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Clinical and laboratory evaluation of phagocyte functions

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After a micro-organism has broken through the first line of defence against infections, i.e., the intact skin, the mucous membranes, and the antimicrobial substances present on them, the invader is confronted with the second line of defence, the leading components of which are the polymorphonuclear and mononuclear phagocytes. Since phagocytes play such a crucial role in the defence against infections, it need hardly be said that abnormal functioning of these cells can have serious consequences for the host.

This article deals with diseases caused by abnormal functioning of the phagocytes themselves, i.e., abnormalities not caused by the absence of opsonins. A brief review is given of the interaction between phagocytes and micro-organisms and the known disorders of phagocyte function. The methods available for the investigation of phagocyte functions are discussed, as well as the problems associated with the assays used to evaluate phagocyte functions and the interpretation of the results. Finally, indications for the investigation of phagocyte functions are described and a step-wise approach to the evaluation of phagocyte function is presented.

INTERACTION BETWEEN PHAGOCYTES AND MICRO-ORGANISMS

The interaction between phagocytes and micro-organisms starts when the phagocytes, guided by chemoattractants, start to move in the direction of the invading micro-organisms. When chemotaxis has brought the phagocytes into contact with the micro-organisms, phagocytosis occurs. Phagocytosis proceeds in two stages, i.e., attachment of the bacteria to the surface of the phagocyte, and ingestion triggered by that attachment. Opsonization of bacteria by immunoglobulins and/or complement is mandatory for optimal phagocytosis [22]. To ingest a bacterium the phagocyte extends pseudopods around it until the plasma membranes meet and fuse, thus forming an internal vacuole containing the micro-organism. Multiple receptor-ligand bindings are obligatory for ingestion, a process which has been compared with the closing ef a zipper [27—28].

TABLE I

Diseases associated with abnormal phagocyte function and their principal signs and symptoms

Disease	Principal signs/symptoms
Chronic granulomatous disease	Granuloma formation Infections with catalase-positive micro-organisms Gingivitis
Myeloperoxidase deficiency	Infections caused by Candida albicans
Chediak—Higashi syndrome	Partial oculocutaneous albinism Recurrent pyogenic infections Gingivitis and periodontitis Granulocytopenia Giant lysosomes
Hyper-IgE syndrome	Eczema Elevated serum IgE levels Recurrent infections of skin, soft tissues, and respiratory tract Eosinophilia, coarse facial features, and broad nasal bridge
Leucocyte adhesin deficiency	Delayed separation of umbilical stump Gingivitis and periodontitis Infections of skin and respiratory tract
Specific granule deficiency	Recurrent skin infections
Acrodermatitis enteropathica	
Kartagener syndrome	Sinusitis Lower respiratory tract infections

During attachment and ingestion, the oxygen-dependent microbicidal system of the phagocyte generates superoxide anions and hydrogen peroxide [2, 12]. More powerful microbicidal substances are formed from hydrogen peroxide and halide ions (chloride and iodide) by the action of myeloperoxidase released from the lysosomes into the phagosome [11, 13, 25]. Phagocytes also have the disposal of a non-oxidative killing mechanism making use of microbicidal factors such as lysozyme, cationic proteins, lactoferrin, and a bactericidal and permeability-increasing factor [7, 29, 30]. Optimal intracellular killing of micro-organisms requires stimulation of the phagocytes by an extracellular receptor-ligand interaction [15, 16]. The last step in the interaction between phagocytes and micro-organisms is the digestion of the latter. Very little is known about this digestive process [7].

DISORDERS OF PHAGOCYTE FUNCTIONS

A number of diseases due to abnormal phagocyte functions are known, each of them having specific signs and symptoms (Tables I and II).

