INTRODUCTION

Macrophage Biology

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Abstract

The importance of macrophages in the host response to infection has been recognised for decades. However, the macrophage has a range of phenotypes, functions and activation states and consequently the study of macrophage biology is complicated by the heterogeneity of these cells. An understanding of basic macrophage biology is required to understand the mechanisms of evasion, invasion and subversion of macrophage defences by protozoan pathogens. Herein we review the origins of macrophages, differences in macrophage phenotypes, mechanisms of macrophage based killing and subversion of this killing by pathogens.

What Is a Macrophage?

Phenotypically and functionally tissue macrophages are an extremely heterogeneous group of cells derived from circulating monocytes. They range in appearance from the dendritic-like microglial cells to the less aborised Kupffer cell. Fortunately, in humans there exists an intracellular membrane marker by which the majority of macrophages can be identified called CD68 (macrosialin in mouse). It has long been known that macrophages are an important component of the innate immune response, but it is increasingly apparent that they are involved in tissue homeostasis, regulation of haematopoiesis, chronic inflammation, atherosclerosis, wound repair and tissue remodelling, as well as killing of invading micro-organisms.

Although macrophage function depends, at least in part, on location, developmental state and in vitro culture conditions, there are some properties that are conserved amongst almost all macrophage populations studied to date. One of the most distinctive properties of macrophages is their ability to ingest particles via phagocytosis. Macrophages are able to recognise both pathogens and noninfectious agents using a variety of germ line-encoded pattern recognition receptors including lectins, toll-like receptors, and receptors for N-formyl methionine containing peptides. Macrophages are involved in safe apoptotic cell clearance and remove small numbers of potentially dangerous micro-organisms via phagocytosis without inducing a strong pro-inflammatory response. Should they fail to clear perceived threats, an acute inflammatory response is mounted. This results in the secretion of a variety of cytokines, chemokines and antimicrobial agents. Secretion of these mediators can result in autocrine activation of the macrophage by binding of cytokines to cytokine receptors or recruitment of cells involved in the adaptive immune response via secretion of chemokines. The macrophage destroys invading micro-organisms using an arsenal of antimicrobial effector mechanisms that encompass enzymatic degradation, oxidation, nutrient limitation and antimicrobial peptides. Upon internalisation and digestion of the pathogen, the macrophage presents foreign antigens to primed T lymphocytes, thus amplifying the adaptive immune response. When macrophage-based
clearance is insufficient, prolonged or chronic inflammation may occur. Macrophages are intimately linked with a number of chronic infections and inflammatory conditions such as the formation of atherosclerotic plaques, and conditions such as rheumatoid arthritis. The role of macrophages in host defence has been extensively reviewed in references 1-5.

Herein we describe the origins of macrophages, differences in macrophage phenotypes, mechanisms of macrophage based killing and subversion of this killing by intracellular parasites.

**Macrophage Origin**

Although it is clear that myeloid cells are derived from precursor cells found in the bone marrow, their developmental pathway is not entirely resolved. Confounding this is the fact that studies of leukocyte development are much more easily performed in the mouse and thus there is some confusion as to differences between human and mouse leukocyte development. It is generally accepted that CD34+ precursors give rise to monocytes, granulocytes, erythrocytes and thrombocytes. Cells that express both CD34 and the receptor for macrophage colony stimulating factor (M-CSFR) give rise exclusively to myelomonocytic cells. Circulating CD34+ monocytes are found in the blood and it is these circulating monocytes that give rise to tissue macrophages. In human peripheral blood there are at least four subsets of monocytes that are characterised by their levels of expression of CD64 (FcγRII), CD14 and CD16 (FcγRIII). In response to stimuli these monocytes give rise to terminally differentiated cells, macrophages and dendritic cells.

Monocytes that express high levels of CD14, CD64 and little or no CD16 (CD14+/CD64+/CD16-) comprise greater than 80% of the circulating monocyte population in healthy individuals. These monocytes produce high levels of pro-inflammatory cytokines when they are stimulated with bacterial components. Their response to a variety of chemokines in vitro is presumed to be an important factor in migration to peripheral tissues during the course of infection and inflammation; subsequently they are able to differentiate into macrophages with excellent anti-microbial activity and the capacity to interact with both B and T lymphocytes.

Monocytes expressing CD16 as well as CD64 and CD14 (CD14+/CD64+/CD16+) constitute less than 10% of circulating monocytes in humans. These cells produce high levels of pro-inflammatory cytokines, low levels of IL-10, have a very high phagocytic capacity and participate in antibody dependent cellular cytotoxicity (ADCC). They are believed to be precursors to “resident” macrophages. Upon in vitro culture with cytokines, CD14+/CD64+/CD16+ expressing cells differentiate into either macrophages or dendritic cells with a distinctive DC1 phenotype and are increased in patients with Kawasaki disease and influenza and decreased in patients with rheumatoid arthritis.

