Macrophage Function Disorders

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Based in part on the previous version of this Encyclopedia of Life Sciences (ELS) article, Macrophage Function Disorders by Siamon Gordon.

Macrophages respond to alterations in their microenvironment by producing a wide variety of products that mediate inflammation, immunity and tissue homeostasis or injury. Macrophage recognize pathogen-associated molecular patterns (e.g. bacterial products) and endogenous ligands (e.g. apoptotic cells) through a broad and adaptable range of pattern-recognition receptors. The consequence of this recognition is generally effective clearance via phagocytosis; however, when this is not effective macrophages may become inappropriately activated and initiate an inappropriate inflammatory response. Although primary deficiencies of macrophage function in human disease are relatively uncommon, there is increasing evidence that even subtle genetic changes in macrophage function contribute to altered responses to both acute infections and in many major acquired disease processes such as autoimmunity, inflammatory diseases and cancer.

Advanced article

Article Contents

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Introduction

The macrophages of the body represent a widely dispersed family of cells, many of which are highly phagocytic, and display considerable heterogeneity, depending on their tissue microenvironment and stimulation by a range of infectious and antigenic agents. They are relatively long-lived, biosynthetically active cells and express diverse surface receptors and secretory products. They adapt readily to changes in their milieu and help to maintain homeostasis locally and systemically. If unable to deal adequately with an infectious or injurious stimulus, macrophages initiate a chronic inflammatory process which contributes to persistent tissue damage; they can also mediate acute, sometimes massive, responses from other cell types and organ systems. **See also**: Inflammation: Chronic; Macrophages

Although primary deficiencies of macrophage function are relatively uncommon, macrophages contribute to, and play a central role in, many major disease processes. These include storage diseases, chronic infections and immunological disorders, as well as responses to metabolic injury (**Table 1**). Macrophages may participate in the pathology of disease by producing secretory products or by altering functional phenotype, which can be characterized by changes in expression of their receptors (for further information, see Taylor *et al*., 2005). Herein we discuss a subset of macrophage functions, focussing especially on their role in infectious and chronic disease, and highlight recent advances in human genetics that contribute to our understanding of macrophage function disorders.

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Immune Activation

Macrophages and their close antigen-presenting cell (APC) relations, dendritic cells, play an important role in innate immunity, as well as in the acquired response. Macrophages and dendritic cells capture particulate and soluble antigens at body surfaces and at other sites outside secondary lymphoid organs through a range of recognition receptors, including mannose and scavenger receptors. Ligation of these and other receptors by microbial ligands can induce the rapid migration of APCs to draining lymph nodes, for induction or suppression of an immune response. Langerhans cells in skin and other complex epithelia are particularly important in this regard and respond to lipopolysaccharide (LPS) and cytokines such as tumour necrosis factor α (TNF-α), by migration and differentiation into dendritic cells. Although the role of macrophages per se in immune induction is poorly understood, its contribution to oxidative and other effector mechanisms in cellular and humoral immunity is without question. Macrophages also contribute to host defence to viruses and other microorganisms, as well as to

Table 1 Diseases in which macrophages play a significant role

Type	Example	Mechanism
Storage	Gaucher disease	Genetic lysosomal hydrolase deficiency
	Haemosiderosis	Secondary Fe^{2+} accumulation in reticulo-endothelial system
	Haemochromatosis	Primary Fe^{2+} overload (also other tissues)
Chronic inflammation	Silicosis Asbestosis	Nondegradable, bioactive particle accumulation in lung and pleura
	Rheumatoid arthritis	Autoimmune destruction
	Inflammatory bowel disease	Defects in intracellular sensing of bacteria lead to a skewed cytokine response
Infection	Septic shock (Gram-negative, Gram-positive)	Release of vasoactive mediators and cytokines, especially tumour necrosis factor α and interleukin 1β
	Tuberculosis	Cellular immunity activates Macrophage, via interferon γ
	Malaria	Uptake of parasitized erythrocytes and release of mediators
	AIDS (acquired immunodeficiency syndrome)	Human immunodeficiency virus (HIV) infects Macrophage and CD4 T cells leading to failure of cellular immunity
	Dengue virus	Antibody-dependent enhancement leads to increased infection of FcγR expressing cells
Metabolic injury	Atherosclerosis	Oxidized lipoproteins induce foam cell formation in major arteries
	Alzheimer's disease	Nondegradable amyloid peptide induces Macrophage secretion of neurotoxic products
Malignancy	Cancer	Macrophage within the tumour microenvironment facilitate angiogenesis, extracellular matrix remodelling and promote tumour cell motility

autoimmune tissue injury by antibody and complement-dependent cytotoxicity and clearance, involving fragment crystalline (Fc) and complement receptors, often working in collaboration. **See also**: Antigen-presenting Cells; Cytokines; Inflammatory Mediators; Natural Killer (NK) Cells; T Lymphocytes: Helpers

The phenotype of macrophage activation covers a spectrum from classic immune activation, which is essential for cellular immunity and antimicrobial resistance, to deactivation, which is characterized by prominent anti-inflammatory effects and is essential for the resolution of inflammation. Interleukin 4 (IL-4) and IL-13 induce a distinct, alternate type of activation in which major histocompatibility complex (MHC) class II antigen expression and mannose receptor-mediated endocytosis are enhanced, favouring humoral immunity. **Figure 1** describes the four major forms of activated macrophages. **See also**: Cytokines as Mediators of Disease; Lymphoid System; Tumour Necrosis Factors

Classical activation

Exposure to interferon γ (IFNγ) and a toll-like receptor (TLR) agonist (e.g. microbes or their products) results in macrophages that are said to be classically activated. These macrophages express high levels of MHC and CD80/86, which increases their capacity as APCs. Immune activation by IFNγ is central to host resistance to many intracellular pathogens as it induces the production of nitric oxide synthase (iNOS) and superoxide radicals as well as a number of proteolytic enzymes (**Figure 1a**). Failure to produce or respond to this cytokine as a result of recessive genetic defects results in persistent or disseminated mycobacterial and other infections in mice and humans and life-threatening opportunistic infections in acquired immune deficiency syndrome (AIDS). The macrophage contributes to the initial infection, dissemination and persistence of human immunodeficiency virus type 1 (HIV-1), and may be responsible for neuropathology. Known factors that influence infection of macrophages by different HIV strains include CD4 and chemokine coreceptors for viral entry. **See also**: AIDS: Clinical Manifestations