Abnormal chemotaxis is the funda-

TABLE II

Disorders of phagocyte function and related diseases

Abnormal phagocyte function	Disease
Chemotaxis	Hyper-IgE syndrome Chediak—Higashi syndrome Specific granule deficiency Acrodermatitis enteroparhica Kartagener syndrome
Phagocytosis	Leucocyte adhesin deficiency
Intracellular killing	Chronic granulomatous disease Myeloperoxidase deficiency Chediak—Higashi syndrome Specific granule deficiency
Adherence	Leucocyte adhesin deficiency Specific granule deficiency

TABLE III

Methods for the investigation of phagocyte function

Cell function	Methods
Chemotaxis	*in vivo:* Rebuck skin window *in vitro:* agarose assay Boyden chamber
Phagocytosis	*in vivo:* clearance *in vitro:* increase cell-associated bacteria/particles determined: — microscopically — by radiolabeling of bacteria decrease extracellular bacteria — determined microbiologically
Intracellular killing	Direct assessment by counting of *in vitro:* total number of viable bacteria number of cell-associated bacteria Indirect measurement by determination of *in vitro:* H_2O_2 production O_2 consumption chemiluminescence NBT test
Adherence	Direct determination of *in vitro:* aggregation adherence Indirect determination of *in vitro:* receptors

mental disorder in the hyper-IgE syndrome, the Kartagener syndrome, and acrodermatitis enteropathica. Chemotaxis and intracellular killing are abnormal in the Chediak-Higashi syndrome and specific granule deficiency.

Chronic granulomatous disease and myeloperoxidase deficiency are cha-

racterized by abnormal intracellular killing. The only disease caused by impaired phagocytosis and adherence of phagocytes is leucocyte adhesin deficiency. For detailed information about these diseases, see references 21 and 31.

Impaired functioning of phagocytes can be due to a primary abnormality of the phagocyte or be secondary to such conditions as pregnancy, malignancy, and hyperglycaemia, or the use of certain drugs.

Besides abnormal functioning, a shortage of phagocytes, (granulocytopenia and monocytopenia) and a shortage of opsonins (a- and hypogammaglobulinaemia) can lead to infection and related problems. These two aspects of phagocyte dysfunction will not be discussed here.

Methods for the investigation of phagocyte functions

The methods available for the investigation of phagocyte functions are summarized in Table III. Chemotaxis can be studied *in vivo* by using the Rebuck skin window. A small area of the skin is abraded and then covered with a coverslip which after a given time is examined for the presence of phagocytic cells. This approach makes it possible to quantify the locomotion of leucocytes [23]. For the study of chemotaxis *in vitro*, two methods are available, one measuring the movement of cells toward a chemoattractant in agarose and the other measuring movement over a filter placed between two compartments, one holding the cells and the other a chemoattractant [32].

Phagocytosis can be evaluated by assessing the clearance from the circulation of intravenously injected material, for example colloids, bacteria, macromolecules, or opsonized red cells. With this approach the functional state of the Kupffer cells in the liver and of the macrophages in the lungs and spleen can be determined. However, the clearance of particulate matter also depends on the flow rate of the blood, the presence or absence of opsonizing factors, the degree of adherence of the material to vessel walls, and changes in the composition of the population of phagocytic cells in the liver [17]. Phagocytosis can be studied *in vitro* by measuring the increase in cell-associated bacteria or inert particles. Microscopical techniques to determine the number of cell-associated bacteria or inert particles. Microscopical techniques to determine the number of cell-associated bacteria or particles are in general time consuming and insufficiently accurate unless the number of cells is very large. The alternative is to use radioactive-labelled bacteria and measure cell-associated radioactivity, but this radioactivity does not necessarily represent the uptake of bacteria. If adhesion of bacteria to the phagocytes is normal but not ingestion, abnormality cannot be detected by this method.

A simple alternative, although it too does not allow discrimination between abnormal adhesion and ingestion for the detection of phagocytosis, is to measure the change in the number of

extracellular bacteria microbiologically. With this approach, phagocytosis and intracellular killing can be assessed seperately [17].

Intracellular killing of phagocytes can be assessed by measuring the decrease of the number of viable bacteria; the total number of viable bacteria or the number of viable cell-associated bacteria can be determined [17]. This type of experiment can be designed such that phagocytosis and intracellular killing are assayed simultaneously. This kind of assay is sometimes referred to as a phagocytosis or opsonophagocytosis assay and sometimes as a killing, opsonokilling, or intracellular killing assay. Intracellular killing of bacteria by phagocytes can be assessed with a method calling for a period for phagocytosis followed by the removel of non-phagocytosed bacteria by washing and differential centrifugation of the cells, which are then re-incubated in the presence of serum, the rate of killing being expressed as the decrease in the number of viable cell-associated bacteria [17].