Monocytes that do not express CD64 but have low to intermediate levels of CD14 and high levels of CD16 (CD14dim/CD64-/CD16+ or CD14low/CD64low/CD16+) constitute less than 10% of circulating monocytes. These monocytes have increased costimulatory activity and CD45 expression, but produce very little type 1 interferon or other pro-inflammatory cytokines. They have weaker phagocytic responses and ADCC but display an enhanced ability to interact with T or B lymphocytes and express higher chemotactic activity than their CD14+/CD16- counterparts, especially in response to the endothelial cell tethered chemokine, fractalkine. The ability of these cells to transmigrate in response to fractalkine (due to expression of CX3CL1, which is not expressed on CD16- cells) indicates that they may be precursors to tissue macrophages. These cells differentiate into myeloid antigen presenting cells and are believed to play a part in the Th1 response in vivo. Elevated numbers of these cells are found in HIV infected patients, and those suffering from pararheumatic systemic vasculitis and sepsis.

Although these characterisations are helpful for making broad generalisations, there are a number of monocyte/macrophage subsets that aren't easily classified. For example, macrophages of the intestinal mucosa have a distinct receptor expression profile that does not include CD14, complement receptors, or Fc receptors (see below). This illustrates the conclusion that there are no clear guidelines to identify and classify subsets of macrophages. For a thorough review on monocyte and macrophage heterogeneity see reference 14.
Macrophage Biology

Activation States of Macrophages

Macrophage biology is plagued by confusion concerning the terminology of macrophage activation states. In the absence of pro-inflammatory or infectious stimuli macrophages have a number of homeostatic functions including the engulfment of apoptotic cells, erythrocyte clearance, and constitutive tissue repair. The macrophage’s response to infection must be tailored to the microbial threat and it has been discovered, primarily from in vitro studies, that the type of microbial or inflammatory stimulus results in the production of macrophages with varied functions. To date four major classes of immunologically acquired macrophage activation have been proposed, classical, innate, alternative, and deactivated.

Classical Activation

The concept of macrophage activation came about as a result of the observation that macrophages treated with bacterial components and interferon-γ (IFN-γ) developed an enhanced ability to destroy a wide range of ingested pathogens. IFN-γ is produced by CD4+ and CD8+ T lymphocytes, NK cells and possibly by the infected macrophages themselves. IFN-γ alone does not confer this ability; rather it primes the macrophages for activation. The second signal is a bacterial component, generally LPS. Some studies suggest that it is the bacterial stimulation of TNF-α that provides the secondary signal rather than the LPS itself, although this has not been completely characterised.15 Classically activated macrophages have an increased ability to present antigen due to an enhanced expression of MHC class II and CD80/CD86 (B7.1/B7.2) and increased production of iNOS. They have an enhanced ability to destroy intracellular pathogens due to an increased respiratory burst, and acquire the ability to mediate diverse inflammatory effects in the host by secreting a variety of cytokines.

The importance of IFN-γ in parasite infection was demonstrated in vivo when it was found that antibody mediated neutralisation of IFN-γ in infected mice caused them to die more rapidly and have increased parasite loads.16-19 Subsequent experiments with mice defective in expression of IFN-γ or its receptor demonstrated that they were more susceptible to a variety of intracellular bacterial or protozoan pathogens.20-23 IFN-γ-induced activation is a contributor to the pathology of rheumatoid arthritis, delayed-type hypersensitivity and may contribute to atherosclerosis.24-26

Innate Activation

Classical activation requires two steps, exposure to IFN-γ and to a bacterial products and results in a macrophage with altered phenotypic and functional properties. It has recently been shown that exposure to bacterial components, such as LPS or CpG, alone results in macrophages with altered phenotypes and functional properties. For example, it has been demonstrated that macrophages treated with LPS or CpG have an enhanced ability to produce IL-12 in response to a second exposure to LPS due to expression of the macrophage receptor with collagenous structure (MARCO).27 TLR agonist-induced expression of MARCO has also been linked to an enhanced ability of the macrophage to bind and clear Neisseria.28 A complete description of the receptors involved in innate activation and a full description of the functional properties of these cells has yet to be completed.

