

Progress report

Macrophage activation, chronic inflammation and gastrointestinal disease

The involvement of macrophages in chronic inflammatory conditions has been the subject of considerable experimental investigation in recent years. Macrophages are able to secrete a wide range of biologically active compounds. They are important in the control of infection and, through their immunological and non-immunological receptors, macrophages interact with other cell types and a wide variety of proteins.

Monocyte proliferation is apparent in a number of disorders, including collagen diseases, many infectious diseases, neo-plastic conditions and chronic inflammatory gastrointestinal diseases.¹ Generalised proliferation of mature macrophages is present in chronic granulomatous conditions such as tuberculosis, brucellosis and other infections by obligate intracellular parasites as well as in berylliosis and sarcoidosis. Other examples of macrophage proliferation include storage diseases – for example, Gaucher's disease, neoplastic macrophage proliferation and monocyte-macrophage dysfunction syndromes. The latter syndromes include chronic granulomatous disease² and various lymphoproliferative disorders.³ Macrophage dysfunction may also result from adrenocorticosteroid administration or exposure to ionising radiation.¹

Macrophage cell function has been extensively investigated in disorders such as lepromatous leprosy, miliary tuberculosis, disseminated fungal infections and in Hodgkin's disease. Intrinsic or acquired functional defects, however, have not been convincingly proved in these conditions.⁴

Recent information on the tissue damaging potential of compounds released from activated macrophages has focused interest on a wide variety of chronic inflammatory conditions where macrophages may potentiate tissue damage in a non-specific manner.⁵ Early work in this area concentrated on rheumatic diseases where the results strongly supported the concept of macrophage protease involvement in the promotion of inflammation.⁶ These ideas have been more recently extended to various alimentary conditions,^{7–10} including acute or chronic liver disease and inflammatory bowel disease.

The purpose of this review is to highlight recent advances concerning macrophage function, kinetics, activation, and heterogeneity and to consider how far macrophages may be involved in the promotion of inflammation in a variety of gastrointestinal diseases. General information will be presented, followed by a detailed review of the literature relevant to alimentary disease.

Macrophages

ORIGIN AND KINETICS

Monocytes in the blood constitute a mobile pool from which tissue

macrophages may be derived. The capacity of both monocytes and tissue macrophages to proliferate is limited, and therefore the provision of adequate numbers of macrophages, especially at an inflammatory focus is dependent upon delivery of blood monocytes. Circulating blood monocytes are derived from a rapidly proliferating precursor pool of cells in the bone marrow, termed promonocytes. The same basic stem cell may give rise to either monocytes or granulocytes and differentiation is controlled by specific colony stimulating factors derived from monocytes, lymphocytes or endothelial cells.¹¹

Under normal conditions, peripheral blood monocytes circulate for 24–100 hours and randomly migrate into the tissues.^{12 13} When tissue macrophages are removed from the body and reinjected, they tend to localise in their original sites – for example, Kupffer cells localise to the liver.¹⁴ The factors influencing monocyte localisation in any particular tissue, however, is poorly understood. The question of how peripheral macrophage populations are replaced under steady state conditions is not conclusively settled.¹⁵ Studies concerning the origin of liver macrophages in the mouse have indicated that about half of all blood monocytes eventually become Kupffer cells and that the turnover time of Kupffer cells is 21 days.¹⁶ The current consensus¹⁷ is that both in the normal steady state and in inflammatory situations macrophages present in the tissues are largely derived from blood monocytes. A small and variable proportion may arise from local proliferation, especially during acute inflammation.^{15 18 19}

Three changes in monocyte kinetics have been observed during inflammation.²⁰ Firstly, premature monocytes are released from the bone marrow; secondly, there is a temporary shortening in cell cycle time in the precursor pool and, thirdly, more stem cells appear to be diverted to monocytopenesis. All these changes result in an increased monocyte pool and presumably increased numbers of derivative cells at the site of inflammation. The magnitude of these changes depends on the inflammatory stimulus and the consumption of macrophages at the site of inflammation. For instance, there are more marked changes in tuberculosis compared with sarcoidosis, which are examples of high and low turnover granulomatous conditions.²¹

MACROPHAGE FUNCTION

The mononuclear phagocyte system is involved in five major areas: (a) defence against microorganisms, (b) removal of dead or damaged cells, cell debris and inorganic material, (c) regulation of haematopoiesis, (d) cooperative and effector functions in the immune response, (e) synthesis of biologically active compounds such as complement components, prostaglandins, interferon and neutral proteases.

In this review, the secretory functions of the macrophage in relation to activation and inflammation will be considered in detail and other aspects of macrophage function will only be briefly outlined. These other functions of the macrophage may, however, be highly relevant to infections, allergic and autoimmune mechanisms in inflammatory bowel and liver disease, and to the development of immunological abnormalities such as the hyperglobulinaemia of liver disease.²²

DEFENCE AGAINST MICROORGANISMS

Macrophages are the principal cells involved in killing intracellular parasites such as mycobacteria,²³ toxoplasma²⁴ and cryptococci.²⁵ Macrophage activation was a term used to describe the enhanced bactericidal properties of macrophages previously exposed to intracellular bacteria.²⁶ This term has now adopted a wider meaning.

Apart from activation, macrophages need to exhibit efficient chemotaxis, phagocytosis and intracellular killing in order to eliminate these microorganisms. Monocyte chemotaxis is slow in comparison with neutrophil movement. Mediators of chemotaxis include complement components (C5b), lymphokines and derivatives of phospholipids.²⁷ Inhibitors of chemotaxis (interacting with the cells) and inactivators (interacting with the chemical mediators) are present in serum in different disease states.^{28 29} Once macrophages arrive at an inflammatory site, they remain there under the influence of a lymphokine, migration inhibition factor which activates the cells and results in increased adherence. This molecule is probably identical to macrophage activating factor.

