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Editorial

# The alveolar macrophage

## The macrophage

### CLASSIFICATION

Almost one hundred years ago Metchnikoff identified a large cell capable of phagocytosis which he called the macrophage.[1] For many years its role was interpreted as that of a scavenger of debris and microbes; but in recent years attention has focused on the origin, structure, and function of the macrophage. It has become clear that the macrophage and closely related non-lymphoid mononuclear cells participate in a wider range of activities, which are central to the continued well being of the host.

Originally classified as belonging to the rather nebulous "reticuloendothelial system," the macrophage has come to be regarded as part of the mononuclear phagocyte system.[2] All the cells included in this system, from the bone marrow stem cell to the tissue macrophage, are classified as belonging to a single system based on similarities of cellular morphology and function. Even this scheme requires some modification with the identification of the veiled cell, interdigitating cell, and the Langerhans cell, all of which are derived from bone marrow like the macrophage and all of which have important individual roles in the immune response as antigen presenting cells.[3] They are not macrophages, however, and will not be discussed in detail. With light microscopy alone it may be difficult to distinguish these other cells from macrophages and it is impossible to distinguish between different forms of macrophages (exudate, resident, and activated). Preoccupation with light microscopic appearances led to the persistence of an oversimplified view of the macrophage and a general unawareness of the different cell types that are present. This in turn precluded an understanding of the differing functional roles fulfilled by the cells in various chronic inflammatory states. Only by ultrastructural analysis using the electron microscope, the application of sophisticated cell surface receptor recognition markers, and the identification of intracellular enzymes can the various cell types (macrophage, interdigitating cell, etc) be differentiated and more informed assessment of their function made.

Address for reprint requests: Dr RM du Bois, Royal Free Hospital, London NW3 2QG.

The lung is unique in providing access to large numbers of macrophages by use of the technique of bronchoalveolar lavage. In no other organ can a large yield of cells be obtained by an atraumatic technique. This ideal means of studying the macrophage and related non-lymphoid cells has produced important information about the immunological mechanisms and biochemical features of the macrophage in lung disease.

### ORIGIN OF THE MACROPHAGE

Although the origin of the pulmonary macrophage has been the subject of keen debate in recent years, there now seems little doubt that the cells are derived from bone marrow. Animal studies[4] and, more recently, studies on patients who have received bone marrow transplants[5] have confirmed the origin of the cells. A small percentage of cells are, however, capable of further division within the interstitium and alveolus, once the target organ has been reached.[4] The average life span for an alveolar macrophage in man has been estimated to be of the order of 81 days, but a population of more long lived cells seem likely to be sited within the interstitium.

### TERMINOLOGY

As blood monocytes migrate into tissue there is a process of structural and functional evolution. The monocyte may become a resident macrophage or, at sites of inflammation, an exudate (inflammatory) macrophage. These two types of tissue macrophage may be distinguished by ultrastructural analysis and by markers such as peroxidase content (greater in exudate macrophages) and $\beta$ galactosidase content (higher in resident macrophages). On the other hand, the term activation refers to a series of changes within the macrophage by which it acquires a new or additional functional capacity in response to stimulation by factors in the local milieu. The nature of the stimulating agents is diverse and activation takes forms that vary with the nature of the stimulus. The term has been much abused over recent years and it has often been assumed that activation for one particular function results in activation for all possible macrophage functions. This is not the case and the term must now be used with reference to the stimulus inducing the change and the functional capacity of the cell after the change.

For example, a two hour pulse of the lymphokine macrophage activating factor induces enhanced expression of Ia antigen on the surface of the macrophage, making it capable of antigen presentation. On the other hand, continual exposure to macrophage activating factor activates the cell to produce hydrogen peroxide, which is necessary for bacterial killing and antibody dependent cytolysis. This diversity of response is important not only for understanding pathogenic mechanisms in which macrophages take part but also for the future development of therapeutic tools by which these changes might be modified.

Certain structural attributes of the macrophage are associated with a particular function. For example, the expression of DR antigen on the surface of the macrophage is necessary for antigen presentation to T lymphocytes. Thus recognition of structural differences in the cell in tissue allows reasoned interpretation of functional potential.