IFNγ-activated macrophages also contribute to tissue injury in delayed-type hypersensitivity reactions, by enhanced cytocidal activity and mediator production. Such macrophages are primed to respond to LPS and other microbial ligands by massive release of cytokines such as TNFα, contributing to direct endothelial injury and septic shock. The effector mechanisms are regulated by opposing receptor and signalling pathways of which several have been recently studied intensively. These include pro-inflammatory cascades (CD14, TLRs, nuclear factor-κB (NF-κB)) and possible anti-inflammatory, downregulatory

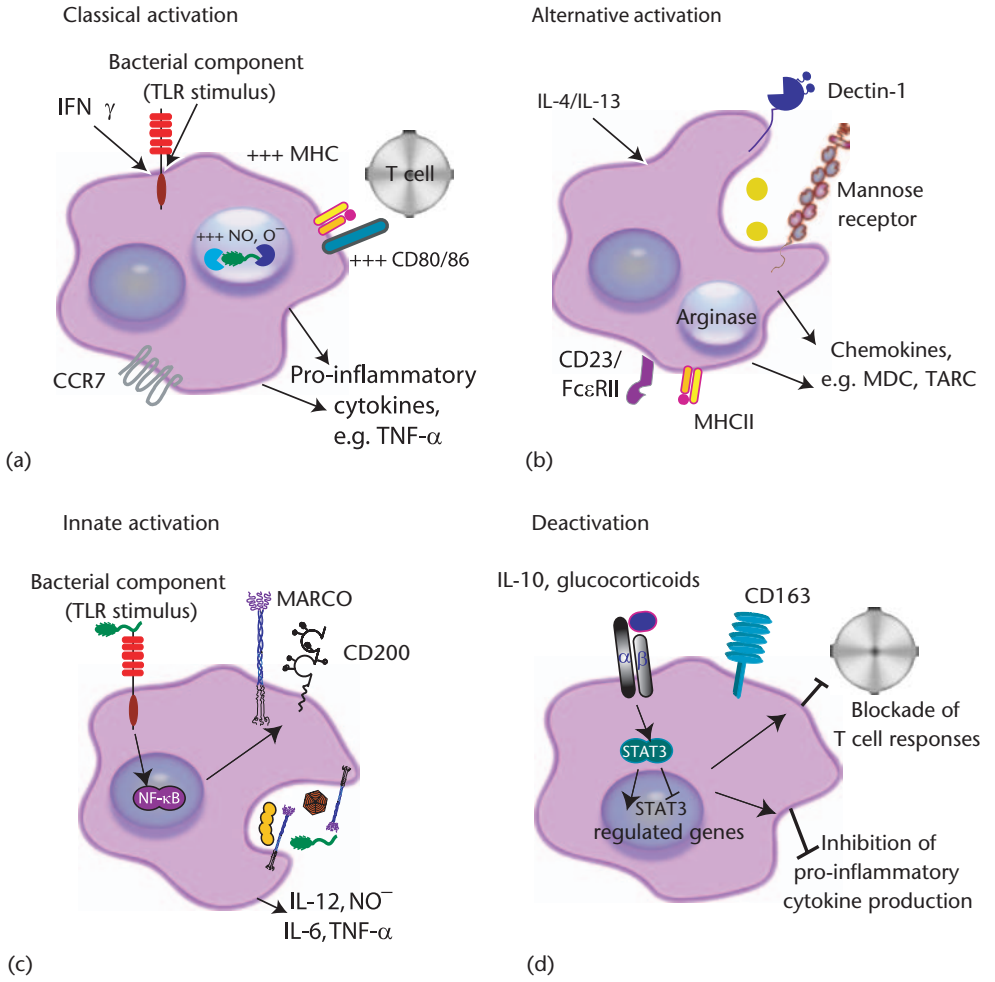

Figure 1 Macrophage activation phenotypes.

mechanisms involving scavenger and other receptors which promote LPS clearance. **See also**: Hypersensitivity: T Lymphocyte-mediated (Type IV)

Alternative activation

The presence of a parasitic infection or certain protein antigens (allergens) results in T cell polarization to a Th2 phenotype and the subsequent production of IL-4 and IL-13. Exposure to IL-4 or IL-13 results in alternatively activated macrophages (**Figure 1b**). These cytokines enhance endocytosis, due in part to expression of the phagocytic receptors mannose receptor and dectin-1, and have increased MHC class II expression. The production of arginase may compete for substrate with iNOS and thus reduces the capacity of these cells to kill intracellular pathogens. The role of the alternatively activated macrophage *in vivo* has not been completely characterized; however, it is implicated in the host response to parasites in addition to allergic asthma, fibrosis and may promote healing of inflammatory reactions, and the induction of humoral responses.

Innate activation

The presence of IFNγ is not characteristic of all infectious diseases and thus there is a hypothetical niche for a macrophage subset that is altered in response to microbial pathogens in the absence of a secondary cytokine stimulus. Recently it has been discovered that macrophages exposed to TLR agonists increase the expression of several receptors independent of a secondary stimulus. The scavenger receptor MARCO (macrophage receptor with collagenous structure) increases the phagocytic capacity of the macrophage towards broad classes of bacteria (which can be completely unrelated to the initial stimuli) and bacterial products. The presence of this receptor skews subsequent cytokine production in response to bacterial products, although this has not been fully characterized. The role of

these 'innately activated' macrophages is only beginning to be explored (**Figure 1c**).

Deactivation

The presence of IL-10 or other anti-inflammatory mediators, such as glucocorticoids, can induce a state of deactivation in macrophages that have been exposed to bacterial products (**Figure 1d**). IL-10 is produced during the course of infection and inflammation and acts in an autocrine manner to reduce pro-inflammatory cytokine production, which results in a decrease in T cell activation. The importance of IL-10 is confirmed in IL-10 knockout mice, which have increased mortality in response to a number of experimental infections due to overwhelming inflammatory responses. Systemic treatment with IL-10 has been proposed as a therapy for a number of diseases characterized by chronic inflammation including inflammatory bowel disease, rheumatoid arthritis, chronic viral infections and fibrosis, with limited success. For further information on macrophage activation, see Martinez *et al.* (2008).