Cells that have been exposed to a chemoattractant show enhanced expression of surface complement receptors and are thus better able to adhere to opsonised particles.³⁰ Subsequent particle ingestion is an energy dependent process involving the activation of actin binding protein, which leads to actin polymerisation to microfilaments. These microfilaments provide the forces for pseudopod formation and particle engulfment.³¹ Particle ingestion depends on the surface receptors involved and the state of activation of the cell.

Macrophages possess multiple mechanisms for killing or degrading ingested organisms. These include the generation of hydrogen peroxide³² and other oxygen-derived products.³³ Myeloperoxidase and other peroxidase activity³⁴ have been shown in these cells. Lysosomal fusion with the phagocytic vacuole suggests that cationic proteins, hydrolases and catalases are also important in bacterial degradation.^{35 36}

REMOVAL OF DAMAGED CELLS, DEBRIS AND INORGANIC MATERIAL

Changes in erythrocyte surface membranes caused by immunoglobulin coating, physical or chemical injury, surface carbohydrate alterations or aging result in erythrophagocytosis by the mononuclear phagocyte system.³⁷ Alveolar macrophages clear particulate matter from inspired air.³⁸ Various inorganic materials such as beryllium, barium salts, zirconium and silica are localised within tissues, often within macrophages. This eventually results in granulomatous and excessive fibrotic reactions.

REGULATION OF HAEMATOPOIESIS

The formation of granulocyte and monocyte colonies in culture is dependent on substances with specific colony stimulating activity. Circulating monocytes and macrophages are the main source of colony stimulating activity in man.³⁹ Granulocytes are able to produce compounds such as lactoferrin, with colony-inhibiting activity. These compounds block the resting but not the activated production of colony stimulating activity by monocytes.⁴⁰ Further control of marrow stem cell proliferation is possibly provided by macrophage production of prostaglandin E, which limits stem cell proliferation and is produced in response to raised levels of

colony stimulating activity.⁴¹ Macrophages therefore control the proliferation of their own and other progenitor cells through both positive and negative feedback systems. Bone marrow macrophages have been shown recently to influence the growth of early and late committed erythroid precursors through the synthesis of soluble factors.⁴²

COOPERATIVE AND EFFECTOR FUNCTIONS IN THE IMMUNE RESPONSE

Over the last decade a great deal of experimental work has increased the understanding of these complex and central functional roles of the macrophage. It is beyond the scope of this article to discuss in depth the immunoregulatory role of the macrophage and the reader is referred to a recent review by Unanue.⁴³

In summary, mononuclear phagocytes have been shown to exert a fine control on the early events that lead to antigen stimulation of T and B lymphocytes. They regulate the extent to which lymphocytes are stimulated, and respond as effector cells to responses from stimulated lymphocytes. Macrophages are important in early events through their ability to take up antigen, to express Ia (Dr) and to secrete lymphostimulatory molecules. The central role of Ia (Dr) antigen expression and macrophage-T cell interactions is now widely accepted. In late events, macrophages function as effector cells in terms of microbicidal and cytotoxic activity as well as in the modification of lymphocyte responsiveness. Their efficiency in these events depends on the state of activation of the macrophage.

SYNTHESIS OF BIOLOGICALLY ACTIVE COMPOUNDS

There are a wide number of products synthesised and released by macrophages. These can most usefully be divided into various broad categories as indicated in Table 1.⁴⁴ The production of these compounds varies widely in different circumstances and is detailed more specifically in the sections on macrophage activation and on macrophage secretion and its relationship to inflammation.

MACROPHAGE ACTIVATION

The ability of macrophages to function efficiently depends on their state of activation. The term 'activated macrophage' was introduced by Mackaness²⁶ to describe the enhanced bactericidal properties of macrophages previously exposed to intracellular bacteria. In recent years this term has been applied to describe a wide range of functional changes in the macrophage, not necessarily resulting in increased bactericidal activity. In biochemical terms, the secretion of plasminogen activator, a neutral proteinase, seems to correlate well with macrophage activation as originally defined.⁴⁵ The induction of the synthesis and secretion of plasminogen activator may depend on a number of sequential metabolic changes.⁴⁶ The latter may occur earlier than those changes required for full microbicidal activity. At present, therefore, it is best to describe macrophage activation in terms of the function under study, such as bactericidal, tumouricidal, phagocytic, or metabolic activity and in terms of the agent used to induce macrophage activation.⁴⁷

A number of agents are able to activate macrophages, and these are tabulated in Table 2. Possible interactions between these agents and

Table 1 *Secretory products of macrophages**

1	<i>Enzymes that affect extracellular and connective tissue proteins</i>
	Lysosomal enzymes
	acid hydrolases
	cathepsins
	esterases
	lipases
	Neutral proteinases
	plasminogen activator
	collagenase
	elastase
	Angiotensin-converting enzyme
	Fibronectin
	Procoagulant
2	<i>Products involved in defence processes</i>
	Complement components (C1, C2, C3, C4, Factors Band D)
	Interferon
	Lysozyme
	α_2 -macroglobulin
	α_1 -antitrypsin
3	<i>Modulators of cell function and cytotoxicity</i>
	Lymphostimulatory molecules
	Colony-stimulating factors
	Angiogenesis factor
	Prostaglandins
	Leukotrienes
	Thymidine
	Cyclic nucleotides
	Arginase
	Oxygen-derived products

* This table is not intended to be comprehensive, but covers the major groups of products known to be released by macrophages.