In view of the absolute number of non-lymphoid mononuclear cells of monocyte lineage present in tissue and the variety of functions that these cells may perform in response to the varying nature of the stimulation (see below), the word macrophage as it is currently used is clearly an umbrella term for a vast, heterogeneous population of cells.

## FUNCTION OF MACROPHAGES

One of the earliest series of experiments on macrophage function was undertaken by Mackaness in the 1960s.[6] He described the part played by the macrophage in cellular immunity to intracellular parasites. Macrophages obtained from animals infected with intracellular parasites are morphologically different from normal cells. They contain an enlarged Golgi apparatus, more free ribosomes, and an elaborate endoplasmic reticulum. The cells spread more avidly in vitro, have an enhanced phagocytic ability and increased content of lysosomal enzymes, and become effective microbicidal cells. More recently, the capacity for the macrophage to destroy cells as well as microbes has become established (for review see 7), and in the last decade the versatility of the macrophage in terms of secretory products, interactions with other immune and inflammatory cells, and the expression of cell surface receptors has been recognised. The macrophage is known to be capable of secreting more than 50 secretory products and the expression of more than 30 surface receptors has been described, although an individual cell may not possess all of these capabilities. The secretory products include hydrolytic enzymes, proteolytic enzymes, antiproteases, complement factors, arachidonic acid metabolites, reactive oxygen species, fibronectin, and monokines; while many receptors and surface antigens such as Fc, complement, and histocompatibility antigens have been identified. These attributes endow macrophage like cells with enormous functional capacity, which is reflected by the part these cells play in a wide range of biological activities.

Much of our understanding of the cells of the mononuclear phagocyte system comes from animal studies, particularly those on the mouse and the rabbit, and some caution is necessary in applying information obtained from animal macrophages to man. It is not proved that functional capacity is identical in macrophages obtained from different animals or even in macrophages obtained from different sites within the same animal.

### *Methods of studying macrophage function*

The study of macrophage function in vitro requires a source of free macrophages, since cells obtained by tissue extraction are difficult to process and the processing may affect the subsequent behaviour of the cells in culture.

The mouse peritoneal cavity was one of the earliest sources of macrophages and cells could be retrieved by peritoneal lavage. Cells were obtained in varying states of activation or stimulation by applying various intravenous or intraperitoneal inflammatory stimuli in vivo before lavage. The effects of these stimuli on macrophage morphology in culture, secretion of a range of lysosomal and other enzymes, phagocytic capacity, and microbicidal enhancement were then observed. In addition, different agents were applied in vitro to cultures of macrophages which had received no in vivo stimulation, and the changes in the same morphological and functional characteristics were noted. Research of this kind has produced information which has advanced understanding of the cell biology of chronic inflammation and provided experimental methods that could be applied to human macrophages.

Studies of human alveolar macrophages may be divided into two main types. Firstly, macrophages from patients with known diseases can be cultured and the spontaneous secretion of compounds into cell culture media may be assessed (for example, neutrophil chemotactic factors). The second approach has been to obtain macrophages from the lungs of normal volunteers and to apply varied stimuli to cultures of these cells in vitro. An example of this sort of study is the application of immune complexes to cell cultures and the observation that this stimulus induces the macrophage to secrete neutrophil chemotactic factor. With these two types of study several important macrophage secretory products have been identified. Furthermore, cell

surface receptors can be identified immediately after lavage and changes in receptor expression monitored during culture in the presence or absence of stimuli (for example, Fc component of immunoglobulin, C3b component of complement).

Other macrophage functions which can be studied with a lavage population are phagocytosis of bacteria or particulate material (such as latex particles or zymosan), bactericidal capacity, and—perhaps of most interest—interactions with other inflammatory cells (for example, the effect of the macrophage on lymphocyte transformation in response to an antigen stimulus: the ratio of macrophages to lymphocytes has been shown to be a crucial factor in determining whether the macrophage effect is enhancement or suppression—as macrophage numbers increase, a suppressor response develops[8]).