Macrophage Functions

Macrophages have a variety of pattern-recognition receptors (PRRs) that are expressed on the cell surface and intracellularly, localize to specific compartments such as the endosomes or to the cytosol. PRRs recognize conserved molecular patterns that are found on a broad range of microbes, both pathogenic and harmless. These conserved molecular patterns (called pathogen-associated molecular patterns or microbe-associated molecular patterns) are often elements of the cell wall for microbes or deoxyribonucleic acid/ribonucleic acid (DNA/RNA) sequences in the case of viruses and some bacteria. Some host molecules such as heat shock proteins or uric acid crystals are also detected via PRRs. It is hypothesized that the presence of these endogenous PRR agonists are 'danger signals' that are only released during tissue damage that is a result of, or resembles the damage caused during infection. Once a macrophage detects microbes, their products or a danger signal, an inflammatory response is initiated that may induce the production of pro-inflammatory cytokines, chemokines or activation and maturation of the macrophage. Genetic studies in mice and the rare cases in which a mutation is found in humans have demonstrated that these receptors are vital to host defence against infectious disease; however, it appears they also contribute to autoimmunity, chronic inflammatory conditions and conditions such as septic shock, in which an overactive immune response can be fatal. For more information on PRRs. **See also**: Pattern Recognition Receptor

TLRs (Toll-like receptors)

The TLRs recognize a broad range of microbial products, some synthetic agents and certain host-derived molecules (**Table 2**). TLR3, 7 and 9 are expressed in the endosomal compartments where they detect microbial DNA (TLR9), which becomes available upon phagocytosis and

Table 2 Selected pattern-recognition receptors and their ligands

Localization	Receptor	Ligands
Cell surface	TLR1	Lipopeptides
	TLR2	Lipopeptides, peptidoglycan, lipoteichoic acid, zymosan, porins, lipoarabinomannan
	TLR4	Lipopolysaccharide, host protein (e.g. heat shock protein, fibrinogen), viral protein (e.g. F protein, Env protein), Taxol
	TLR5	Flagellins
	TLR6	Lipoteichoic acid, zymosan
	TLR10	Unknown
	TLR11[a]	Uropathogenic *E. coli*
Endosomal	TLR3	dsRNA, poly(I:C), siRNA, viral RNA
	TLR7	ssRNA, synthetic agonist R848
	TLR9	CpG-ODN, unmethylated DNA
Cytosolic	NOD family[b]	MDP (MurNAc-L-Ala-D-isoGln),
	NALP family[b]	Bacterial/viral RNA, pore toxins, ATP, uric acid crystals, MDP
	NAIP[b]	Flagellin
	RIG-I	5′-triphosphate dsRNA
	MDA5	dsRNA

[a]mouse only.
[b]ligands for many members of this family have not yet been identified.

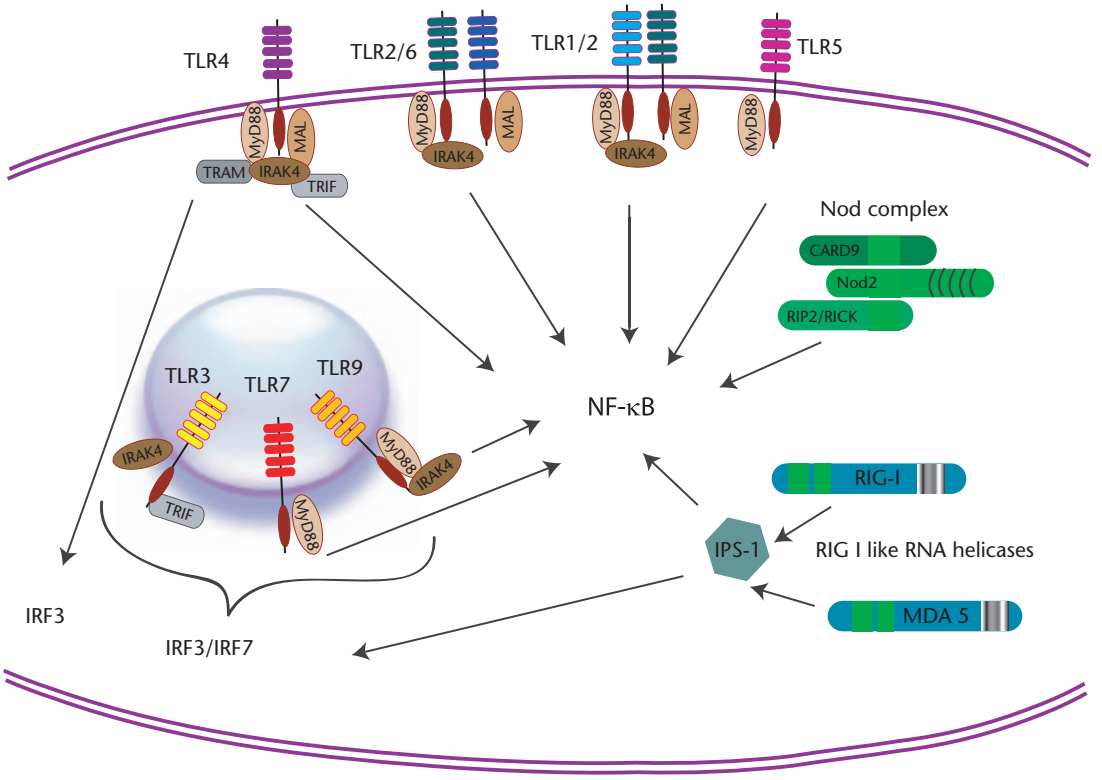

Figure 2 TLRs, NLRs and RLHs: cellular localization and signalling. The localization of pattern recognition receptors depends on their ligands and signalling properties. The surface expressed TLRs include TLR4, TLR2, TLR1, TLR5 and TLR6 whose ligands include bacterial surface proteins and lipids. These receptors may signal from the cell surface or from the phagosome after endocytosis. The intracellular TLRs include TLR3, TLR7 and TLR9 which recognize viral or bacterial nucleic acids. With the exception of TLR3 all TLRs share the adaptor protein MyD88 and through recruitment of a variety of adaptor proteins and signalling molecules, activate the transcription factor NF-κB to initiate transcription of various pro-inflammatory genes. The TLRs which are localized to the endosome also activate the transcription factors IRF-3 and IRF-7 which are essential for the induction of the type I interferons (IFN-α/β), crucial components of the anti-viral response. TLR4 is unique among the surface-expressed TLRs because it is associated with additional adaptor proteins (e.g. TRAM, TRIF) and as a consequence is also able to induce IRF-3 and type I interferon production. The cytoplasmic sensors, the RLH and the NLRs recruit additional adaptor proteins via protein–protein interactions, upon binding to their ligands. The NLRs have been demonstrated to induce NF-κB activation whereas the RLHs have been demonstrated to activate both NF-κB and IRF3/7. As a result of the different patterns of receptor localization and adaptor protein recruitment, a tailored immune response can be initiated in response to a range of pathogens.

destruction of microbial pathogens, or single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA) (TLR3 and 7, respectively), characteristic of viral infection. The remaining TLRs (TLR1, 2, 4, 5, 10, 11, 12) are expressed on the cell surface where they may detect whole bacteria or their components.