receptors on the macrophage plasma membrane have been recently reviewed.⁵³ Alterations in cyclic AMP, cyclic GMP and phosphatidylinositol turnover are related to changes in macrophage activity. Many agents have been used to increase the yield of macrophages from the peritoneal cavity. Cells thus elicited show some of the features of activated macrophages, but not all the biochemical changes associated with fully activated cells are found.⁵⁴ These elicited cells can be fully activated *in vitro* by agents normally only effective *in vivo*.⁵⁵ The induction of cytotoxicity for tumour cells⁵⁵⁻⁵⁷ and the secretion of plasminogen activator⁴⁶ may require the operation of at least two signals. *In vivo*, it is probable that macrophages are influenced by more than one group of activating molecules, and once initiated the sequence proceeds in a stepwise manner dependent upon the appropriate activation signals and the presence of responsive precursors.⁵⁸ It has been suggested that this

Table 2 *Macrophage activating agents*

1	Lymphokines ⁴⁸
2	Polyanions (endotoxin, ds-RNA, ⁴⁹ glucan)
3	Complement components (especially C3 _i) ⁵⁰
4	Immune complexes ⁵¹
5	Interferon ⁵²

sequence may provide a protective mechanism restricting the development of changes which might otherwise result in local tissue injury.⁵⁹ A hypothetical sequence is shown in Figure 1. After activation, many macrophage properties are altered, and these are briefly tabulated in Table 3.

MACROPHAGE SECRETION AND CHRONIC INFLAMMATION

Macrophages comprise a significant component of any chronic inflammatory infiltrate. These cells modify their pericellular environment and influence the function of other cells within the inflammatory lesion; see Table 1 as a guide to the categories of secretory products.

1 ENZYMES AFFECTING EXTRACELLULAR AND CONNECTIVE TISSUE PROTEINS

Lysosomal enzymes

There are a large number of hydrolytic lysosomal enzymes, which are usually localised as intracellular enzymes within the lysosomes. With maturation and, especially activation the number of cytoplasmic lysosomes and the activity of associated enzymes increases, reflecting increased synthesis.⁶⁰ The amount of enzyme released can vary from a relatively small percentage after a phagocytic challenge in normal cells to extensive release (80%) in activated cells.⁶¹ Selective release has been shown to be initiated by agents that provoke chronic inflammation,^{61 62} such as zymosan, chrysolite asbestos,⁶³ antigen-antibody complexes formed at equivalence⁶⁴ and lymphokines.⁶⁵ Such release is selective in nature, the cells retaining full viability as judged by other criteria. Once initiated, active secretion proceeds independently of the intracellular fate of the ingested material.⁶² Alterations in lysosomal enzyme terminal sugar residues and state of phosphorylation during synthesis may well determine their subsequent transport and storage with mannose-6-phosphate intracellular receptors playing a central role.⁶⁶

The activity of these enzymes in the extracellular milieu will depend on pH, the presence of protease inhibitors and the proximity of the cells to the substrate. Acid hydrolases, in particular cathepsins, can degrade collagen, proteoglycan and basement membranes.⁵ Subcutaneous injection of acid hydrolases can produce chronic inflammation.⁶⁷

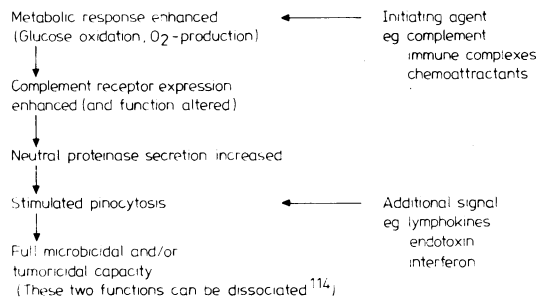


Fig. 1 Schematic representation of macrophage activation sequence.

Table 3 *Some properties of activated macrophages*

<i>Morphological</i>
Increased size and adhesiveness
Enhanced spreading, membrane ruffling
<i>Biochemical</i>
Increased glucose oxidation
Enhanced protein synthesis
Secretion of lysosomal enzymes
Increased neutral proteinase production
<i>Surface changes</i>
Increased number of Fc-receptors
Altered function of complement receptor
Decreased expression of lectin-like receptors
Increased responsiveness to chemotactic stimulation
<i>Functional</i>
Enhanced ability to kill intracellular pathogens
Increased cytotoxicity against tumour cells

Adapted from Ogmundsdottir and Weir;⁵³ Hopper, Wood, and Nelson.⁵⁹

NEUTRAL PROTEINASES

These enzymes are synthesised and secreted by macrophages, especially in the activated state.⁶⁸⁻⁷⁰ This appears to be a two stage process involving initial macrophage activation, followed by a phagocytic stimulus.⁴⁶ Virtually all the production of these enzymes is for export. Collagenase and elastase are able to degrade their appropriate substrates if the amount released exceeds the local concentration of protease inhibitors. Plasminogen activator may amplify the inflammatory reaction by promoting the generation of coagulation factors, tissue kinins and complement cleavage products as well as by activating collagenase.⁶ There is convincing experimental evidence for the ability of these enzymes, either independently or in conjunction with lysosomal hydrolases, to degrade connective tissue matrices.^{71 72} The deleterious effects observed on connective tissues in chronic inflammation may well follow the release of these enzymes from macrophages.

It is widely accepted that the inability to neutralise liberated leucocyte proteases results in tissue damage in lung, liver or other tissues in individuals with α_1 -antitrypsin deficiency.^{73 74} These clinical observations in α_1 -antitrypsin deficiency strongly suggest that the release of these enzymes is relevant to human clinical disease, although there is no proven association with inflammatory bowel disease.⁷⁵

The production of angiotensin converting enzyme,⁷⁶ fibronectin⁷⁷ and procoagulant⁷⁸ by macrophages has been shown. The significance of these findings in relation to inflammation remains unclear. Angiotensin converting enzyme production is one of few macrophage secretory products shown to be induced by corticosteroid exposure.⁷⁶

2 PRODUCTS INVOLVED IN DEFENCE PROCESSES

As indicated in Table 1, many complement components are synthesised by macrophages. There is considerable heterogeneity among different macrophage populations in their ability to synthesise these components,

probably reflecting different states of activation and maturity.⁷⁹ The cleavage product of C3, C3b, is an important molecule in macrophage activation and amplifies the local activation of macrophages at sites of inflammation (Fig. 2).

