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 radiochromium, 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. C5α) 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 certifies 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 (Elsbach & 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-arthritic drugs that contain thiol groups (d-penicillamine, sodium aurothioglucose and tiopronin) have recently been shown to act by scavenging hypochlorite so preventing its action (Cuperus et al., 1985). Indeed many of the anti-arthritic drugs act by inhibiting neutrophil functions.

(ii) Lysozyme extracted from neutrophils can be
assayed by a turbidometric assay that quantitates the
fall in optical density of a standard solution of
Micrococcus lysodeikticus in comparison with a stan-
dard 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.

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

\[ \text{H}_2\text{O}_2 + \text{I}^- \rightarrow \text{OI}^- + \text{H}_2\text{O} \]

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 neutro-
phil 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

\[ \text{H}_2\text{O}_2 + \text{OCl}^- \rightarrow \text{H}_2\text{O} + \text{Cl}^- + ^1\text{O}_2 \]

whilst blue wavelength emission 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 reac-
tion 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
condition (CGD) produce no CL during phagocytosis and
patients with MPO deficiency have a delayed lower CL
response. In fact a mixture of MPO, hydrogen perox-
ide 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; Eamon 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 for-
mation (Uchida et al., 1985).

CL can be used to define any form of neutrophil

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<thead>
<tr>
<th>Table 1</th>
<th>Neutrophil or opsonic defects and CL</th>
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<tbody>
<tr>
<td><strong>Stimulus</strong></td>
<td><strong>Function being tested</strong></td>
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<tr>
<td>FMLP</td>
<td>respiratory burst enzymes of neutrophils</td>
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<tr>
<td>opsonized zymosan</td>
<td>oxidative burst after phagocytosis</td>
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<td>normal pooled serum with neutrophils</td>
<td>tests the neutrophil phagocytic capacity</td>
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<tr>
<td>patient's own serum with neutrophils</td>
<td>tests opsonization ability</td>
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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 opsonized 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


