Phagocytes, the first-line cells of the body's defence mechanisms against invading pathogens, kill microorganisms by means of lysosomal degradative enzymes and highly toxic reactive oxygen intermediates. The reactive oxygen compounds are produced, in a process called the 'respiratory burst', by the NADPH oxidase complex in phagocytes. In a process called the 'respiratory burst', activated phagocytes reduce molecular oxygen to superoxide via a special electron transport system (NADPH-oxidase). Superoxide radicals form hydrogen peroxide in a dismutase reaction catalyzed by the superoxide dismutase enzyme (SOD). Hydrogen peroxide serves as a substrate for the myeloperoxidase (MPO) reaction, in which a variety of highly toxic metabolites, including hypochlorite, are generated. These processes generate phagocyte chemiluminescence (CL), a process that can be predicted.

Abstract. Phagocytes, the first-line cells of the body's defence mechanisms against invading pathogens, kill microorganisms by means of lysosomal degradative enzymes and highly toxic reactive oxygen intermediates. The reactive oxygen compounds are produced, in a process called the 'respiratory burst', by the NADPH oxidase complex in plasma membranes, and by myeloperoxidase in phagolysosomes after degranulation. These processes generate highly reactive oxygen metabolites. Killing processes can take place inside the cell in phagolysosomes as well as outside the phagocyte. In a process called the 'respiratory burst' activated phagocytes reduce molecular oxygen to superoxide via a special electron transport system (NADPH-oxidase). Superoxide radicals form hydrogen peroxide in a dismutase reaction catalyzed by the superoxide dismutase enzyme (SOD). Hydrogen peroxide serves as a substrate for the myeloperoxidase (MPO) reaction, in which a variety of highly toxic metabolites, including hypochlorite, are generated. These processes produce electronically excited oxygen compounds which, on relaxation, emit photons, giving rise to phagocyte chemiluminescence (CL).

Introduction

Phagocytosing leukocytes constitute the first line of the body's defence mechanism against invading microbial pathogens. Neutrophils (polymorphonuclear leukocytes, PMNL) are the first cells to invade a site of inflammation following an infection. In an inflammatory response the neutrophils are followed later by activated monocytes, macrophages and - especially in the case of parasitic infection - also by eosinophils. Phagocytes kill microorganisms by means of lysosomal degradative enzymes, such as proteases, and highly toxic reactive oxygen metabolites. Killing processes can take place inside the cell in phagolysosomes as well as outside the phagocyte.

This paper describes the conditions for the measurement of CL, and reviews the activity of phagocytes from individuals undergoing stress or disease. The capability of phagocytes to emit photons reflects remarkably well the activity of the disease can be estimated. Physiological changes, e.g. in the reproductive cycle, can also be predicted.
states which, on relaxation to the ground state, emit photons. This emission is referred to as phagocyte chemiluminescence (CL).

Lucigenin and luminol amplify the CL emission by factors of $10^2$ to $10^3$ and $10^3$ to $10^4$, respectively. Lucigenin has a high specificity for the superoxide radical, and thus lucigenin CL reflects the activity of the NADPH-oxidase complex, whereas luminol CL is dependent on MPO activity.

Neutrophils, eosinophils and monocytes have both NADPH-oxidase and MPO activity, and when activated they generate both lucigenin- and luminol-enhanced CL. Macrophages, when primed or activated, are able to generate lucigenin CL, but in the course of maturation the MPO content decreases and thus mature macrophages have a diminished luminol-enhanced CL response.

The commonly used activators include opsonized or unopsonized zymosan, a chemotactic peptide n-formyl-methionine-leucyl-phenylalanine (fMLP), immune complexes, the membrane perturber phorbol myristate acetate (PMA), and calcium ionophore A23187. Zymosan is a cell wall preparation of Saccharomyces cerevisiae containing glucan and mannan, which are recognized by the complement receptor 3 complex (CR3). CR3 also recognizes C3bi and possibly fbrinogen. The binding is dependent on the divalent cations calcium and magnesium. In the opsonization process zymosan attaches to complement compounds and immunoglobulins (Ig). Opsonized zymosan is recognized partly by CR3, partly by CR1 which binds to C3b, and partly by FcγRII and FcγRIII receptors which bind to the Fc portion of the IgG molecules attached on zymosan particles. The FcγRI receptor apparently mediates the antibody-dependent cell-mediated cytotoxicity (ADCC) reaction. Expression of receptors on cell membranes, changes in their functional capacity, signal transduction, phagocytosis, and degranulation all participate in the CL response. Defects in these processes attenuate the response.

Recently, attention has been focused on the hazards of and the damage caused by phagocyte infiltration into tissues and by release of reactive oxygen intermediates. The myocardium is infiltrated within minutes from the onset of infarction, the kidneys in certain types of glomerulonephritis, and the lungs in several pathological conditions. The hyper- or hypoactivity of phagocytes is a decisive factor in the pathogenesis of many diseases like rheumatoid arthritis. The next sections describe the measurement of phagocyte CL and the beneficial and detrimental consequences of the production of reactive oxygen species by phagocytes.

**Method**

Phagocytic cells are generally isolated from blood treated with anticoagulants or from other biological fluids using standard gradient centrifugation methods. On many occasions the buffy coat obtained from blood after erythrocyte sedimentation can be used as a source of phagocytes without further separation.

Phagocytic cell activities can also be measured in ex vivo state simply by diluting whole blood or other body fluids enough to get rid of the inhibitory amounts of plasma and red cells. If the blood samples are not dilute enough, the opsonins in the plasma can interfere, especially when unopsonized particles are used as stimulants. It should be noted here that ex vivo cells are not necessarily in the same functional state as the cells after isolation steps where activation processes may take place. Cooling and rewarming should be avoided because of altered receptor expression.

It has been claimed that blood cells other than phagocytes (lymphocytes, NK cells) were also able to emit CL, but in all cases investigated so far the contaminating phagocytes have been shown to be the actual source. B-lymphocytes transformed by the Epstein-Barr virus might be an exception.

Researchers nowadays seldom carry out phagocyte CL tests without amplifiers: luminol and lucigenin are generally used. Lucigenin reacts with superoxide anion and needs to be reduced to become luminescent. It is therefore considered to be dependent on NADPH oxidase activity. Luminol, on the other hand, has been shown to be oxidized in the myeloperoxidase reaction. When using luminol in the millimolar range one needs less than a thousand phagocytic cells (as in the case in whole blood tests) to get reliable signals. The number of isolated cells used in routine tests varies, generally around $10^5$. If adhesion is not being specially studied, gelatin (or other proteins) should be used to prevent aggregation and the adhesion of the cells to the walls of the measuring vials. Hank’s balanced salt solution is probably the most frequently used buffer. If other buffers are used one should pay attention especially to their content of divalent cations.

Liquid scintillation counters are not recommended as measuring devices because of poor temperature control. Modern luminometers with strict temperature controls, multiple sample capabilities (up to 96 in microtiter plate readers), and computerized data processing are the instruments of choice.

**Defects in phagocyte functions**

An individual suffering from recurrent infections, which are often severe and may eventually be fatal, may have a defect (often one of genetic origin) in one of the crucial functions of phagocytic cells. Chronic granulomatous disease (CGD) is a rare disorder in which the patients suffer from severe recurrent infections with bacteria and fungi, owing to an inability of their phagocytes to kill catalase-positive microorganisms. This is caused by the failure of CGD leukocytes to produce sufficient amounts of superoxide and hydrogen peroxide during phagocytosis. The defect has been