Once ligands are bound to their respective TLR an inflammatory response is mediated via the recruitment of a limited number of adaptor proteins (e.g. MyD88, TRIF, TRAM, Mal/TIRAP) and the subsequent activation of transcription factors such as NF-κB (**Figure 2**). Although there are common themes in gene transcription, specificity in response to particular TLR stimuli is a result of recruitment of different adaptor proteins and subsequent differences in the kinetics and types of transcription factors that are activated. The resulting transcriptional response includes genes for pro-inflammatory cytokine production, cell surface receptors and co-stimulatory molecules and can result in increased capacity to present antigen to T cells, enhanced phagocytosis and recruitment of additional leucocytes. **See also**: Toll-like Receptors

NLR family (nucleotide-binding domain, leucine-rich repeat containing)

The NLRs are intracellular sensors of pathogens and endogenous danger signals. In humans, there are 22 NLRs including a subfamily of proteins (NOD1 and NOD2 – nucleotide-binding oligomerization domain containing 1 and 2) that sense breakdown products of bacterial peptidoglycan (PGN) and three subfamilies (NALPs (NACHT, leucine-rich repeat and PYD (pyrin domain) containing 1), IPAF (ICE-protease-activating factor) and NAIPs (neuronal apoptosis inhibitor protein)) that are

involved in the formation of inflammasome complexes. The inflammasomes are cytosolic protein complexes that are involved in caspase-1 activation, required for the subsequent processing of the pro-inflammatory cytokines IL-1β, IL-18 and possibly IL-33. Although the ligands for all the NLRs have not yet been identified, it appears they are required for sensing cytosolic pathogen-associated molecular patterns (PAMPs) (**Table 2**) such as those released by the intracellular pathogens *Listeria monocytogenes*, and *Shigella flexneri* and viruses which replicate in the cytosol. The NLRs are especially important in host defence to cytosolic pathogens and function synergistically with TLRs to enhance the immune response Carneiro *et al*. (2008); Martinon *et al*. (2007).

RLHs (retinoic acid-inducible gene I (RIG-I)-like helicases)

A second class of PRRs that recognize the cytosolic products of viral replication (i.e. dsRNA) are the RLHs, which include RIG-I (retinoic acid-inducible gene I), MDA5 (melanoma differentiation-associated gene 5) and LGP-2 (laboratory of genetics and physiology 2). These are cytosolic sensors that are especially important in cells of myeloid origin (e.g. macrophages and myeloid dendritic cells) in which they induce a strong type 1 interferon response. The helicase domain of the RLHs binds directly to dsRNA and subtle differences in the modifications of the dsRNA confer differing specificity to either RIG-I or MDA5. For example, RIG-I recognizes RNA that is 5′-triphosphorylated and recognizes Flaviviruses, Orhomyxoviruses, Paramyxoviruses and Rhabdoviruses. Although the exact ligand for MDA5 has not been identified, it appears to be specific for the Picornaviruses, possibly because instead of 5′-triphosphorylated RNA, they cap their RNA by covalently linking it to a protein, VPg (virion protein genome-linked protein). LGP-2 lacks a key domain (caspase recruitment domain – CARD) required for downstream transcriptional activation and thus is a negative regulator of RIG-I and MDA5. The result of dsRNA binding to these PRRs is activation of the transcription factors IRF3 (interferon-regulatory factor 3) and IRF7 and a robust inflammatory response.

Pattern-recognition receptors in disease

The TLRs are essential for host defence towards infection but have also been implicated in autoimmunity, septic shock and chronic inflammation. Very few Mendelian disorders have been identified in genes involved in TLR responses; however, the few that have been identified include two mutations in the NF-κB signalling pathway and one in IRAK4, a TLR adaptor protein. There have been a number of more subtle changes in function of the TLRs or downstream adaptors identified that are the result of single nucleotide polymorphisms. Some of these have been associated with an increased risk of developing specific diseases (e.g. tuberculosis, malaria, invasive *Staphylococcus aureus* infection), whereas others are associated with resistance to septic shock or the development of chronic disease.

The NLRs are essential for activation of the pro-inflammatory cytokine IL-1β. A number of genetic conditions in which IL-1β production is dysregulated have been associated with mutations in NALP1. For example, familial vitiligo, an autoimmune disease in which melanocytes are destroyed, is associated with NALP1 mutations. Although the exact pathology is not clear, it may be that dysregulated IL-1β results in inappropriate activation of cytotoxic T cells.

The three closely related syndromes, familial cold autoinflammatory syndrome, Muckle–Wells syndrome and chronic infantile neurological cutaneous and articular syndrome/neonatal onset multisystemic inflammatory disease (CINCA) are all a result of defects in the NALP3 gene. These diseases are characterized by periodic fever, increase in the serum levels of acute-phase proteins, joint inflammation, skin rashes and eventually amyloidosis. The mutations result in increased activation of the inflammasome and subsequent overproduction of IL-1β, even in the apparent absence of stimuli.

To date little is known about human genetic deficiencies in the RLHs, however, a number of viruses have developed evasion strategies by targeting components of the RLH signalling pathways with decoy molecules or proteases that abrogate RLH signalling. To restore RLH-mediated immune responses, drug companies are developing therapies that inactivate these viral evasion strategies.

The localization of PRRs depends on their ligands and signalling properties (**Figure 2**). The surface-expressed TLRs include TLR4, 2, 1, 5 and 6 whose ligands include bacterial surface proteins and lipids. These receptors may signal from the cell surface or from the phagosome after endocytosis. The intracellular TLRs include TLR3, 7 and 9 which recognize viral or bacterial nucleic acids. With the exception of TLR3, all TLRs share the adaptor protein MyD88 and through recruitment of a variety of adaptor proteins and signalling molecules, activate the transcription factor NF-κB to initiate transcription of various pro-inflammatory genes. The TLRs which are localized to the endosome also activate the transcription factors IRF3 and IRF7 which are essential for the induction of the type I interferons (IFNα/β), crucial components of the anti-viral response. TLR4 is unique among the surface-expressed TLRs because it is associated with additional adaptor proteins (e.g. TRAM, TRIF) and as a consequence is also able to induce IRF3 and type I interferon production. The cytoplasmic sensors, the RLH and the NLRs recruit additional adaptor proteins via protein–protein interactions, upon binding to their ligands. The NLRs have been demonstrated to induce NF-κB activation whereas the RLHs have been demonstrated to activate both NF-κB and IRF3/7. As a result of the different patterns of receptor localization and adaptor protein recruitment, a tailored immune response can be initiated in response to a range of pathogens.

