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 & Airault, 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. 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 (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-arthritis drugs that contain thiol groups (D-penicillamine, sodium aurothiomalate, aurothiogluconate 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.

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 *Kl. 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 (λ_{max} 634 and 703 nm) is thought to represent the formation of singlet oxygen

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 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 I).

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

Table I Neutrophil or opsonic defects and CL

<i>Stimulus</i>	<i>Function being tested</i>
FMLP opsonized zymosan	respiratory burst enzymes of neutrophils oxidative burst after phagocytosis
normal pooled serum with neutrophils	tests the neutrophil phagocytic capacity
patient's own serum with neutrophils	tests opsonization ability

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-arthritis 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-arthritis 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

- ALLEN, R.C., STJERNHOLM, R.L. & STEEL, R.H. (1972). Evidence for the generation of an electronic excitation state in human polymorphonuclear leucocytes and the participation in bactericidal activity. *Biochemical and Biophysical Research Communications*, **42**, 679.
- AMBRUSO, D.R., ALTENBURGER, K.M. & JOHNSTON, R.B. (1979). Defective oxidative metabolism in newborn neutrophils: discrepancy between superoxide anion and hydroxyl radical generation. *Pediatrics*, **64**, (suppl) 722.
- ANDERSON, B.R. & AIRAULT, H.J. (1979). Important variables in granulocyte chemiluminescence. *Proceedings of the Society for Experimental Biology and Medicine*, **162**, 139.
- BAGDADE, J.D., STEWART, M. & WALTERS, E. (1978). Impaired granulocyte adherence in patients with poorly controlled diabetes. *Diabetes*, **27**, 677.
- BARBOW, A.G., ALLRED, C.D., SOLBERG, C.O. & HILL, H.R. (1980). Chemiluminescence by polymorphonuclear leukocytes from patients with active bacterial infection. *Journal of Infectious Diseases*, **141**, 14.
- BOYDEN, S. (1962). The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. *Journal of Experimental Medicine*, **115**, 452.
- BREEDVELD, F.C., LAFEVER, G., DE VRIES, E., DAHA, M.R. & CATS, A. (1984). C3 receptors on granulocytes from patients with rheumatoid arthritis. *Clinical and Experimental Immunology*, **57**, 557.
- CAMPBELL, A.K., HOLT, M.E. & PATEL, A. (1984). Chemiluminescence in medical biochemistry. In *Recent Advances in Clinical Chemistry*, Vol III, Alberti, K. & Price, G. (eds). Churchill Livingstone: Edinburgh, London.
- COHEN, R., SCIUTTO, P., BROWN, G.L. & POLK, H.C. (1984). Failure of opsonisation as a sign of lethal sepsis. *Journal of Infectious Diseases*, **149**, 651.
- CUPERUS, R.A., MUIJERS, A.O. & WEVER, R. (1985). Myeloperoxidase from human leukocytes: a therapeutic mechanism for drugs in rheumatoid arthritis. *Arthritis and Rheumatism*, **28**, 1228.
- DUSWALD, K.H., JOCHUM, M., SCHRAMM, W. & FRITZ, H. (1985). Released granulocyte elastase: an indicator of pathobiochemical alterations in septicemia. *Surgery*, **98**, 892.
- EASMON, C.S.F., COLE, P.J., WILLIAMS, A.J. & HASTINGS, M. (1980). Measurement of opsonic and phagocytic function by luminol dependent chemiluminescence. *Immunology*, **41**, 67.
- ELSBACH, P. & WEISS, J. (1983). A re-evaluation of the role of oxygen dependent and oxygen independent microbicidal systems of phagocytes. *Reviews of Infectious Diseases*, **5**, 843.
- HANSEN, N.E. & ANDERSON, V. (1973). Lysozyme activity in human neutrophil granulocytes. *British Journal of Haematology*, **24**, 613.
- HÖRL, W.H., STEINHAVER, H.B. & SCHOLLMAYER, P. (1985). Plasma levels of granulocyte elastase during haemodialysis. *Kidney International*, **28**, 791.
- HOUDE, C.J. & GRAY, B.H. (1986). Effects of a bactericidal protein from human polymorphonuclear leucocytes on *Ps. aeruginosa*. *Infection and Immunity*, **52**, 90.
- JAMES, D.W., BETTS, W.H. & CLELAND, L.G. (1983). Chemiluminescence of polymorphonuclear leucocytes from rheumatoid joints. *Journal of Rheumatology*, **10**, 184.
- JOHNSON, C.M., RHODES, K.H. & KATZMANN, J.A. (1984). Neutrophil function tests. *Mayo Clinic Proceedings*, **59**, 431.
- KELLERMAN, J.S., BROWN, G.L., LAMONT, P.M. & POLK, H.C. (1985). Characterisation of neutrophil iodination for the assessment of phagocytic and opsonic function in septic patients. *American Journal of Surgery*, **150**, 301.
- KITAHARA, M., SIMONIAN, Y. & EYRE, H.J. (1979). Neutrophil myeloperoxidase. *Journal of Laboratory and Clinical Medicine*, **93**, 232.
- KLEBANOFF, S.J. & CLARK, R.A. (1977). Iodination by human polymorphonuclear leucocytes. *Journal of Laboratory and Clinical Medicine*, **89**, 675.
- LEIGH, P.C., VAN DEN BARSELAAR, M.T., DUBBLEDEMAN-REMPT, I. & VAN FURTH, I. (1980). Kinetics of intracellular killing of *Staph. aureus* and *E. coli* by human granulocytes. *European Journal of Immunology*, **10**, 750.
- LEIGH, P.C., VAN DER BAARSELAAR, M., DAHA, M. & VAN FURTH, R. (1981). Participation of immunoglobulins and complement components in the killing of *Staph. aureus* and *E. coli* by granulocytes. *Infection and Immunity*, **33**, 714.
- LENTNEK, A.L., SCHREIBER, A.D. & MACGREGOR, R.R. (1976). Induction of augmented granulocyte adherence by inflammation. *Journal of Clinical Investigation*, **57**, 1098.
- MILLS, E.L., THOMPSON, T. & BJØRSTEN, B. (1979). The chemiluminescence response and bactericidal activity of polymorphonuclear leucocytes from newborns and their mothers. *Pediatrics*, **63**, 429.
- MILLER, D.S. & BECK, S. (1975). Polymorphonuclear phagocytosis: a rapid radioactive method. *Journal of Laboratory and Clinical Medicine*, **86**, 344.