*Antimicrobial function*

The antimicrobial function of the macrophage requires, firstly, the recognition of organisms by the macrophage and, secondly, their subsequent destruction. The exact mechanisms whereby macrophages can identify organisms are not clearly understood, but the presence on the surface of the macrophage of receptors for the Fc component of IgG and the C3b component of complement is almost certainly required for the phagocytosis of opsonised bacteria. Other receptors, such as those for the mannose receptor and non-specific surface receptor recognition systems, probably also play a part. After becoming attached to the surface bacteria are phagocytosed by a complex process which includes the invagination of part of the surface membrane to form a phagosome within the cytoplasm of the cell. The production of a phagosome requires an intricate arrangement of microfilaments, including actin and myosin.[9]

The fate of the ingested organism varies—the result may be complete destruction or its persistence as an intracellular parasite. Associated with phagocytosis is the "respiratory burst," which generates reactive oxygen species such as superoxide, hydrogen peroxide, singlet oxygen, and hydroxyl radicle.[7] The respiratory burst is an increase in cell metabolism that follows phagocytosis, consisting of an increased oxygen consumption by the cell and increased glucose metabolism via the hexose monophosphate shunt pathway. Associated with these changes is an increase in the production of superoxide anions by the phagocyte. Though its exact biochemical nature is not known, the generation of superoxide is probably due to an oxidase system associated with the cell membrane. As this oxidase system oxidises, preferentially, NADPH to NADP it has been called NADPH oxidase. The generation of superoxide may be linked to increased hexose monophosphate shunt activity as NADP is necessary for the oxidation of glucose-6-phosphate to 6-phosphogluconate, which is an early reaction in the hexose monophosphate shunt pathway. Hydrogen peroxide may be produced by further reduction of superoxide and this then reacts with another molecule of superoxide to generate hydroxyl radical. In addition, the interaction of hydrogen peroxide with halide anions generates hypohalous acids.

These oxygen derived reagents are necessary for microbicidal and possibly antibody mediated tumour cell lysis, but they are also potent agents that may cause peroxidation of cell membrane lipid components and therefore cellular damage. Inhibitors of oxygen radicals are necessary to maintain tissue homeostasis. Superoxide dismutase catalyses the conversion of superoxide to hydrogen peroxide, and this is degraded by catalase and also glutathione peroxidase. These enzymes are present within alveolar macrophages. There are other non-specific oxygen radical scavengers, such as ascorbic acid; but the relative importance in vivo of these scavengers is unknown. (For reviews see 7 and 9.)

It must be emphasised that the mechanism whereby a macrophage is activated to kill micro organisms probably differs for different organisms. The activation for the killing of rickettsiae, for example, requires an initial priming by lymphokines followed by a second signal from, for example, endotoxin.[10] The identification of the variety of factors that can prime or trigger killing is currently the subject of active research. Irrespective of the exact mechanisms of microbe killing, fusion of lysosomes with phagosomes allows the phagocytosed material to be degraded by lysosomal enzymes.

*Antitumour activity*

The macrophage has the capacity to become cytotoxic to tumour cells in two ways. The first is a direct effect of cell to cell contact and the second is antibody dependent. The direct mechanism of macrophage induced tumour cytotoxicity involves the binding of macrophage to tumour cells and then the secretion of a protease, cytolytic protease, capable of lysing the tumour cell.[11] In the process whereby the macrophage becomes activated to secrete cytolytic protease it is primed with a lymphokine (currently thought to be $\gamma$ interferon), and a second signal (for example, traces of endotoxin) stimulates enzyme release. In antibody dependent tumour killing the tumour cells must first be coated by antibody, as this form of tumour cell lysis depends on recognition by the macrophage of the Fc fragment of immunoglobulin. The final step in tumour lysis by this mechanism is unclear but may depend on the

formation and release of hydrogen peroxidase.[12]