Phagocytosis

Macrophages are actively phagocytic and destructive, and can produce either pro- or anti-inflammatory cytokines which enhance or depress a subsequent immune response. There are striking differences in the inflammatory and immunologic consequences of uptake of microorganisms versus apoptotic cells. Understanding the relationship between effective clearance of pathogens and apoptotic cells will contribute to our understanding of tolerance, autoimmunity and host resistance. For a more detailed description of phagocytosis. **See also**: Dendritic Cells (T-lymphocyte Stimulating); Phagocytosis; Phagocytosis: Enhancement

Phagocytosis of TLR agonists

Surface-bound TLRs recognize bacterial patterns concurrently and may initiate pro-inflammatory signalling from either the surface or the phagosome. This signalling activates phagosome maturation, which is characterized by recruitment of specific phagosomal proteins (e.g. LAMPs (lysosomal-associated membrane protein), vacuolar adenosine triphosphatases (ATPases)) and a subsequent drop in pH. The drop in pH activates various proteases that result in degradation of the microbe and is accompanied by generation of the reactive oxygen and nitrogen species that are required for microbial destruction. The low pH is also required for processing antigens and loading MHC class II molecules that are present in the phagolysosome and thus for initiation of the adaptive immune response (**Figure 3**).

Phagocytosis of apoptotic cells

Apoptosis, also known as programmed cell death, is a highly regulated process by which cells die and are cleared by an immunologically silent method. Apoptosis occurs during embryonic development during tissue development and remodelling, when excess cells are generated (e.g. during negative selection of T cells), during certain diseases or infections and as a result of cellular senescence. Although many cell types, including nonprofessional phagocytes can remove apoptotic cells, macrophages are particularly well adapted for this function.

One of the first stages of apoptosis is the expression and release of 'find me' signals that are detected by macrophages and other cell types. These are secreted mediators that include the lipid lysophosphatidylcholine (LPC) and may also include nucleotides such as ATP and uridine triphosphate (UTP). The receptors required to detect these signals have not been identified. Better characterized are the 'eat me signals' expressed on apoptotic cells. These can be lipids which would not ordinarily be exposed on the cell surface (i.e. normally contained on the inner leaflet of the plasma membrane), or surface changes in the charges of glycoproteins. The most well-known 'eat me' signal is phosphatidylserine (PS). To date there is conflicting evidence as to whether there is a particular receptor for PS itself (although a number of candidates have recently been identified), however, it is clear that there are a number of 'bridging molecules' such as complement C1q and other plasma-derived proteins, that bind the newly exposed PS and are recognized by phagocytic receptors such as integrins and scavenger receptors (**Figure 4**).

Although the phagosome containing the apoptotic cell undergoes acidification and the apoptotic cells are destroyed by proteolytic enzymes, without the additional inflammatory signal provided by the TLRs, antigens are not loaded onto MHC class II and there is no pro-inflammatory response. In fact, apoptosis is characterized by the production of the anti-inflammatory cytokines TGF-β and IL-10 (**Figure 4**).

Defects in the clearance of apoptotic cells are associated with systemic lupus erythematosus (SLE), whereas a strong apoptotic response is associated with delayed onset of graft versus host disease in the context of transplantation.

Autophagy

Autophagy is the process of breaking down cytoplasmic components (e.g. organelles) by isolating them in vesicles that fuse with the lysosomes (**Figure 5**). At first this process was believed to be a constitutive housekeeping event designed to recycle old or damaged organelles; however, it is now known that autophagy can be increased by pro-inflammatory cytokines such as IFNγ and is involved in host defence against pathogens such as some viruses (e.g. herpes simplex) and bacterial pathogens (e.g. *Mycobacterium tuberculosis*, *L. monocytogenes*, etc.). Autophagy protects the hosts from infectious disease not only by destroying infected organelles but also by exposing antigens to MHC class II molecules and thus initiating an adaptive immune response. Although the mechanisms of action are not entirely clear, it has also been demonstrated that autophagy may be involved in the removal of aggregated proteins such as those that are associated with neurodegenerative diseases, in suppressing tumour formation and may promote autoimmunity by inappropriately presenting 'self' antigens via MHC class II. **See also**: Lysosomal Degradation of Proteins

Role in Pathogenesis and Disease

Macrophages illustrate well the interplay between intrinsic cellular properties (especially genetically determined) and environmental influences, including infections, toxins, drugs and pollutants. Both contribute to the complex patterns of gene expression which underlie cellular responses such as growth, phagocytosis and endocytosis, adhesion, migration, secretion and cell–cell interactions (trophic or cytocidal). 'Disease' results from either a primary deficiency of macrophages, or a reaction to extracellular abnormalities, resulting in under- or overactivity, or dysregulation of homeostatic pathways. Macrophages may be absent, present at an inappropriate place and

