phagocytosis</td>
</tr>
<tr>
<td>normal pooled serum with neutrophils</td>
<td>tests the neutrophil phagocytic capacity</td>
</tr>
<tr>
<td>patient’s own serum with neutrophils</td>
<td>tests opsonization ability</td>
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</tbody>
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FMLP = formyl, methionine, leucine, phenylalanine, Chemotactic peptide.
activation whether it involves membrane activation and ingestion of a bacterium, a virus, or osonized zymosan or immune complexes. Thus neutrophils taken from rheumatoid synovial fluids show increased CL (James et al., 1981) as one might expect.

It has been mentioned that the anti-arthritic drugs that contain thiol groups work by scavenging hypochlorite and preventing its formation by MPO (Cuperus et al., 1985). Indeed amelioration of symptoms by many anti-arthritic drugs seems to depend on inhibition of neutrophil (and macrophage) actions.

CL has been used to characterize the diminished bactericidal capacity of the neutrophils of the neonate (Quie & Mills, 1979; Mills et al., 1979; Shigeoka et al., 1981). The newborn can combat normal bacterial challenges but nevertheless CL is poor. It turns out that newborn neutrophils can produce superoxide but hydroxyl radical formation is defective (Ambruso et al., 1979).

References


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