There have been few studies of the functional capacity of human alveolar macrophages to kill tumour cells, although Swinburne *et al*[13] have shown that, at a macrophage to target cell ratio of 20:1, human alveolar macrophages were capable of 100% cytotoxicity for a human lung adenocarcinoma cell line. Vose[14] isolated macrophages from human lung tumours and found that they could express cytolytic activity against fresh tumour target. Lemarie *et al*,[15] measuring alveolar macrophage chemotaxis in samples from a large number of individuals with various forms of lung disease, found that chemotaxis was significantly less in samples obtained by lavage from the neighbourhood of a tumour than in samples obtained from patients with other lung disease. It is not clear from this study whether the macrophage defect was intrinsic or whether a tumour factor could have influenced macrophage chemotaxis. Current evidence from human studies suggests therefore that macrophages can kill tumour cells but that a macrophage defect exists in patients with lung tumours. The exact nature of this defect, or whether it is primary or secondary to tumour factors, is not clear. Whether or not the macrophage can be restored to full tumoricidal capacity is not known, but with increasing knowledge and understanding of the stages concerned in macrophage activation for tumour cytolysis it may eventually become possible to prime and trigger cytotoxicity in vivo.

*Role of the macrophage in immunity*

The generation of an immune response to protein antigen requires the activation of helper T cells. This depends on the presentation of antigen to the T cell by an accessory cell. Such accessory cells are drawn from the mononuclear phagocyte system.[16] The antigen to be presented may be processed by the macrophage and then "presented" to the lymphocyte on the cell surface of the macrophage. An essential prerequisite of antigen presentation is the concomitant expression of Ia self antigens, which are coded by the major histocompatibility complex. This dual antigen expression is vital to the activation of the T lymphocyte and without it immune responsiveness is lost. There are several factors which can increase the expression of Ia antigen, particularly T cell lymphokines—for example, $\gamma$ interferon,[17] while class E prostaglandins and $\alpha$ fetoprotein can both reduce the expression of Ia antigens.[16 18]

In man the DR antigen is the equivalent of the Ia antigen in the mouse. There have now been several reports that alveolar macrophages obtained by lavage from patients express the DR antigen and that this expression is enchanced in such disorders as fibrosing alveolitis and sarcoidosis.[19] There have, however, been no studies that have been capable of relating expression of the DR antigen to function, and the functional significance of this expression is still unclear.

The use of monoclonal antibodies to other surface antigens on the macrophage will allow phenotyping of the macrophage subsets, both in tissue[20] and in free cell suspension (DA Campbell and colleagues, unpublished findings). Such techniques will not only provide differentiation of distinct subgroups but enable these subgroups to be separated so that proper functional studies can be performed. In a series of experiments on open lung biopsy tissue from patients with fibrosing alveolitis, Campbell *et al*[21] have demonstrated the presence of several phenotypically distinct macrophages within the tissue. The interstitial macrophages almost universally express the DR antigen, whereas within lymphoid aggregates the presence of non-lymphoid mononuclear cells bearing distinct antigens typical of dendritic reticulum cells in association with T helper lymphocytes and B cells has shown that true germinal centres have developed within the lung of patients with this disorder. High levels of immunoglobulins, circulating immune complexes, antinuclear factor, and rheumatoid factor have been found not only in the blood but also in lavage fluid from patients with this disorder. This raises the intriguing possibility that, in addition to being the target organ, the lung may be the origin of these immunoglobulins and complexes.

## Human alveolar macrophages

### INTERSTITIAL LUNG DISORDERS

Since the report by Reynolds *et al*[22] of the variety of local inflammatory cells obtained by lavage of the lungs of patients with interstitial lung disease, the technique of bronchoalveolar lavage has beome a valuable tool in the development of our understanding of these diseases and is in use in many centres throughout the world. The technique has been used most intensively to identify further the cells which produce the inflammatory response in fibrosing alveolitis and sarcoidosis.[23 24] An excess of neutrophils and eosinophils is found in the former, whereas the latter disease is characterised by an excess of lymphocytes in the lavage fluid. This has given rise to the idea that fibrosing alveolitis is a "neutrophil alveolitis" and sarcoidosis a "lymphocyte alveolitis."[25 26] These terms identify quite appropriately which cells are present in abnormal proportions in bronchoalveolar lavage from patients with these disorders, but there is a danger that their use may obscure the importance of the other inflammatory cells that are present. For example, the

eosinophil may be plentiful in fibrosing alveolitis and excess lymphocytes may also be found in the lung lavage returns.[23] Furthermore, in sarcoidosis that has advanced to a fibrotic state the neutrophil is also prominent in bronchoalveolar lavage fluid.[27]