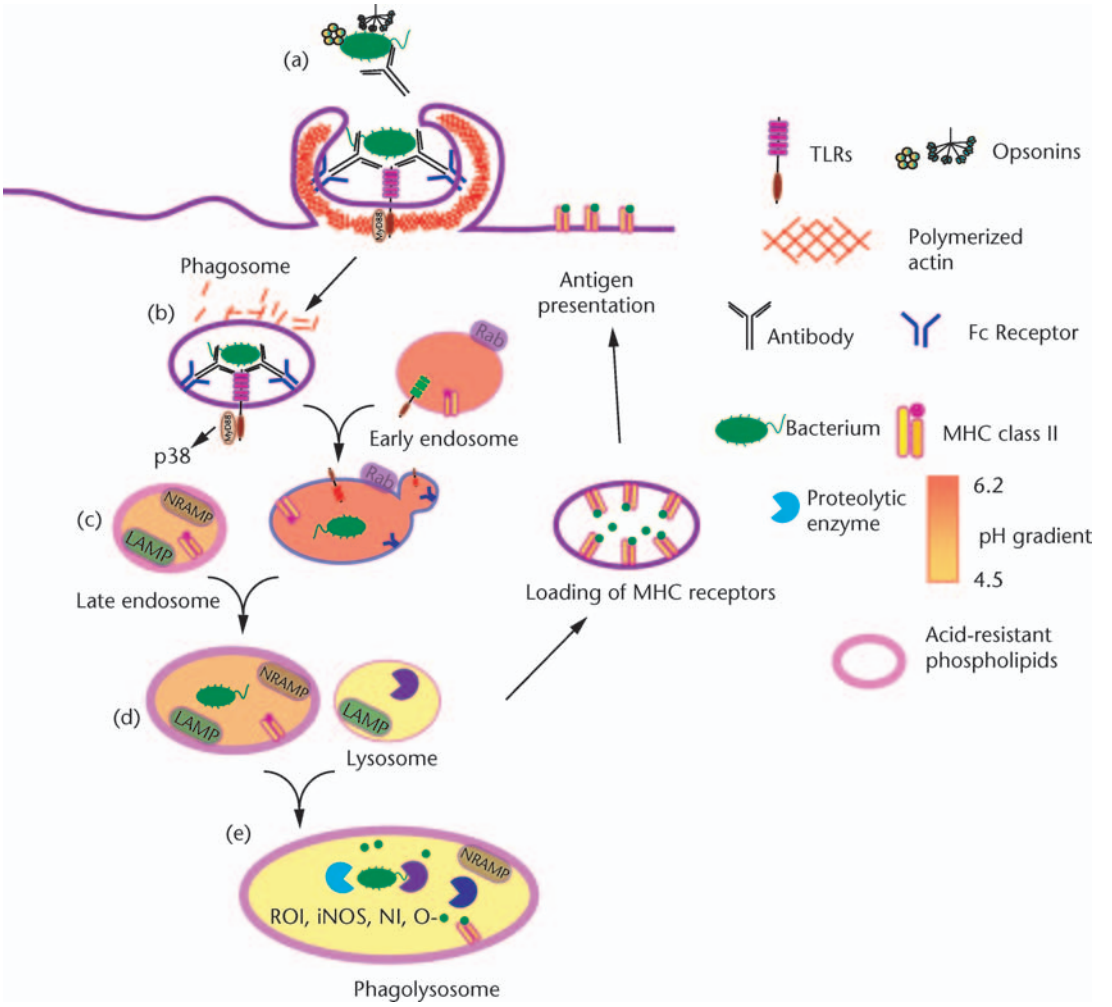

Figure 3 Phagocytosis of TLR ligands. (a) Microbes are coated with a variety of opsonins including complement, pentraxin 3 and antibodies. A number of receptors are involved in initial recognition of microbes and induction of pro-inflammatory signalling (e.g. the toll-like receptors (TLRs), and especially TLR2, 4 and 5) but these receptors are not phagocytic. Receptors involved in phagocytosis include the complement receptors, Fc receptors and others. (b) Phagocytosis mediated by this method occurs via the 'zipper' mechanism (i.e. sequential binding between the Fc receptors and their ligands along the circumference of the microbe). Phagocytic receptor ligation initiates a signalling cascade that results in activation of the Rho-GTPase family of signalling proteins, actin polymerization and extension of the plasma membrane. TLR signalling from the phagosome results in activation of p38 and this activation results in endosome maturation. (c) Fusion with the early endosome results in a slight drop in pH that results in the uncoupling of receptors with their ligands. Receptor recycling is facilitated by the Rab proteins, which also confer the ability to undergo subsequent fusion. The developing phagosome now contains MHC class II and TLRs, which signal from within the developing endosome. (d) Fusion with late endosomes results in the addition of LAMP proteins, the accumulation of acid-resistant phospholipids and a subsequent drop in pH. (e) Upon fusion with lysosomes the low pH results in the activation of a number of proteolytic enzymes. These are necessary for both direct antimicrobial activity as well as the creation of peptides for presentation via MHC class II.

increased or decreased in number due to faulty production or survival. Abnormalities can arise from inappropriate differentiation, recruitment or activation. The role of macrophages in a disease process is easily missed in the absence of a single organ location, striking disease association or ready access to tissues, other than blood (where circulating monocytes are relatively infrequent and not representative of tissue populations).

The mouse model has long been useful in identifying genes involved in macrophage function; however, recent advances in human genetics have identified a number of genes associated with primary immunodeficiencies or macrophage function disorders in humans. Large-scale genome-wide association studies have also been informative in detecting more effects of single nucleotide polymorphisms, especially in chronic diseases such as diabetes and atherosclerosis. Here we refer to selected examples of macrophage function disorders, with emphasis on genetic disorders, to illustrate their role in important diseases.

Infectious disease

Tuberculosis has been a major human disease for thousands of years and thus there has been considerable

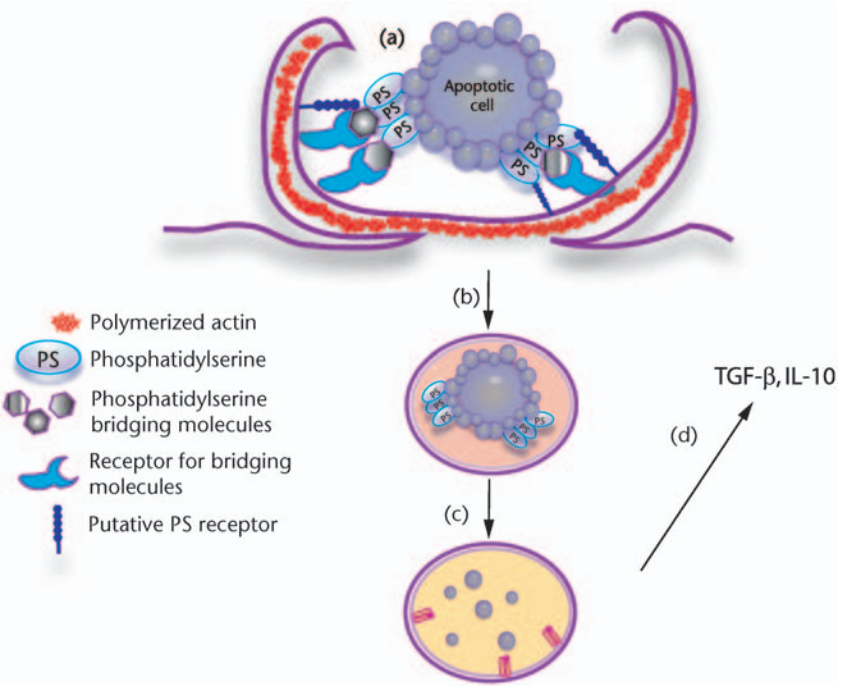

Figure 4 Phagocytosis of apoptotic cells. (a) Apoptotic cells express lipids such as PS that are normally confined to the inner membrane of the plasma membrane in addition to glycoproteins that have distinctive modifications of their charges. This results in binding by a number of 'bridging molecules' (e.g. C1q, MFGE8 (milk fat globule-EGF factor 8)) that are normally found within the plasma. These are recognized by receptors (e.g. complement receptor 3, possibly integrins and PS receptor) present on phagocytic cells and initiate phagocytosis via a ruffling mechanism. (b) The vacuole containing the apoptotic cell undergoes acidification and (c) the cell is degraded but antigens are not loaded onto MHC class II molecules. (d) Although it is not clear by which signalling mechanism, the anti-inflammatory cytokines TGF-β and IL-10 are produced. These prevent the macrophage from becoming activated and from initiating an adaptive immune response.