Macrophages synthesise classical or Type I (wide pH stability) interferons^{80 81} and are essential cooperative cells for the production of Type II (acid labile) interferons by T lymphocytes.⁸²

Lysozyme is a major secretory product of both monocytes and macrophages, but the precise role of this enzyme remains uncertain. Synthesis and secretion remain remarkably constant under varying experimental circumstances,⁸³ and this is a useful cell-specific marker for mononuclear phagocytes in culture. Polymorphonuclear neutrophils are the only other cells that produce large amounts of lysozyme, which has some bacteriolytic activity.⁸⁴ Recent evidence suggests that it is a potent inhibitor of polymorphonuclear neutrophil chemotaxis and oxidative metabolism and may thus modify the inflammatory response.⁸⁵ There is no evidence, however, that lysozyme plays an important role in chronic inflammation.

Mononuclear phagocytes synthesise α_2 -macroglobulin⁸⁶ and α_1 -antitrypsin.⁸⁷ This appears to be more prominent in mature tissue macrophages. These proteins are able to inhibit the activity of plasminogen activator, elastase, collagenase, and lysosomal hydrolases. Simultaneous release of proteases and protease inhibitors, together with phagocytosis of protease-inhibitor complexes, may help to control local protease activity.

3 MODULATORS OF CELL FUNCTION AND CYTOTOXICITY

The release of lymphostimulating molecules has been recently reviewed by Unanue.⁴³ Colony stimulating factor release has already been mentioned in the context of regulation of haematopoiesis.

Through the release of a factor or factors involved in vascular growth, activated macrophages may mediate microvascular proliferation.⁸⁸

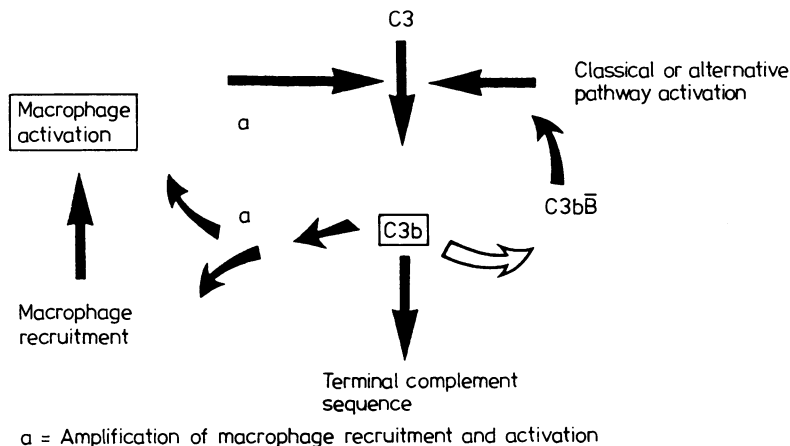


Fig. 2 Complement component (C3b) involvement in macrophage activation.

ARACHIDONIC ACID OXYGENATION PRODUCTS

Macrophage membranes contain a high proportion of their total fatty acid content as arachidonic acid (25%). This compares with a few per cent in other cell types. Phagocytic stimuli such as zymosan⁸⁹ and antigen antibody complexes⁹⁰ but not latex particles lead to the release of arachidonic acid by an inducible phospholipase. Cyclooxygenase and lipoxygenase then compete for the released arachidonic acid leading to the production of prostaglandins, hydroxyeicosatetraenoic acids and leukotrienes.⁹¹⁻⁹² The major prostaglandin products are PGE₂ and 6-keto-prostaglandin F1 α . These products appear to be responsible for many of the inhibitory effects of macrophages (Table 4), which can be prevented by inhibiting prostaglandin synthesis with indomethacin.⁹³

There appear to be distinct regulatory mechanisms influencing the production of either prostaglandins or leukotrienes.⁹² Under certain conditions, large quantities of leukotriene C (slow-reacting substance) are released by macrophages, suggesting that macrophages are involved in the vascular changes of immediate hypersensitivity reactions. Prostaglandins and leukotrienes also contribute to the inflammatory process through their influences on vascular tone and permeability.

The production of thymidine and arginase may have some importance in the cytostatic properties of macrophage-conditioned media.⁹⁴

OXYGEN-DERIVED FREE RADICALS

In response to activation of macrophages by particulate or soluble inflammatory mediators, macrophages undergo a respiratory burst. This is associated with increased oxygen consumption and increased glucose metabolism *via* the hexose monophosphate shunt. In conjunction with these events macrophages secrete superoxide-anion (O₂⁻), hydrogen peroxide (H₂O₂) and possibly hydroxyl radical (OH), together with singlet oxygen (¹O₂).⁹⁵⁻⁹⁶ Superoxide-anion is the major product (90%) of initial oxygen consumption, hydrogen peroxide being derived by dismutation of O₂⁻.⁹⁷ The production of oxygen metabolites by macrophages is dependent on the site of isolation, the state of activation and the extent of differentiation of the cells.⁹⁸

Primarily, these products are involved in the microbicidal activity of phagocytes. There is evidence, however, showing that oxygen metabolites released from activated macrophages damage endothelial cells, fibroblasts,⁹⁹ tumour cells¹⁰⁰ and platelets. In addition, free radicals may

Table 4 *Effects of macrophage attributed to prostaglandin production*⁴³

Inhibitory

Mitogen-induced lymphocyte proliferation
Lymphokine production
Natural killer cell generation and activity
Killing by cytolytic T cells
Antibody-dependent cell cytotoxicity
Mixed leucocyte reaction

Stimulatory

Collagenase production
T cell osteoclast-activating factor production

promote inflammation through the inactivation of serum inhibitors of protease activity resulting in increased tissue damage.^{101 102}

A recent review by Janoff and Carp¹⁰³ has discussed the experimental evidence supporting the view that oxidants released locally by leucocytes are able to inactivate α_1 -antitrypsin and mucus protease inhibitors. Proteases liberated at the sites of inflammation may then remain free to attack the tissue structures if high enough concentrations of oxidants are present. Superoxide also causes depolymerisation of hyaluronate, increasing its susceptibility to degradation by lysosomal enzymes.¹⁰⁴ Thus, oxygen metabolites, either by themselves or in conjunction with lysosomal glycosidases and other proteases can injure the structural and cellular matrix of tissues.