Alternative Activation

Early on it was observed that the antigen presenting cells obtained from mice with experimental nematode infections (in which there is a Th2 cytokine environment) were able to process and present antigen without inducing T cell proliferation.29 Subsequently it was found that exposure to the Th2 associated cytokines, IL-13 and IL-4, resulted in macrophages with enhanced expression of the mannose receptor and MHC class II, but which were not able to induce T cell proliferation. Increased expression of the mannose receptor is associated with endocytosis and antigen presentation, although perhaps less efficiently than classically activated cells.30 There is also an increased flow of internalised particles and ligands to lysosomes. It has been demonstrated that alternatively activated macrophages are important in clearance of parasitic and
extracellular pathogens, but unlike classically activated macrophages they do not display an increased oxidative burst and thus are not as efficient in killing intracellular pathogens.30,31

The importance of alternatively activated macrophages in parasitic and protozoan infections is now well established. In vivo models of Schistosoma mansoni, trypanosome and Leishmania infection demonstrate that there is a complex interplay between the production of Th1 and Th2 cytokines and the subsequent development of macrophage subsets. It appears that an initial Th1 response (characterised by elevated levels of IFN-γ and IL-12) is required to control the initial stages of infection by T. cruzi, T. brucei, and S. mansoni, however the cytokine balance shifts during the course of disease to a Th2 response.32-34 It is generally believed that this shift to a Th2 bias is required for clearance and resolution of the infection as animals defective in producing Th2 cytokines and thus alternatively activated macrophages do not survive. There is also evidence that for some protozoan pathogens the shift to a Th2 mediated response may result in dissemination of the parasite throughout the host.35

Alternatively activated macrophages do not make substantial amounts of nitric oxide (NO) because of their induction of arginase, an enzyme that counteracts the harmful effects of NO. Arginase does contribute to polyamine and proline biosynthesis, and promotes cell growth, collagen formation, and tissue remodelling. It has been proposed that this subclass of macrophages may play a primary role in wound repair, angiogenesis, fibrogenesis, synthesis of the extracellular matrix and granuloma formation.36 Alternatively activated macrophages also appear to have an anti-inflammatory function, and they have been demonstrated to decrease T cell proliferation and produce the anti-inflammatory cytokine IL-1 receptor antagonist and IL-10.37 Consistent with this analysis, these cells have a slight decrease in LPS-induced respiratory burst and cytokine production compared to classically activated macrophages.

Alternatively activated macrophages are important in Th2-mediated diseases such as asthma, allergy and in the resolution of infectious disease and parasitic infection. The process of alternative activation has been reviewed in reference 38.

Deactivation

Activated macrophages have potent biological functions that are essential for the host’s response to infection. However, once infection is resolved it is essential to end the pro-inflammatory program. Exposure to a number of anti-inflammatory molecules such as cytokines (e.g., IL-10, TGF-β), receptor ligation (e.g., CD200 - CD200R), steroids, or uptake of apoptotic cells can induce a “deactivated” phenotype. These cells can be identified by the expression of CD16339 and they have a reduced expression of MHC class II, a decreased respiratory burst and pro-inflammatory cytokine production, as well as enhanced anti-inflammatory cytokine production.

Types of Macrophages

Macrophage subpopulations can be divided a number of ways. There are phenotypic and functional distinctions between macrophages found at different locations throughout the body and between resident and recruited macrophages. The distinction between resident and recruited macrophages is particularly murky due to the difficulty in distinguishing the two sets in vivo. Although it has long been known that circulating monocytes migrate to the tissues where they become macrophages40 there is some debate over the role of newly recruited monocytes in the development of resident cells. Originally it was believed that tissue macrophages were derived and replenished exclusively from circulating monocytes, however, transplantation studies in both mice and humans indicate that the replenishment of resident tissue macrophages with donor macrophages is extremely slow. This could occur because of very low levels of recruitment and replenishment by circulating monocytes or because the tissue macrophages of the recipient are capable of self-renewal.41,42 Similar results were found for epidermal Langerhan’s cells.43 Thus it is believed that early in foetal or embryonic development the tissues are populated with cells derived from circulating monocytes. These cells mature into resident macrophages and under steady state conditions replenishment from circulating cells is low. When these
cells are activated by infection or inflammation they are able to enter the draining lymph nodes with the appropriate chemotactic stimulus and move to B and T lymphocyte areas to present pathogens. Under such conditions monocytes enter the tissues to replenish the activated macrophages and these cells become “recruited” macrophages. The monocytes that are recruited to the sites of infection or inflammation may be different from those which replenish resident cells under steady state conditions.