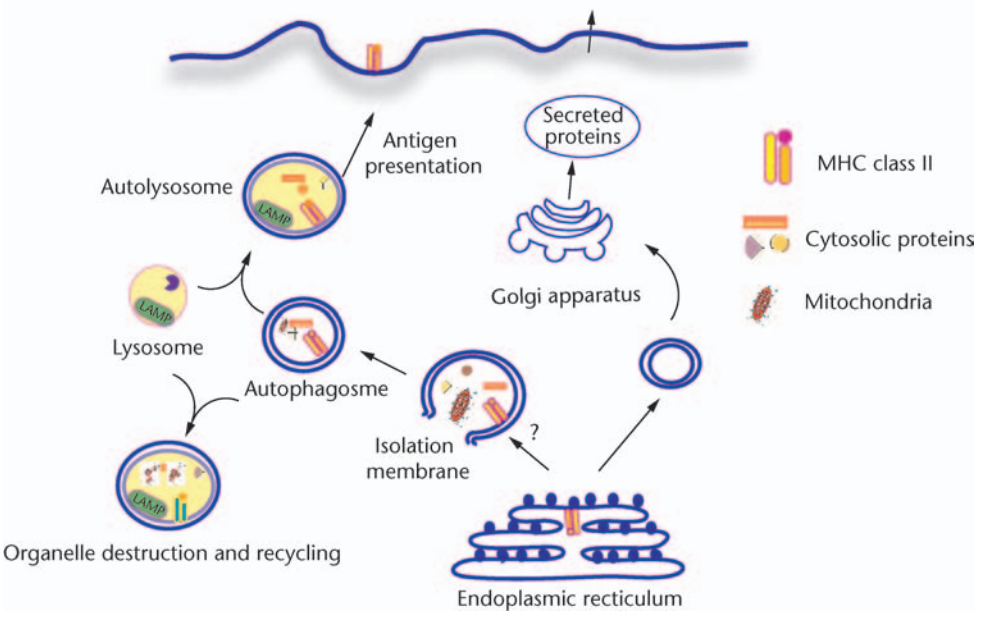

Figure 5 The autophagy pathway. Autophagy is a homeostatic process that can be further enhanced in macrophages in the presence of interferons or by starvation. Cytosolic proteins and organelles are found in single or double membrane vesicles that may be derived from the endoplasmic reticulum or from *de novo* synthesis. These vesicles fuse with lysosomes and the proteins and organelles are degraded and recycled. Conventional wisdom states that endogenous and cytoplasmic proteins are presented by MHC class I molecules whereas exogenous peptides are presented by MHC class II molecules, however, it has now become clear that peptide presentation is altered considerably upon induction of autophagy. The presentation of peptides from intracellular and lysosomal source proteins is increased on MHC-II.

selective pressure on certain human populations, whereas others were unexposed until relatively recently. As such, human genetic variability with respect to susceptibility to tuberculosis has been relatively well studied and can be subdivided into genes that are associated with Mendelian heritance patterns and genetic polymorphisms that contribute, but do not definitively confer susceptibility or resistance.

A number of defects in genes encoding cytokines or their receptors are associated with Mendelian susceptibility to mycobacterial disease. For example, individuals with defects in the IFNγ receptor are highly susceptible to various mycobacterial infections, including the vaccine strain, BCG (Bacillus Calmette-Guérin). The macrophages from these individuals are insensitive to IFNγ and thus do not become activated and are unable to kill the bacteria or form the granulomas that are essential for containment of infection.

Recessive mutations in the cytokine IL-12 or its receptor have also been identified. IL-12 is secreted by macrophages and induces IFNγ production by nuclear killer (NK) and T cells, which is required for activation of the macrophages. Thus these individuals are defective in macrophage activation and granuloma formation. Mutations in the IL-12 receptor can also result in impaired immunity against the pathogen *Salmonella*, which resides within macrophages (Altare, 1998).

Absence or deficiency of a particular macrophage molecule is highly instructive but uncommon. In contrast, subtle variations (i.e. polymorphisms) are common and contribute to an individual's susceptibility to disease. In the case of mycobacterial infections, a number of polymorphisms in candidate genes have been identified. For example, genetic variability in the vitamin D receptor has been associated with susceptibility to tuberculosis in various populations. Alveolar macrophages produce high levels of vitamin D and it has recently been demonstrated that the processing of vitamin D by macrophages is associated with the induction of cathelicidin, an antimicrobial peptide that contributes to the macrophage's ability to kill mycobacteria. As vitamin D is produced primarily in the skin by exposure to the sun, this mechanism has been proposed to explain why 'sunshine therapy' was sometimes an effective treatment for tuberculosis in the pre-antibiotic era. Other polymorphisms that are associated with susceptibility to tuberculosis are found in genes expressed in many macrophages including NRAMP1, which controls cytoplasmic cation levels and is involved in the production of reactive oxygen and nitrogen intermediates, and the genes encoding MHC components (Fernando and Britton, 2006). **See also**: Chronic Granulomatous Disease and Other Disorders of Phagocytosis

As mentioned above the TLRs are important sensors of bacterial components. To date there are not many Mendelian disorders associated with the TLRs. The most frequent are mutations in *IRAK4*, which encodes an important adaptor protein downstream of many TLRs (**Figure 2**). Interestingly, *in vitro* monocytes and macrophages from patients with this mutation are defective in responses to a broad range of TLR stimuli including cell wall components from Gram-negative and positive bacteria, bacterial DNA and, in addition, certain pro-inflammatory cytokines. From these observations, one might expect that these individuals would be broadly immunocompromised, however, *in vivo*, they seem to be susceptible to a narrow range of pathogens, in particular Gram-positive pathogens such as *Streptococcus pneumoniae*. Other Mendelian disorders resulting in immunodeficiency and associated with TLR signalling include mutations in NF-κB (i.e. in genes encoding the adaptor proteins IKKγ/NEMO) which results in X-linked hypohidrotic ectodermal dysplasia with immunodeficiency and a mutation in an adaptor protein downstream of TLR3 (Unc93b), which results in susceptibility to herpes simplex encephalitis (Bustamante *et al*., 2008). Although full-fledged mutations in the TLRs and their signalling pathways are relatively rare, there are a considerable number of polymorphisms that have been associated with infectious disease. These include TLR polymorphisms associated with sensitivity to Gram-negative cell wall components (e.g. LPS), susceptibility to sepsis, meningococcal disease, tuberculosis, pneumococcal infection, fungal and viral infections. This list will no doubt increase with time (Misch and Hawn, 2008).