The possible importance of these interactions has been shown using models of lung injury, where catalase and superoxide dismutase, inhibitors of hydrogen peroxide and superoxide activity respectively, reduce the degree of inflammation whereas antiproteases have little if any suppressive effect on the lung injury.¹⁰⁵ Similar observations for superoxide dismutase have been made in chronic adjuvant arthritis in rats, autoimmune glomerulonephritis in mice¹⁰⁶ and alveolitis.¹⁰⁷ These studies support an important role for oxygen metabolites in both acute and chronic inflammation. Regulation of secretion by macrophages is summarised in Table 5.

MACROPHAGE HETEROGENEITY

Macrophages show a wide functional diversity and their capabilities vary with their state of maturation, their anatomical location and their degree of activation. Many aspects of these differing properties have been reviewed by Hopper and coworkers.⁵⁹ Not only are there the changes related to maturation, but populations of cells that appear otherwise uniform can show marked variation in specific properties.

Metabolic assessment shows differences in specific activities of 5-nucleotidase, acid phosphatase and synthesis of prostaglandins among a

Table 5 Regulation of secretion by macrophages

Neutral proteinases (see Table 1)

- Secretion increases dramatically (>100-fold) after macrophage activation and/or phagocytosis
- Virtually all the synthesised enzyme is secreted
- Secretion may persist if phagocytosed particle is not degraded

Lysosomal enzymes

- Most of the enzyme incorporated into lysosomes
- Small proportion released during phagocytosis
- Increased synthesis apparent in activated macrophages
- Activated macrophages may liberate a large proportion of the newly synthesised enzyme
- Enhanced secretion may persist in the activated macrophage

Lysosyme

- Bulk of enzyme secreted
- Level of synthesis uninfluenced by state of activation of the macrophage

Prostaglandins, leukotrienes, oxygen-derived free radicals

- Increased release (40fold) rapidly develops after membrane interaction
 - Rapid decline in release if stimulus is removed
 - Repeated stimuli result in repeated bursts of increased release
-

population of otherwise normal human monocytes.¹⁰⁸ Under the same experimental conditions, the surface expression of immunological (C₃b) receptors may be enhanced,³⁰ whereas glycoprotein receptor expression may decrease.¹⁰⁹ Surface expression of Ia antigens varies markedly among different macrophage populations. Splenic macrophages and circulating monocytes show a high degree of expression, whereas peritoneal and hepatic macrophages show much lower expression.¹¹⁰ Secretory activity of individual cells has been shown to vary widely.¹¹¹

Alveolar macrophages have higher hydrolytic enzyme concentrations, a greater response to migration inhibitor factor, greater elastase production and lower Fc receptor expression than peritoneal macrophages.⁵⁹ Peripheral blood monocytes show less secretory activity than Kupffer cells isolated simultaneously.¹¹² Chemotactic responsiveness appears earlier and is more rapid in monocytes compared with resident macrophages, although a larger proportion of the latter cells may eventually respond.¹¹³

Using different experimental conditions, there may be alterations in the functional expression of activation. The induction of antimicrobial and antitumour activity can be completely separate.¹¹⁴ The interrelationship between macrophage subpopulations and other cells remains to be determined. Much of the available evidence indicates that recently recruited and activated macrophages are in many ways functionally superior to resident macrophages.

These variations in macrophage function and activity are obviously relevant in inflammatory bowel and liver disease. Newly recruited macrophages may well function quite differently when compared with old resident cells, and these aspects need to be considered when assessing any data on cells isolated from tissues.

Gastrointestinal disease

INFLAMMATORY BOWEL DISEASE

Both Crohn's disease and ulcerative colitis are chronic inflammatory disorders of the intestine of unknown aetiology. Macrophages are prominent in the inflammatory cell infiltrate in both conditions, but in Crohn's disease a hallmark of the condition is the granuloma, where macrophages are particularly conspicuous. Although the presence of granulomata might suggest a greater involvement for macrophages in Crohn's disease, kinetic studies have shown little difference between this condition and ulcerative colitis. Meuret and coworkers have studied patients with severe active Crohn's disease and ulcerative colitis.¹¹⁵ They have shown a monocytopoietic proliferative activity of 1.4 in Crohn's disease and 1.7 in ulcerative colitis (normal subjects, 1.0). This compares with figures of 3.5 in active tuberculosis and 1.1 in sarcoidosis, examples of high and low turnover granulomatous conditions.