Tissue macrophages generally have stellate morphology and high endocytic ability (including nonspecific uptake of particles and Fc receptor-mediated uptake). Although they proliferate very slowly they have active RNA and protein synthesis. Resident tissue macrophages have important homeostatic functions and clear protein aggregates (e.g., protease-inhibitor complexes), physiological molecules (e.g., lysosomal hydrolases), denatured molecules (e.g., modified lipoproteins) and apoptotic cells from the intracellular spaces in either an immunologically silent or tolerogenic fashion. These cells are also important sentinels for clearance of invading micro-organisms.

Despite the heterogeneity of macrophages there are obvious functional divisions between different subsets and it is useful to characterise macrophage subpopulations on the basis of location. Herein we briefly summarise the characteristics of macrophage subpopulations that are most frequently associated with parasitic infections. Although we omit discussion of macrophage subpopulations of the lung, brain and bone, macrophages at these locations have all been shown to harbour protozoan parasites and contribute to pathology in rare instances. For reviews on these cell types see references 44-47.

Kupffer Cells

Resident macrophages of the liver are termed Kupffer cells. The liver is an essential and active component of the innate immune response. Following infection at extrahepatic locations, local macrophages produce the cytokines IL-1, TNF-α and IL-6. Detection of IL-6 causes hepatocytes to produce a number of acute phase proteins that are responsible for the systemic effects of inflammation, and enhancing opsonic phagocytosis and complement activation. Extra-hepatic cytokines are detected by Kupffer cells; the cells become activated and have enhanced anti-microbial properties, although resident Kupffer cells have a less vigorous respiratory burst and are thus less efficient at killing certain pathogens than other types of macrophages.49

Kupffer cells express high levels of phagocytic receptors. These include Fc receptors by which they remove soluble IgG complexes and antibody coated particles or micro-organisms, complement receptors by which they remove complement coated bacteria and erythrocytes and scavenger and toll like receptors by which they remove bacteria and endotoxin from the circulation. Their avidity for clearing erythrocytes results in the characteristic accumulation of iron in these cells.

Kupffer cells may be further subdivided on the basis of their location in the liver into cells of the periportal, midzonal and perivenous regions. Macrophages at different locations have different capacities for secretion of TNF-α, prostaglandin E, nitric oxide and IL-1. The Kupffer cells of the periportal region have the greatest phagocytic activity and highest lysosomal enzyme activity which is believed to be because this is the entry point for blood, and thus the first contact point for any blood borne pathogens. Kupffer cells are involved in both clearance and transmission of pathogens as they have been demonstrated to harbour a number of protozoan pathogens.50-52 For example, these cells may be especially important in the systemic spread of *Plasmodium falciparum*. Sporozoites move through the liver via the blood stream and are phagocytosed by Kupffer cells due to recognition of at least two proteins, circumsporozoite protein (CSP) and thrombospondin-related adhesive protein (TRAP).53,54 The sporozoites appear to be able to survive in the vacuoles of the macrophage and to exit the macrophage at a later time point.53,55,56 At this point the parasites invade neighbouring hepatocytes and cause their destruction, resulting in many of the symptoms of disease. The role of Kupffer cells in malaria is reviewed in reference 57 and the immunobiology of the liver is reviewed in reference 58.
Protozoans in Macrophages

Splenic Macrophages

The spleen is a unique lymphoid organ involved in clearance of pathogens and senescent erythrocytes from the blood as well as antigen presentation and activation of an adaptive immune response. Macrophages of the spleen are generally subdivided on the basis of location; however, it is important to note that the architecture of mouse and human spleen is quite different and that the majority of our knowledge of splenic macrophages comes from murine studies. In the few comparative studies that have been performed it appears as though receptor distribution and function of different macrophage populations vary between humans and mice. Nevertheless, the spleen functions as both a site of clearance of blood-borne pathogens and of interactions between antigen-presenting cells and B and T lymphocytes in both humans and mice. The summary below is based primarily on mouse studies.

The white pulp is a specialized area of lymphocyte accumulation that contains B and T lymphocytes. The white pulp is separated from the red pulp, which is the major area of erythrocyte clearance, by the marginal zone. The marginal zone contains marginal zone B lymphocytes and dendritic cells as well as two types of macrophages, the marginal zone macrophages that are located adjacent to the red pulp, and the marginal zone metallophilic macrophages that are located adjacent to the white pulp. The macrophages of the marginal zone are involved in the clearance of apoptotic cells and microorganisms as well as the maintenance of B lymphocytes. Like Kupffer cells, these macrophages are involved in turnover of erythrocytes and recycling of iron. The function of the marginal zone metallophilic macrophages is not entirely clear, although it is believed that they are involved in the response to viruses as they produce high levels of IFN-α and IFN-β.