Chronic disease

Atherogenesis

Monocytes are recruited relatively early and selectively to major arteries in response to local accumulation of lipoproteins, e.g. in hyperlipidaemia. Production of oxygen metabolites may exacerbate lipoprotein oxidation and promote its uptake by macrophages via a range of scavenger receptors, resulting in foam-cell formation. Such macrophages can interact with local endothelium, smooth muscle cells and fibroblasts, as well as T lymphocytes to initiate and perpetuate a modified form of chronic inflammation resulting in atheroma formation. Cell death, growth and migration of smooth muscle cells can all follow, mediated by macrophage products. Obstructive lesions may be repaired by fibrous tissue, or exacerbated by clotting induced by macrophages (and platelets) and by plaque rupture induced by macrophage metalloproteinases. Macrophages can also contribute to weakening and rupture of arterial walls, as in aneurysm, and of the heart, after myocardial infarction as part of an immune or inflammatory process. **See also**: Ischaemic Heart Disease; Lipoprotein Metabolism: Structure and Function; Ozone and Reactive Oxygen Species

Although there is a strong environmental component to the development of heart disease, there is also ample evidence for a genetic contribution. Polymorphisms in genes expressed in macrophages such as those associated with cholesterol uptake and efflux (and thus foam-cell formation) have been implicated in development of chronic heart disease as have a number of other genes known to be

expressed by macrophages, but without a clearly defined function.

Inflammatory bowel disorders e.g. Crohn disease

Inflammatory bowel diseases are associated with chronic inflammation of the gastrointestinal tract and may result in ulceration. Various theories of the aetiology of Crohn disease, which is characterized by inflammation of the ileum and colon, have been proposed, including a possible microbial or infectious cause or an excessive inflammatory response to the normal contents of the bowel. More recently, it has been proposed that it is actually a reduced inflammatory response that results in decreased recruitment of leucocytes and reduced production of inflammatory mediators. For reasons that are not yet known the bowel contents cause transmural lesions and result in macrophage-mediated granuloma formation. One of the characteristic features of Crohns disease is defective neutrophil accumulation, both *in situ* and in *ex vivo* experiments using human blood-derived cells. *In situ*, this appears to be due to reduced production of the neutrophil chemokine IL-8 by macrophages. Without the influx of neutrophils, it appears as though uptake of transmural bacteria is mediated by macrophages and these macrophages produce altered and enhanced amounts of pro-inflammatory (Th1) cytokines that may contribute to the inflammatory milieu.

Although there is overwhelming evidence that there are genetic elements associated with susceptibility to Crohns disease and other inflammatory bowel disorders, these disorders are clearly polygenic and as such the genetic contribution is difficult to pin down conclusively. The genes most closely associated with susceptibility to Crohn's include those that encode NOD2, an intracellular PRR (**Figure 2**), and two genes involved in autophagy (IGRM and ATG16L1) (**Figure 5**). Although it is not entirely clear how these genes contribute to susceptibility to inflammatory bowel diseases, it is believed that they result in an altered ability to detect intracellular pathogens either by APCs or epithelial cells results in a dysregulated inflammatory response. Polymorphisms in other genes including the IL-23 receptor are associated with inflammatory bowel disorders in general and may result in defective adaptive immune responses (Marks and Segal, 2008). For further information, see 'Genetics of Inflammatory Bowel Disease' by CW Lees, G Ho and J Satsangi.

Cancer

Cancer is increasingly viewed as having a modified inflammatory component, and as with many inflammatory diseases, macrophages can be present in high numbers. In mouse models in which macrophages are almost entirely ablated due to a loss of function mutation in the gene encoding colony-stimulating factor 1 (CSF-1), tumour progression is stalled and metastasis ablated. Indeed, in human patients, a high density of tumour-associated macrophages indicates a poor prognosis. Macrophages are associated with even early stage tumours and their presence is essential for such processes as angiogenesis, extracellular-matrix breakdown and remodelling (which is required for subsequent motility) and intravasation. In cases where chronic inflammation is believed to be involved in the aetiology of cancer, macrophages may contribute to malignancy by releasing DNA-damaging free radicals in addition to a host of growth and proliferation factors which may contribute to uncontrolled or dysregulated growth. In other experimental models the cancerous cells themselves recruit macrophages by secreting chemotactic and growth factors. Once recruited, macrophages appear to be strongly associated with hypoxia, angiogenesis and vascularization of the solid tumour, a process that is essential for tumour cell survival and is thus a target for drug development. The final and most harmful stage in cancer progression is metastasis, which again is a macrophage-associated process. Macrophages are associated with regions of basement membrane destruction, an essential pre-requisite for metastasis and through a complicated interplay between chemokines and growth factors produced by both the cancer cells and the macrophages contribute to intravasation. Although it is not entirely clear how macrophages contribute to seeding tumour cells at distant sites, in mouse models in which they are ablated, seeding is decreased (Condeelis and Pollard, 2006).

Concluding Remarks

Given the properties outlined above, it is evident that the mononuclear phagocyte system represents a distributed organ responsible for homeostasis within the host, adapting to local and systemic deviations arising internally as well as from without. The cells are involved in every disease process in which there is persistent tissue injury or metabolic disturbance. They mediate acute as well as chronic inflammation, and promote repair through removal of dead cells and fibrin by phagocytosis and fibrinolysis, induce blood vessel ingrowth (angiogenesis) and modulate fibroblast invasion and production of extracellular matrix. They produce mediators that mobilize systemic responses of the host including fever, release and catabolize stress and other hormones, increase metabolic activity of other cells, including the 'acute response' by hepatocytes, and influence blood flow to tissues and capillary permeability. The macrophages themselves display considerable heterogeneity in their functions, often expressing activators as well as inhibitors of a property, e.g. proteolytic activity, or pro- and anti-inflammatory cytokine production, depending on the evolution of a particular host response. The study of macrophage biology and their involvement in human disease is therefore an avenue for potential therapeutic intervention.

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