The alveolar macrophage remains the most numerous cell obtained from lavage in almost all diseases except hypersensitivity pneumonitis. Morphological examination of macrophages retrieved by bronchoalveolar lavage plays a small part in the diagnosis of diffuse parenchymal lung disease. Iron containing macrophages are found in pulmonary haemosiderosis, characteristic X bodies within macrophages are revealed by electron microscopy in histiocytosis X, and lamellar bodies in lavage fluid and within macrophages are diagnostic of pulmonary alveolar proteinosis. Until relatively recently the functional role of the macrophage in disease has been ignored, but an important series of studies from Crystal's laboratory in the United States has produced evidence suggesting that the macrophage has a central role in the pathogenesis of both fibrosing alveolitis and sarcoidosis (for reviews see 25 and 26). In fibrosing alveolitis macrophages obtained by lavage have been shown to secrete spontaneously both a high and a low molecular weight neutrophil chemotactic factor.[28 29] It is now believed that circulating immune complexes stimulate the macrophage to produce neutrophil chemotactic factors, which in turn attract neutrophils into the lung. Then the neutrophil, it is suggested, discharges oxygen radicles and proteases, which damage the cellular and connective tissue framework of the interstitium. This gives rise to further macrophage activation, thereby amplifying the inflammatory process. As the macrophage is capable of protease secretion and oxygen radicle production, however, the relative contributions of these two cells to tissue damage have not yet been elucidated. The macrophage is also responsible for the production of alveolar macrophage derived growth factor and fibronectin. These are respectively progression and competence factors stimulating fibroblasts to replicate, and their production is triggered by lymphokines. Macrophages obtained by lavage from patients with interstitial fibrosis spontaneously secrete excess quantities of both growth factor and fibronectin.[30 31]

In sarcoidosis the study of lavage macrophages has also aided understanding of the pathogenetic mechanisms underlying the granulomatous response. Lavage macrophages spontaneously secrete interleukin-1,[32] which is thought to activate T lymphocytes to synthesise interleukin-2[33]; this in turn may activate other T lymphocytes to produce lymphokines, including monocyte chemotactic factor. It is suggested that this results in recruitment of monocytes to the inflammatory response and this amplification produces the building blocks on which the granulomas are formed. Macrophages obtained by lavage from patients with sarcoidosis, like those from patients with fibrosing alveolitis, secrete increased amounts of fibronectin[30] and alveolar macrophage derived growth factor.[31] It is not clear why the fibrotic response in sarcoidosis is not as pronounced as in interstitial pulmonary fibrosis. Despite these considerable contributions to understanding derived from lavage studies, important questions still require answers. For example, the relative contributions of neutrophils and macrophages to tissue damage are not known and the control of fibroblast proliferation and collagen synthesis are understood only partially. Furthermore, the complex interrelationships between the various cell types that produce granulomas in one disease and extensive fibrosis in another need further clarification. It is tempting to suggest that with clearer definition of the subpopulations of macrophage it will be possible to subdivide function as well as phenotype and that a clearer picture of the cell biology of these disorders will emerge. One important criticism of the study of lavage macrophages in the exploration of the pathogenesis of interstitial disorders is based on the suggestion that these lavage cells may not accurately reflect the function of cells present in the interstitium. So far this problem has not been addressed satisfactorily, but by using a range of monoclonal antibodies to provide accurate phenotypes of the lavage populations it should be possible to provide an answer.