Splenic macrophages possess a variety of pattern recognition receptors that are of vital importance in clearance of blood-borne pathogens including *Leishmania spp.*, and *Plasmodium falciparum* (reviewed in refs. 61,62). Certain pathogens that are easily cleared from circulation by the macrophages of the liver have virulence factors that prevent facile clearance from the spleen. For example, in experimental models of visceral leishmaniasis, the hepatic component of the infection is self-limiting (probably as a result of granuloma formation); however, amastigote growth in the spleen cannot be contained and results in tissue destruction. Although it is known that the marginal zone macrophages avidly phagocytose amastigotes it is not known whether their inability to clear the parasite is due to differences between hepatic and splenic macrophages such as differences in the mechanism of entry of the pathogen, differences in its ability to suppress cytokine production or some other unidentified mechanism.

The importance of the spleen in the host's response to infection is clear as splenectomised patients have a high risk of severe bacterial infections and must take prophylactic antibiotics. Patients who have undergone a splenectomy are also more likely to suffer from malaria and to have higher titres of parasites within their blood. Thus the macrophages of the spleen are important in host defence towards bacterial and parasitic infection.

Dendritic Cells

The dendritic cell (DC) is the close cousin to the macrophage. Both macrophages and dendritic cells capture and present nonspecific antigens although the dendritic cell also presents self antigens and is involved in the induction of tolerance. The dendritic cell is referred to as an immature dendritic cell (iDC) before it encounters antigenic stimuli. These cells are found in nonlymphoid tissues and, like macrophages, dendritic cells are highly phagocytic, a function that is facilitated by the presence of motile, long dendrite-like processes that are able to sample antigen. In the absence of foreign or inflammatory stimuli immature dendritic cells may take up and process antigen, but do not interact with T cells because they do not express significant amounts of MHC class II or costimulatory molecules on their surface. Should the dendritic cell receive a “danger” signal (e.g., pathogen associated molecules or pro-inflammatory cytokines) it undergoes an activation process which increases expression of MHC class II, of costimulatory molecules (CD80 and CD86) and of selected chemokine receptors that allow it to migrate to
the lymph node where antigen presentation occurs. These dendritic cells are referred to as mature dendritic cells (mDC). In contrast to macrophages, dendritic cells are able to present antigen to both naïve and activated T cells. Dendritic cells activate the adaptive immune response by both presenting antigen to T cells, but also by secreting a number of cytokines and chemokines. Often cytokine production by dendritic cells is far greater than that of macrophages, the result of which is greater recruitment and activation of T cells.

Although it now appears that there may be as many as five classes of DCs we limit this discussion to the best characterised classes of dendritic cells that are most likely involved in the response to protozoan infections. Dendritic cell subtypes of mice and humans are reviewed in reference 66.

The most recent class of circulating precursor cell to be identified is the plasmacytoid DC (pDCs) which is CD64-/CD16+. These cells comprise a very low percent of the total circulating population but despite those low numbers they are essential in the host’s response to viruses. Plasmacytoid cells can also be obtained from the spleen. They express TLR7, TLR9 and TLR11 (in mice) and are not responsive to TLR2 and TLR4 agonists such as LPS and peptidoglycan. Plasmacytoid DCs produce high amounts of IFN-α, but no or little IL-6 or TNF-α. Compared to other DC subsets, plasmacytoid DCs have limited phagocytic capacity, do not participate in ADCC and have very little interaction with either B or T lymphocytes. In general they are not believed to play a role in the host defence against protozoan pathogens, although it has been demonstrated that malaria blood stage schizonts can lead to increased expression of CD86 and stimulate production of IFN-α by pDCs in vitro.67 For a current review on the function of pDCs see reference 68.

Myeloid DCs can also be detected in the circulation and are characterised by the expression of markers such as CD13, CD11c and CD33. Upon stimulation with pathogen associated microbial ligands via TLR1, TLR2, TLR5 and TLR8 these cells do not produce IFN-α or -β but rather the pro-inflammatory cytokines, IL-6 and TNF-α. Myeloid DCs produce high levels of IL-12 in response to protozoan pathogens in both toll like receptor-dependent69,70 and -independent fashion.71 In contrast to pDCs, these cells produce predominantly homeostatic chemokines72 and have a higher capacity to migrate towards chemokines such as MCP-1, RANTES and IP-10 produced during the course of protozoan infection.73 It is believed that under the correct conditions circulating myeloid DCs can migrate to the tissues where they differentiate into tissue DCs.