It has been suggested that a basic defect in macrophage function might underlie the clinical differences between Crohn's disease and ulcerative colitis.¹¹⁶ Although there is an increased monocytopoiesis in ulcerative colitis when compared with subjects with Crohn's disease,¹¹⁷ phagocytic activity of monocytes is not different between the conditions^{118 119} and bacterial killing appears to be normal. It should be noted that in both these studies monocytes showed enhanced phagocytosis of either *Candida albicans*¹¹⁸ or

*Staphylococcus aureus*¹¹⁹ when compared with normal or disease controls. In both Crohn's disease and ulcerative colitis, random motility of monocytes is increased¹¹⁸ and one study¹²⁰ shows a greater increase in subjects with Crohn's disease. Chemotaxis of monocytes towards either zymosan-activated serum¹¹⁸ or casein¹²⁰ was significantly increased in ulcerative colitis and there was no defect in Crohn's disease when compared with normal controls. There is some evidence to suggest the presence of serum inhibitors of chemotaxis in Crohn's disease^{121 122} which may explain these variable results. Alternatively, patients with ulcerative colitis show a higher monocyte turnover¹¹⁵ and therefore younger, more mobile cells may be present in the circulation.

In general, these studies confirm the presence of circulating monocytes which are functionally more active in inflammatory bowel disease with no evidence of a major macrophage defect in either condition. Small differences are probably explained by differences in experimental design.

In vivo assessment of macrophage function has been difficult. Migration of leucocytes into skin windows has been extensively studied¹²³⁻¹²⁶ and although a reduced mobilisation of leucocytes, predominantly polymorphonuclear leucocytes, has been shown, macrophage accumulation appears normal. Studies concerning the numbers of cells at the inflammatory site have shown increased numbers of macrophages in both conditions¹²⁷ and in one study significantly greater numbers in Crohn's disease.¹²⁸ Together with the previous data, this suggests that more macrophages are destroyed or migrate to the lumen in ulcerative colitis. Crohn's disease would appear to be a more indolent condition with less macrophage destruction or loss.

Other evidence concerning the state of monocyte activation has come from studies on monocyte enzyme production in these conditions. Monocytes isolated from subjects with inflammatory bowel disease have higher lysosomal enzyme activities (N-acetyl- β -glucosaminidase, β -glucuronidase) than corresponding controls,^{8 129 130} indicating monocyte activation.⁴⁴ The increase in cell-associated enzyme activity correlates with disease activity, especially in ulcerative colitis, where disease activity is more easy to define.¹²⁹ Supernatant enzyme release by cultured monocytes is also raised, and this is increased further following exposure of the cells to endotoxin or zymosan,⁸ known macrophage activating agents. There appear to be some differences in the influence of various agents on the time course of enzyme release, in that immune complexes may initiate early release⁹ and prolonged stimulation of synthesis, whereas endotoxin may only stimulate synthesis.^{8 9}

Increased serum activity of lysosomal enzymes has also been observed in inflammatory bowel disease,¹²⁹ but this does not correlate with disease activity. Serum lysosomal enzyme activity may arise from many other cells apart from macrophages. For all these observations there were no striking differences between ulcerative colitis and Crohn's disease. One recent study has indicated that the measurement of changes in enzyme activities in circulating monocytes underestimates the corresponding changes in macrophages isolated from the site of tissue injury.¹¹² Thus the potential for greatly increased release of these enzymes, especially after exposure to various macrophage activating agents at the site of tissue injury, is apparent. Using ultracytochemical and immunohistological techniques,

Otto and Gebbers^{131 132} have shown the release of lysosomal enzymes (peroxidase, acid phosphatase) into the interstitial tissues in both Crohn's disease and ulcerative colitis. They speculate that this might be of pathogenetic significance in connection with the hypothesis of Weissmann,^{133 134} who originally suggested that lysosomal enzymes promote tissue damage. Perhaps the most striking observations in this respect are those of Abraham and his colleagues,¹³⁵ who have shown that in the carrageenan-induced model of colitis in the guinea pig the tissue damage and ulceration are accompanied by an increase in the local release of macrophage lysosomal enzymes. They propose that these events are causally related. If this has any relevance to human disease, it would have important implications, as carrageenans are widely used in processed food.

Neutral proteinase secretion by cultured monocytes in inflammatory bowel disease has also been investigated. These enzymes may be even more important than lysosomal enzymes in promoting tissue damage as they are active at physiological pH and there is good experimental evidence to support their role in tissue damage.⁷¹ Doe and Dorsman¹³⁶ have measured plasminogen activator release by cultured monocytes. They have shown a markedly enhanced release of this protease by monocytes from patients with Crohn's disease and ulcerative colitis compared with age matched controls. The increased release was greater in the Crohn's subjects and there was some correlation with disease activity. These findings are similar to those reported for lysosomal enzymes. Apart from the direct influence on their specific substrates, some neutral proteinases (for instance, plasminogen activator, a serine esterase) are able to promote injury through the generation of plasmin with subsequent fibrinolysis, complement activation, kinin generation and the initiation of the coagulation cascade.⁶

Studies by Rachmilewitz and coworkers have shown increased levels of transcobalamin II in peripheral blood monocytes of patients with inflammatory bowel disease,¹³⁷ and this is further confirmation of monocyte activation in these conditions. It should be noted that these workers found even higher levels in subjects with shigellosis, indicating that the changes are in no way specific. More recent studies by the same group have shown enhanced prostanoid (PGE₂, PGI₂, TXA₂) production by inflamed rectal mucosa in inflammatory bowel disease,¹³⁸ and these prostanoids originate from intestinal inflammatory cells.¹³⁹ Studies using peripheral blood monocytes suggest that these changes at the site of inflammation reflect increased numbers of monocytes present. PGE₂ secretion by individual monocytes did not differ between normals, Crohn's or ulcerative colitis.¹⁴⁰ Differences were noted between Crohn's disease and ulcerative colitis when using peripheral blood mononuclear cells, PGE₂ and TXB₂ release being higher in active Crohn's disease, but this largely reflected the differing proportions of phagocytic cells in the peripheral blood mononuclear preparations.