- MINKENBERG, I. & FERBER, E. (1985). Measurement of membrane NADPH oxidase by means of chemiluminescence. *Journal of Immunological Methods*, **79**, 57.
- NELSON, R.D., QUIE, P.G. & SIMMONS, R.L. (1975). Chemotaxis under agarose. *Journal of Immunology*, **115**, 1650.
- ODEBERG, H., OLSSON, I. & VENGE, P. (1975). Neutrophil leucocyte esterase measured with artificial substrate. *Laboratory Investigation*, **32**, 57.
- PETERSON, P.K., VERHOEF, J., SABATH, L.D. & QUIE, P.G. (1977). Kinetics of phagocytosis and bacterial killing by polymorphs. *Journal of Infectious Diseases*, **136**, 502.
- QUIE, P.G. & MILLS, E.L. (1979). Bactericidal and metabolic function of polymorphonuclear leucocytes. *Pediatrics*, **64** (suppl), 719.
- SCHOPF, R.E. & MUTTAR, J. (1984). Measurement of the respiratory burst by nitro-blue tetrazolium reduction and by chemiluminescence. *Journal of Immunological Methods*, **67**, 109.
- SHIGEOKA, A.O., CHARETTE, R.P. & WYNON, M.C. (1981). Defective oxidative metabolic responses of neutrophils from stressed neonates. *Journal of Pediatrics*, **98**, 392.
- SIEGER, W.B. & WALDMAN, R.H. (1977). Phagocytic function in patients with nosocomial infections. *Journal of the Reticuloendothelial Society*, **22**, 55.
- STEVENS, P. & YOUNG, L.S. (1977). Quantitative granulocyte chemiluminescence in the rapid detection of impaired opsonisation of *E. coli*. *Infection and Immunity*, **16**, 796.
- TERNOVITZ, T. (1985). Human monocyte and neutrophil chemotaxis *in vitro* employing 51-chromium labelled leucocytes. *Acta Pathologica Scandinavica*, **93C**, 189.
- TRUSH, M.A., WILSON, M.E. & VAN DYKE, K. (1978). The generation of chemiluminescence by phagocytic cells. *Methods in Enzymology*, **57**, 462.
- UCHIDA, T., KARNO, T., HOSAKA, S. (1985). Phagosomal superoxide formation measured by luminol binding microspheres. *Journal of Immunological Methods*, **77**, 55.
- VEL, W., NAMAVAR, F., VERWEIJ, A., PUBBEN, A. & MACLAREN, D.M. (1984). Killing capacity of human polymorphonuclear leukocytes in aerobic and anaerobic conditions. *Journal of Medical Microbiology*, **18**, 173.
- WILSON, M.E., TRUSH, M.A., VAN DYKE, K. & KYLE, J.M. (1978). Luminol dependent chemiluminescence of opsonophagocytic dysfunctions. *Journal of Immunological Methods*, **23**, 315.
- YAMAMURA, M., BOLER, J.C. & VALDIMARSSON, H. (1977). Phagocytosis measured as inhibition of uridine uptake by *Candida albicans*. *Journal of Immunological Methods*, **14**, 19.
- ZGLICZYNSKI, J.M., OLSZOWSKA, E., OLSZOWSKI, S., STELMASZYNSKA, T. & KWASNOWSKA, E. (1958). Possible origin of chemiluminescence in phagocytosing neutrophils: reaction between chloramines and hydrogen peroxide. *International Journal of Biochemistry*, **17**, 515.