With respect to parasitic infection the macrophages and dendritic cells of the skin and the gut are especially important. Because of the interplay between macrophages and dendritic cells at these sites in response to infection we summarise their properties on the basis of their location.

**Macrophages/Dendritic Cells of the Skin**

The resident cells of the epidermis are crucial in the elimination of pathogens that are transmitted by insect bites or other breaches of the skin. There are two populations of dendritic cells in skin, the Langerhan’s cells which are characterised by the expression of CD207 (Langerin) and dermal dendritic cells which are characterised by expression of CD208 (DC-SIGN). Dermal DCs have been implicated in binding *Leishmania* amastigotes and *Schistosoma mansoni* egg antigens.74 Dermal dendritic cells are located at the capillaries and the reticular dermis whereas Langerhans cells are located at the basal and supra-basal layers of the epidermis.75 The long processes of these cells are uniquely adapted to capturing antigen, which is mediated by expression of C-type lectins and Fc receptors. These DCs present antigen in the context of both MHC class I and class II. Tissue macrophages and dendritic cells of the skin appear to have different abilities to phagocytose particulate matter and pathogens. Langerhans cells phagocytose 0.5 - 1 μm beads whereas macrophages ingest larger particles (>3.5 μm). There are differences in the types of pathogens preferentially phagocytosed by different subsets of skin DCs and macrophages.76,77 The role of dendritic cells in the skin has been reviewed in reference 78.
There is a complex interplay between tissue macrophages and dendritic cells during the course of parasitic infection. In cutaneous *Leishmania* infection the skin is inoculated with promastigotes that are ingested, and generally destroyed by resident macrophages via the production of reactive oxygen and nitrogen species. In vitro studies suggest that macrophages do not necessarily become activated or increase surface expression of various surface markers. Sequential activation of skin dendritic cells via ingestion of amastigotes is required to present antigen and to clear infection. CD4+ T lymphocytes must be involved in order to produce the Th1 promoting cytokines IL-12, IFN-γ, and TNF-α. The pathology of *Leishmania* infection of the skin is reviewed in reference 80.

**Macrophages and Dendritic Cells of the Gut/intestine**

The gut consists of many different immunological niches including Peyer’s patches, mucosal lymphoid follicles and the lamina propria. Antigen presenting cells can be found in all these areas. Macrophages of the mucosa and intestine are uniquely adapted to cope with the high antigenic and bacterial load of the gut. Although these cells are derived from CD14 expressing circulating monocytes they do not express CD14 or other bacterial recognition receptors and as such are essentially nonresponsive to stimulation with bacterial products. The inability of these macrophages to respond to bacterial stimuli by producing cytokines such as IL-1, IL-6, IL-10, RANTES, TGF-β, and TNF-α has lead to the suggestion that they as they develop from circulating monocytes that develop "inflammatory anergy". It is believed that recruited CD14+ expressing monocytes develop this phenotype upon exposure to local cytokines such as TGF-β and that this is an essential adaptation to deal with the high load of predominantly commensal bacteria of the intestine. It should be noted that these macrophages are not defective in their ability to phagocytose or destroy phagocytosed bacteria. They do not express a number of other surface markers including CR3 and LFA-1 and receptors for IgA, IgG, but do express high levels of MHC class II and HLA-DR indicating that they have antigen presenting capacity. Macrophages may be found throughout the intestinal tract but appear to most common in the lamina propria. The role of macrophages in the gut and intestine has been reviewed in references 81 and 82.

Dendritic cells are also important antigen presenting cells in the gut and intestine. In addition to their antigen presenting functions these cells are particularly important in inducing the differentiation of regulatory T cells under steady state conditions. Immature dendritic cells are found in the Peyer’s patches and in the lamina propria. It is believed that pathogens and parasites are transported to the dendritic cells of the Peyer’s patches via M cells whereas dendritic cells of the lamina propria may sample pathogens using long dendrites that extend into the lumen of the gut between the tight junctions of the epithelial cells. Subsequent to infection the dendritic cells develop a mature phenotype (e.g., they express MHC class II, CD40, CD80, etc). It is believed that due to the capacity of these cells to migrate they are involved in dissemination of pathogens to distant sites throughout the body. The immunobiology of cells in the gut is described in reference 83.