Indirect assessment of intracellular killing can be done by measuring H_2O_2 production, O_2 consumption, chemiluminescence, or the reduction of nitrobluetetrazolium [3]. These assays measure the activity of the oxidative killing mechanism of phagocytes. Defects in the non-oxidative killing system will remain undetected if these methods are used only to determine intracellular killing.

The ability of phagocytes to adhere to a surface can be determined either by direct observation of adherence of cells to glass or aggregation of the cells, or indirectly by detecting receptors on the cell surface with the use of antibodies.

Pitfalls in the evaluation of phagocyte functions

When one of the assay methods has shown an abnormality of phagocyte function, caution should be exercised in concluding that the patient has a phagocyte function disorder.

For example, one should be well informed about the patient's general condition and any medicaments used. An abnormality of phagocyte function can be temporary, as shown by the well-known example of decreased killing of S. aureus by granulocytes during poor regulation of diabetes mellitus [24]. This abnormality disappears when near-normal blood glucose levels are maintained. Another example of temporary phagocyte function disturbance is seen, especially in monocytes, if the assays are done during an active infection. Under such conditions we have observed decreased intracellular killing of S. aureus by monocytes in patients with hidradenitis, and repetition of the assay after treatment, gave normal results.

By preference the patient should not be taking any kind of drug at the time of the investigations. Many drugs are known to influence phagocyte functions. The use of antimicrobial drugs can pose a problem in two ways. When a microbiological method is used the antimicrobial drug can affect the bacteria and thus influence

the results. Several antimicrobial drugs have an effect on phagocyte function, at least *in vitro*. Tetracyclines and rifampicin impair the chemotactic activity of granulocytes [8, 9, 19], and phagocytosis is inhibited by tetracyclines and bacitracin [6, 8]. Intracellular killing is depressed by sulfamethoxazole and trimethoprim [1, 14]. On the other hand, many antibiotics, e.g. betalactam antibiotics and aminoglycosides, act synergistically with phagocytes, which leads to enhanced killing of bacteria [10, 18, 20, 26].

A warning should be given with respect to drugs used in assays. For example, lysostaphin is frequently used for the removal of non-phagocytosed S. aureus in intracellular-killing assays. However, this drug has been shown to adhere to and penetrate into granulocytes and monocytes, which affects the number of viable cell-associated S. aureus [4, 5].

As already mentioned, caution should be exercised in concluding from abnormal results that there is a phagocyte function disorder, but one must also be cautious in concluding the opposite when phagocyte functions are found to be normal. Usually, one type of micro-organism is used in the investigation of phagocyte functions, S. aureus being the species used most frequently. If the micro-organism used in the assay is not of the same species as the one responsible for the infection in the patient, a normal result could be due to the use of the wrong micro-organism, since the functioning of the patient's phagocytes could be defective for a specific micro-organism. For example, in chronic granulomatous disease only the killing of catalase-positive micro-organisms is abnormal.

It would be logical to perform the assays with the same micro-organism as the species involved in the patient's infections, but for practical and technical reasons this is not feasible in the routine evaluation of phagocyte function.

Indications for evaluation of phagocyte function

The hallmark of phagocyte dysfunction is recurrent bacterial infections. Recurrent pyogenic infections of the skin, soft tissues, or respiratory tract form an indication for the investigation of phagocyte functions, especially when the infections do not respond well to antibiotic therapy and in the absence of local defects that would explain the recurrence of the infections. Suspicion on clinical grounds that a role is played by one of the known forms of phagocyte dysfunction, is another indication for the performance of phagocyte function assays.