## SMOKING AND THE ALVEOLAR MACROPHAGE

Cigarette smoking has several extremely important effects on the alveolar macrophage. Firstly, the numbers of macrophages obtained by lavage are some four to six times greater in smokers than in non-smokers.[34] Morphologically the macrophages contain increased amounts of pigment and characteristic inclusions may be identified by light and electron microscopy. There is an increased resting metabolism of glucose by macrophages from smokers and an increased generation of oxygen radicles. Intracellular levels of lysosomal enzymes such as acid phosphatase, $\beta$ glucuronidase are significantly higher and elastase secretion is increased (for review see 35). Enhanced macrophage secretory capacity may be important in the pathogenesis of certain lung conditions, notably emphysema. Clearly the effects of cigarette smoking on the macrophages retrieved by lavage must be borne in mind in the design of studies comparing macrophages obtained from different groups of patients.

THE ALVEOLAR MACROPHAGE AND EMPHYSEMA

Emphysema is associated with loss of lung elasticity. The fashionable theory of the pathogenesis of emphysema suggests that an imbalance exists between neutrophil secreted elastase (serine elastase) and the major inhibitor of neutrophil serine elastase, $\alpha_1$ proteinase inhibitor ($\alpha_1$ antitrypsin) within lung tissue.[36] Evidence supporting this hypothesis is the presence of excess neutrophils in the lung lavage fluid obtained from cigarette smokers and the finding of inactive $\alpha_1$ proteinase inhibitor in cigarette smokers' lavage fluid. The exact mechanism whereby the neutrophil is stimulated to secrete its serine elastase is not clear.

The macrophage is, however, capable of secreting its own elastase, a metalloenzyme, which differs from its neutrophil counterpart by not being inhibited by $\alpha_1$ proteinase inhibitor[37] and it is of interest that smokers' macrophages obtained by lavage secrete more elastase than those obtained from non-smokers.[38] At present, the major metalloenzyme inhibitor is thought to be $\alpha_2$ macroglobulin, although it seems possible that a tissue metalloenzyme inhibitor may exist within the lung. Alpha$_2$ macroglobulin is notable for its absence from lung lavage fluid despite its presence in serum. Its role in the inhibition, within the lung, of macrophage derived elastase is not known. Macrophages may also release neutrophil elastase and $\alpha_1$ proteinase inhibitor previously phagocytosed as complexes.

Other inhibitors of proteinase activity may be found in lung secretions, especially $\alpha_1$ antichymotrypsin[39] and bronchial mucous proteinase inhibitor.[40] Alpha$_1$ antichymotrypsin is an inhibitor of the neutrophil proteinase cathepsin G and has been shown to be secreted by human alveolar macrophages in vitro.[39] Bronchial mucous proteinase inhibitor is found predominantly in the upper airways and is a powerful inhibitor of neutrophil elastase and cathepsin G.

Finally, there is evidence of antiprotease inactivation in vivo.[40] Inactive $\alpha_1$ proteinase inhibitor has been recognised in the lung lavage fluid obtained from patients who smoke. This inactivation is believed to be due to oxidation of the methionine residue at the active site of the proteinase inhibitor. Cigarette smoke contains oxidant radicals and also stimulates the generation of free oxygen radicals by macrophages. Either or both of these mechanisms may be relevant to the inhibition of $\alpha_1$ proteinase inhibitor in vivo. Furthermore, $\alpha_1$ proteinase inhibitor can be cleaved by the metalloenzyme, and even the formation of complexes of neutrophil elastase with a proteinase inhibitor may not guarantee inactivation as $\alpha_2$ macroglobulin-neutrophil elastase complexes still retain some enzyme activity.

Thus there appear to be several factors that may be relevant to the pathogenesis of emphysema, and the simple theory of an imbalance between neutrophil elastase and $\alpha_1$ proteinase inhibitor is almost certainly not the whole story. Clearly the macrophage could have more than one role in the development of this disease.

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The macrophage has a large range of functional capacity and the advent of bronchoalveolar lavage has focused attention on the human alveolar macrophage. This atraumatic means of obtaining macrophages has facilitated the diagnosis of conditions such as histiocytosis X and alveolar proteinosis, has improved our understanding of several other lung conditions, and still holds promise for the future, particularly if subgroups of macrophages can be identified and separated for functional study.

RM DU BOIS
*Royal Free Hospital*
*London*

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