To our knowledge, no direct studies of the role of oxygen radicals in inflammatory bowel disease have been published. A recent study on ischaemic injury in the cat small intestine, however, has indicated the importance of superoxide radicals.¹⁴¹ Superoxide dismutase and allopurinol pretreatment significantly reduced damage to the villus and crypt epithelium. It is therefore conceivable that macrophage generated

oxygen radicals could promote injury directly in the gut as well as altering the local protease/antiprotease balance.¹⁰³

The presence of increased numbers of activated monocytes in the circulation indicates that these cells have been exposed to circulating mediators such as lymphokines, endotoxin, complement fragments, or immune complexes. How these interactions apply at the tissue level still remains uncertain. It is interesting to observe that the drugs most commonly regarded as being effective in inflammatory bowel disease, glucocorticosteroids, azathioprine and salazopyrine, have profound effects on macrophage kinetics and biochemistry. Glucocorticosteroids induce a marked monocytopenia,¹⁴² reduce monocyte chemotaxis^{143 144} and inhibit the secretion of neutral proteinases.¹⁴⁵ Azathioprine induces a gradually cumulative monocytopenia,¹⁴⁶ the result of decreased monocyte production,¹⁴⁷ and reduces the influx of monocytes into sites of acute inflammation.¹⁴⁶ Salazopyrine and its various products are able to influence monocyte chemotaxis¹⁴⁶ and prostanoid production.^{148 149}

No specific information on the role of macrophages in promoting fibrosis in inflammatory bowel disease is available, but this topic is discussed in general at the end of the section on liver disease. Recent publication of improved methods of isolating macrophages from colonic tissue with reasonable yields¹⁵⁰ should lead to a better understanding of the relationships between monocytes, macrophages, granulomas and inflammatory bowel disease.

ACUTE AND CHRONIC LIVER DISEASE

Many factors and aetiological agents are involved in the development of hepatic damage.¹⁵¹ Most studies on macrophage function in liver disease have concentrated on differences between cirrhosis, of whatever aetiology, and normals. In no particular condition are macrophages regarded as the most prominent infiltrating cells. Nevertheless, they comprise a substantial proportion of the cells in various forms of chronic active hepatitis.

Monocyte kinetic studies in human liver disease have been difficult to interpret¹⁵² and as far as we are aware there are no clear data on the subject. In the normal steady state, around 50% of monocytes leaving the circulation become hepatic macrophages, Kupffer cells.¹⁶ Labelling studies in animals have shown a marked increase in monocyte recruitment into the liver in hepatic inflammation, in response to a variety of stimuli.^{153 154} In various forms of liver disease, circulating monocyte numbers do not differ from normal.¹⁵⁵ It has been well shown, however, that circulating monocyte numbers return rapidly to normal once the initial inflammatory insult has subsided,¹⁵³ and it may be that the sampling time in this particular study has influenced the result, as the majority of subjects did not have active inflammation. Chemotaxis is defective in cirrhotic patients, affecting all leucocytes,^{29 156} and has been attributed to the presence of serum inhibitors of chemotaxis or inactivators of chemotactic factors. This chemotactic defect has been shown for monocytes from patients with cirrhosis^{155 157} and is caused by circulating inhibitory factors, as the same cells function normally in the presence of normal serum. Similarly, there is reduced monocyte bacterial phagocytosis, bacterial killing and spreading in cirrhotic subjects.^{155 158} Although many of these observations have been made in alcoholic liver disease, a number of these studies have included

substantial numbers of patients with liver disease of a different aetiology, and it would seem that these changes reflect the secondary effects of cirrhosis.

Of more relevance to the present review have been the observations on macrophage secretory activity in liver disease. Initially, monocytes from patients with chronic liver disease were shown to release enhanced quantities of lysosomal enzymes,⁸ especially on exposure to zymosan or endotoxin, although a more recent study from the same laboratory has failed to confirm this.¹⁵⁵ As in inflammatory bowel disease, these findings might indicate that macrophages are being exposed in the circulation to activating agents. In acute hepatitis and active cirrhosis, free lysosomal enzyme activity in the liver is raised.^{159 160} Recent studies from Japan would indicate that this enzyme activity could arise from macrophages and not from damaged hepatocytes.¹⁶¹ It may therefore be important to correlate monocyte enzyme activity both with the activity of the liver disease and the histological features. Holdstock and coworkers¹⁵⁵ have provided some information on this point and it is interesting to note that raised monocyte enzyme activity was seen in a group of 'cirrhotics on steroids', in contrast to the other groups of liver disease. The latter included most of the patients with chronic active hepatitis, a group where macrophage infiltration within the liver is more prominent. Overall, enzyme release from monocytes of cirrhotic patients tended to be diminished and was associated with the predominance of an alcoholic aetiology.

Other studies on macrophage enzyme release and hepatic inflammation have involved the use of animal models. Ferluga and Allison, using a mouse model,⁷ first suggested that macrophages recently recruited into the liver could be further activated, resulting in the release of products toxic to parenchymal cells. A rat model using *Corynebacterium parvum* to induce hepatic granulomas and portal tract infiltration has been developed by ourselves, and macrophages isolated from these livers show markedly enhanced concentrations of lysosomal enzyme and neutral proteinase release compared with resident Kupffer cells.^{10 162} Although only a single lysosomal enzyme (N-acetyl- β -glucosaminidase) and neutral proteinase were measured in these studies, it is probable that the production of a wide range of lysosomal enzymes (acid phosphatase, β -glucuronidase, cathepsin D, β -glucosidase, β -galactosidase) and neutral proteinases (collagenase, elastase) are similarly increased by these activated cells.¹⁶³ A sequence of monocyte/macrophage activation in terms of enzyme release can be seen as monocytes are recruited into the liver and exposed locally to macrophage-activating agents.¹⁶⁴ As previously stated, simultaneous measurement of monocyte and tissue macrophage enzyme activity has shown that assessment of the former underestimates the magnitude of the change at the site of tissue injury.¹¹²

No direct proof of the importance of these products in promoting hepatocyte damage is available. The fact, however, that hepatocytes in culture survive longer and show fewer morphological changes if anti-proteases are included in the culture media¹⁶⁵ and the significant association of α_1 -antitrypsin deficiency with human liver disease¹⁶⁶ suggests that the release of these enzymes does have some relevance. Studies on murine viral hepatitis have implicated lysosomal enzyme release in the

initiation and perpetuation of hepatic necrosis.^{167 168} Thus, there is a great deal of circumstantial evidence that these enzymes could promote tissue injury in hepatic disease.