A step-wise approach to the routine evaluation of phagocyte function

When a dysfunction of phagocytes is suspected, the investigation should proceed step by step (Fig. 1). The first question to be answered is whether granulocytopenia or mono-

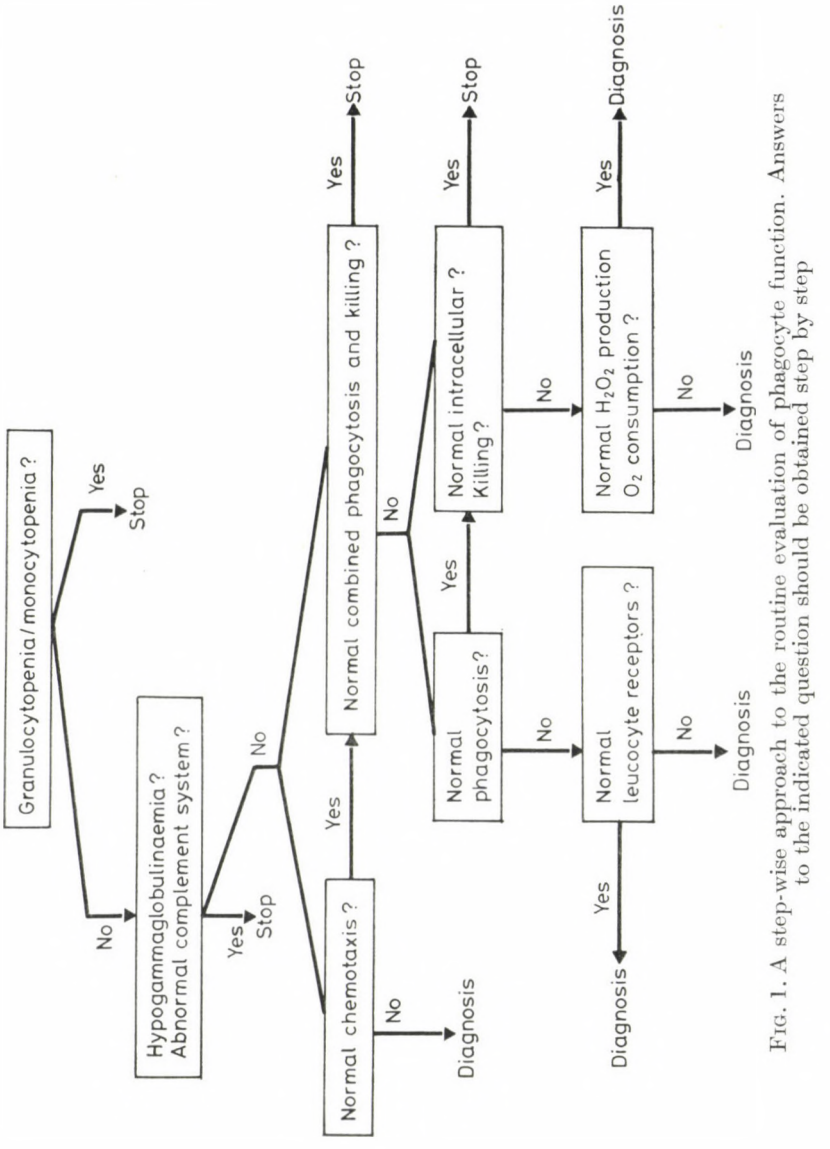

FIG. 1. A step-wise approach to the routine evaluation of phagocyte function. Answers to the indicated question should be obtained step by step

cytopenia is present. If this is the case, it is generally futile to investigate phagocyte functions unless the Chediak-Higashi syndrome is suspected.

The next question is whether opsonins are available. Quantitative determination of immunoglobulin concentrations in the serum, including subclasses of IgG, and evaluation of the complement system should show normal values before an investigation of phagocyte functions is undertaken.

Investigation of phagocyte functions should start with assays that measure cell function directly. The evaluation can start with a method that measures phagocytosis and intracellular killing together. This kind of method is not time consuming, and normal results make further investigations unnecessary. Abnormal results necessitate separate measurement of phagocytosis and intracellular killing, and such assays can be followed by more detailed studies to investigate the mechanism underlying the observe functional defect. If the cause of the phagocyte dysfunction is not found, one has to accept a descriptive diagnosis. This step-wise approach should assure that phagocyte dysfunctions are discovered with a minimum of expense and the least possible burden for the patient.

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