Many protozoan pathogens enter the host via ingestion. *Giardia spp.*, *Cryptosporidium parvum*, *Toxoplasma gondii* and *Entamoeba histolytica* have all been demonstrated to multiply within the gut. Pathogens adhere to the epithelial layers of the intestine and in many cases are able to cross epithelial barriers at which point they may be detected by macrophages and dendritic cells of the intestine. A macrophage and dendritic cell mediated immune response is critical for both the stimulation and activation of macrophages and dendritic cells. Once mobilised, macrophages, neutrophils, and dendritic cells produce IL-12 and initiate a Th1 response. IL-12 production is essential for defence against protozoan pathogens in the gut because it stimulates the production of IFN-γ and activates macrophages. In fact, IFN-γ induced activation of macrophages is so critical for host defence that the ability to
decrease or eliminate its production is an essential virulence determinant for protozoan pathogens.89,90 The mucosal immune response to parasites has been reviewed elsewhere.91,92

**Recognition and Destruction by Macrophages and Subversion by Pathogens**

Despite the fact that the macrophages are exquisitely adapted to destroying intracellular bacterial and protozoan parasites, these infections occur at alarming rates, especially within the developing world. The World Health Organisation (WHO) lists malaria (*Plasmodium spp*), Chagas disease (*Trypanosoma cruzi*), leishmaniasis (*Leishmania spp*), and toxoplasmosis (*Toxoplasma gondii*) as major health risks in developing nations. It is believed that over 12 million people worldwide are affected by leishmaniasis,93 and there are 300 million cases of malaria.94 In order to understand what makes these pathogens so successful it is important to understand the mechanisms of recognition, uptake and destruction of pathogens by macrophages.

The macrophage has a potent ability to recognise, phagocytose and destroy pathogens. The initial binding and recognition process that triggers phagocytosis varies with respect to the micro-organism. There are differences in the outcome of opsonin-dependent phagocytosis and -independent phagocytosis. The importance of opsonic phagocytosis is highlighted by the number of opsonins produced by the host, both constitutively and in response to infection. Opsonins such as C-reactive protein are important in enhancing phagocytosis of a number of intracellular parasites (e.g., *Leishmania* promastigotes). However, phagocytosis mediated by complement activation does not result in a strong oxidative burst from the macrophage and thus some pathogens exploit this mechanism of uptake. For example, *Leishmania* promotes complement-mediated uptake by expressing elongated lipidoglycans on its surface. These lipidoglycans do not prevent complement activation, but the parasite is not lysed because the activated complement is distant from the cell membrane. Furthermore opsonisation allows promastigotes to enter the macrophage through the complement pathway thus evading normal phagosome-lysosome fusion.95

Phagocytosis mediated through Fc receptors generally results in the maturation of the phagosome into an acidic, hydrolytically active compartment and destruction of the pathogen. Intracellular pathogens have a number of conserved strategies for subverting normal Fc receptor mediated uptake. The pathogens *Toxoplasma*, *Plasmodium* and *Eimeria* have a motile invasive stage, called zoites, in which they can use an actinomyosin-based motile system that mediates host cell invasion thus subverting both complement and Fc receptor mediated phagocytosis. *Toxoplasma* uses this system to create vacuoles that exist independently of the normal phagolysosomal pathway and is consequently not exposed to the destructive environment of the phagolysosome. Mechanisms of protozoan invasion of host cells are reviewed in reference 96.

Upon Fc mediated phagocytosis the phagocytic vacuole undergoes numerous maturation steps that are accompanied by continuous remodelling of the phagosome membrane protein composition. Phagosomes sequentially fuse with the early endosomes, late endosomes and lysosomes and the maturation of the phagosome can be tracked by evaluating the accumulation of various surface markers. The pH drops slightly (pH 6.2) upon fusion with the early endosomes. This results in uncoupling of receptor/ligand pairs and receptor recycling mediated by the Rab proteins (Rab4 and Rab11). Upon fusion with the late endosomes the membrane of the phagolysosome accumulates acid resistant phospholipids and is characterised by the expression of Lamp1 and Lamp2. The resulting fusion with lysosomes results in a drop in pH (to 4.7 -5.2) that results in the activation of the proteolytic enzymes such as the cathepsins that are stored within. These enzymes are crucial not only for microbial degradation, but also to generate antigens for presentation by MHC molecules. Oxidative species such as O2- are rapidly produced upon phagocyte activation. The enzyme NADPH oxidase is essential for catalysis of various oxidative species including superoxide, hydrogen peroxide, and halogenated oxygen molecules in a process that is tightly coupled to cytoplasmic membrane and
requires cytoskeletal elements and protein phosphorylation. Nitric oxide species are also involved in antimicrobial killing and are essential for the destruction of a number of intracellular parasites. NO production is catalyzed by nitric oxide synthase from L-arginine and molecular oxygen. Interactions of hydrogen peroxide with myeloperoxidase, reduced iron, or NO lead to formation of additional toxic intermediates such as hypochlorous anion, hydroxyl radicals, nitrogen dioxide and peroxynitrite. The acidification may also be required for the generation of the oxidative burst and subsequent cytokine production.