In chronic liver disease, many factors may be involved in monocyte recruitment into the liver. Recent histological studies have confirmed the transition of monocytes to tissue macrophages within the liver, using glucan to recruit the cells.¹⁶⁹ Macrophage enzyme release will be raised after exposure to activating agents. These agents may arise locally or be present in portal blood. Monocytes have been shown to release more lysosomal enzymes after incubation with portal serum when compared with systemic serum simultaneously obtained.¹⁷⁰ This difference correlated well with the presence of immune complexes (IgG, IgA) in portal blood but not with the presence of endotoxin or complement levels. Monocytes once recruited into the liver could promote further damage on exposure to appropriate activating agents in portal blood.

Whether Kupffer cells are involved in hepatic injury is difficult to determine. Although there is some limited evidence that active Kupffer cell phagocytosis can result in local hepatocyte damage,¹⁷¹ other studies have indicated that blockade of Kupffer cell phagocyte function results in hepatocyte damage.^{172 173} Presumably this is the result of exposure of hepatocytes to various toxic products. Kupffer cell phagocytic function in man is very variable, depending on the disease process. In obstructive jaundice and alcoholic hepatitis, function is commonly depressed, whereas it is increased in other forms of chronic hepatocellular inflammation.¹⁷⁴ Effective Kupffer cell function is depressed in liver disease, either because of portacaval shunting or because Kupffer cells are directly affected by agents such as viruses or alcohol.¹⁷⁵ This may lead to systemic endotoxaemia and immune complex persistence and these may be responsible for some of the manifestations of liver disease such as clotting disorders and renal failure.¹⁷⁶ Although there has been some dispute concerning endotoxaemia in liver disease,¹⁷⁷ most authors agree that this is a common finding.^{176 178} The persistence of endotoxin could lead to complement activation via the alternate pathway, increased plasma lysosomal enzyme activity¹⁷⁹ and macrophage activation. It is interesting to note that Hirata and coworkers have shown that endotoxin administration results in a rapid rise in hydrolytic enzyme activity derived from sinusoidal cells. Degenerative changes occur in the parenchymal cells after this rise in hydrolytic enzyme activity. Thus, blockade of Kupffer cells activity could result in increased enzyme release by recently recruited macrophages. Results from our laboratory indicate that recently recruited macrophages are more active secretory cells than old resident Kupffer cells.¹⁶² A further consequence of altered Kupffer cell function in liver disease is the alterations in cell-mediated and humoral immunity. Some aspects of this have recently been reviewed by Rogoff and Lipsky.¹⁸⁰

No experimental data have been published on toxic oxygen metabolites and hepatic injury, although for other reasons free radical scavengers such as (+)-cyanidanol-3 have been used in liver disease¹⁸¹ with no apparent benefit.

Macrophages are involved in the remodelling of embryonic tissues and through the release of various neutral proteinases can influence connective tissue deposition. A subpopulation of human monocytes (8–20%) can

express fibrin as part of their activity in inflammatory lesions.¹⁸² This can serve as a substrate for fibronectin, which precedes the appearance of collagen in connective tissue remodelling.¹⁸³ Liver macrophages contain fibronectin and Type I collagen.¹⁸⁴ A recent preliminary report suggests that macrophage factors are involved in the recruitment of fibroblasts to the liver during inflammation.¹⁸⁵ This area may warrant further, more intensive study.

Overall, the studies of macrophage function in liver disease have not shown any consistent abnormalities apart from evidence of depressed Kupffer cell function. It is clear that in human liver disease accurate assessment of macrophage activity will only be obtained by studying specific disease groups and avoiding the broad categories that have been studied up to now. In experimental models the potential for enzyme and other secretory product release is apparent, but it is still unclear as to whether this is relevant to any particular condition. As indicated earlier, the association between α_1 -antitrypsin deficiency and human liver disease suggests some role for proteolytic enzymes. The involvement of liver sinusoidal cells, especially fat storing cells and macrophages, in collagen metabolism^{186 187} suggests that these cells may play an important role in fibrosis control and the development of cirrhosis.

Conclusion

There is no doubt that macrophages play a prominent role in a variety of chronic inflammatory conditions. Evidence has been presented to indicate that, especially in inflammatory bowel disease, circulating monocytes are in an activated state. This probably reflects the presence in the circulation of a number of macrophage-activating agents, such as immune complexes, complement components, endotoxin, and lymphokines. Recruitment of these monocytes to the sites of tissue injury is evident both on histological grounds and on kinetic data. The potential for the release of a number of macrophage secretory products at the site of tissue injury has been shown and products such as antiproteases and free radical scavengers are able to modify the inflammatory response both experimentally and in nature. These cells probably contribute to tissue damage in gastrointestinal disease, but it is not evident at present how significant this contribution is. Further studies are required on the modification of inflammation in experimental models, on the relevance of these findings in human disease using immunohistological techniques and on the involvement of macrophages in hepatic fibrosis. Although this review has been concerned with specific areas of macrophage activity, other cellular interactions by macrophages are also of major significance and it is hoped that this report will help to stimulate further work in all these areas.

We should like to thank Mrs Isabella Strachan for her care in preparing this manuscript.

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Received for publication 2 September 1983

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