Once the pathogen has been phagocytosed it has three options. It may either exist in the intralysosomal environment and develop mechanisms to deal with the acidic, hydrolytic environment therein or it may exist in the vacuole but prevent normal maturation from occurring thus remaining protected from the microbicidal properties of the macrophage. Some pathogens escape from the vacuole altogether and live in the more permissive environment of the cytosol.

The majority of intracellular pathogens actively subvert phagolysosome maturation. The pathogen may prevent acidification (e.g., Histoplasma capsulatum, Entamoeba histolytica), remodel the phagolysosome to a more permissive environment (e.g., Salmonella), or arrest the development of the phagosome at an earlier or less destructive stage (e.g., L. donovani, M. tuberculosis).97,98 Pathogens that have developed mechanisms for dealing with life in the lysosome include Leishmania and Coxella. Leishmania resists hydrolysis by having a cell surface of resistant lipidoglycans and can resist antigen presentation by regulating the expression and accessibility of antigenic peptides.99,100

Escape from the phagocytic vacuole is a common theme amongst intracellular parasites including T. cruzi, Listeria, Shigella and Rickettsia. Pathogens have a number of mechanisms by which they escape the phagosomal membrane such as the production of pores (Listeria spp.), lysis (Shigella flexneri), and as of yet unidentified mechanisms (Rickettsia). Once the pathogens have escaped they are able to replicate in the more permissive environment of the cytosol.

The macrophage has elaborate mechanisms to deprive the pathogen of essential components for survival such as iron and amino acids. In an unactivated state macrophages express the transferrin receptor by which they bind and internalise extracellular iron. Once they become activated by IFN-γ they down-regulate the transferrin receptor thus decreasing stores of intracellular iron. IFN-γ induced activation also activates the enzyme indoleamine 2, 3-dioxygease (IDO), which catalyses the degradation of L-tryptophan and thus limits the availability of this amino acid to intracellular organisms. Survival in the phagosome is the intracellular parasite’s most pressing issue, but once the immediate threat of degradation is dealt with the parasite must acquire scarce nutrients and avoid detection by the immune system. Activated macrophages prevent survival by sequestering free nutrients in the cytosol; however, Leishmania expresses nucleotidases on their surface in order to extract the purines from the host that they require for growth. The theme of extracting nutrients from a hostile environment is a common one used by C. burnetti, which has an active system for recruiting nutrients at an acidic but not neutral pH.

The secretion and presence of cytokines have a number of indirect effects on macrophage killing. Besides being essential for macrophage activation, IFN-γ has a number of indirect effects that enhance anti-microbial activity. Exposure to IFN-γ induces the production of a number of chemokines such as IP-10/CXCL10 and CXCL11 which result in the recruitment of additional leukocytes with antimicrobial activity. Chemokines also contribute to the enhancement of antibacterial activity. RANTES, MIP-1α, MIP-1β increase the uptake and cause the intracellular destruction by macrophages of trypomastigotes and rickettsia by inducing NO production.101

Intracellular parasites also subvert host processes by inhibiting or promoting macrophage signalling. This results in disruption of normal host processes such as apoptosis (e.g., T. gondii)102 and pro-inflammatory cytokine production (e.g., T. gondii).103 Abrogation of pro-inflammatory cytokine production alone is not enough to ensure protection and some parasites alter cell
signalling in such a way that the balance between Th1 and Th2 production is skewed, thus inhibiting the host’s normal anti-parasitic response. This may be done by altering signalling pathways through disruption or degradation of key signalling components or more directly by direct degradation of pivotal cytokines such as IL-12. For excellent reviews on the subversion of host cell defences by parasites see references 107-109.

Conclusion

The macrophage is of crucial importance in host defence towards infectious disease. There is much work to be done on understanding of the subtleties of the macrophages response to infectious disease. First we must characterise macrophage heterogeneity and the intricacies of functional differences between subtypes and activation states and secondly we must investigate subtle differences in macrophage function and susceptibility between individuals. It is becoming apparent that differences at the genetic level, including subtle polymorphisms in genes encoding macrophage receptors, effector molecules and signalling pathways, may contribute to the host’s predisposition to infectious disease. This knowledge will be essential in order to translate in vitro observations to understanding of pathogenesis in vivo. Recent advances in the study of infection by protozoa have provided insight into how these pathogens subvert host defences and have illustrated that the macrophage is the essential target for eradication of these pathogens. Increased understanding of these mechanisms is required to develop novel macrophage-based therapies.

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