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The role of Macrophage as APCs,Phagocytosis, and a source of ROS, as Potential Therapeutic and Preventive Target in Cancer and Autoimmunity

Dr. ZelalemKirosBitsue, EmnetAschalewFekede, Prof. Abbas Mirshafiey
United States of Africa Health Organization "AHO",
Department of Immunology, International Campus, Tehran University of Medical Sciences
Teklehaimanot General Hospital

Abstract: Macrophages, the most plastic cells of the haematopoietic system, are found in all tissues and show great functional diversity. They have roles in development, homeostasis, tissue repair and immunity.

Macrophages are a heterogeneous population of innate myeloid cells involved in health and disease. They are the most functionally diverse (plastic) cells of the hematopoietic system, found in all tissues, and their main function is to respond to pathogens and modulate the adaptive immune response through antigen processing and presentation. Further functions of macrophages center on the induction and resolution of inflammation, as well as tissue repair (Mosser DM and Edwards JP 2008). Macrophages have different functions and transcriptional profiles, but all are required for maintaining homeostasis. This involves phagocytosis of debris and pathogens, dead cell clearance, and matrix turnover.

Macrophages and their fused morphologic variants, the multinucleated giant cells, which include the foreign body giant cells (FBGCs) are the dominant early responders to biomaterial implantation and remain at biomaterial-tissue interfaces for the lifetime of the device. An essential aspect of macrophage function in the body is to mediate degradation of bio-resorbable materials including bone through extracellular degradation and phagocytosis.

Events in the foreign body response include protein adsorption, adhesion of monocytes / macrophages, fusion to form FBGCs, and the consequent modification of the biomaterial surface.

In this article, I describe the Macrophage origins, Macrophages in development, Biology of Macrophages, Signaling molecules involved in M1/M2 polarization, and Macrophage and Immunity as well as the role of macrophage as potential therapeutic target for Cancer and Autoimmunity.

Key Words: Macrophage, immunity, Phagocytosis, ROS, Immunoregulatory, Cancer and Autoimmunity

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1. Introduction

Macrophages, which were originally identified by Metchnikoff on account of their phagocytic nature, are ancient cells in metazoan phylogeny. In adult mammals, they are found in all tissues where they display great anatomical and functional diversity. In tissues, they are organized in defined patterns with each cell occupyingits own territory, a type of tissue within a tissue. Although several attempts have been made to classify macrophages, the most successful definition is the mononuclear phagocytic system (MPS), which encompasses these highly phagocytic cells (professional phagocytes) and their bone marrow progenitors. In the MPSschema, adult tissue macrophages are defined as end cells of the mononuclear phagocytic lineage derived from circulating monocytes that originate in the bone marrow. However, this definition is inadequate as macrophages have several origins during ontogeny and each of these different lineages persist into adulthood(1).Other functional classifications of macrophages have included binary classifications that refer to inflammatory states. These include the activated macrophage and alternatively activated macrophage (AAM) categories, and the derivative M1 and M2 categories for these types of macrophage in the non-pathogen-driven condition(2),(3). These two states are defined by responses to the cytokine interferon-c (IFN-c) and activation of Toll-like receptors (TLRs), and to interleukin-4 (IL-4) and IL-13, respectively. Although this classification is a useful heuristic that may reflect extreme states, such as that of activated macrophages during immune responses mediated by T helper cells that express IFN-c (TH1) or of AAMs during parasitic infections(2),such binary classifications cannot represent the complex in vivo environment formostmacrophage types, in which numerous other cytokines and growth factors interact to define the final differentiated state. Indeed, transcriptional profiling of resident macrophages by the Immunological Genome Project show that these populations have high transcriptional diversity with minimal overlap, suggesting that there are many unique classes of macrophages(1). Macrophages have roles in almost every aspect of an organism's biology; from development, homeostasis and repair, to immune responses to pathogens. Resident macrophages regulate tissue homeostasis by acting as sentinels and responding to changes in physiology as well aschallenges from outside. During these homeostatic adaptations, macrophages of different phenotypes can also be recruited from the monocyte reservoirs of blood, spleen and bone marrow(4), and perhaps fromresident tissue progenitors or through local proliferation(5),(6).Unfortunately, in many cases these homeostatic and reparative functions can be subverted by continuous insult, resulting in a causal association of macrophages with disease states, such as fibrosis, obesity and cancer. Thus,macrophages are an incredibly diverse set of cells that constantly shift their functional state to new metastable states ('set points') in response to changes in tissue physiology or environmental challenges. They should not even be considered as one cell type but should be subdivided into different functional subsets according to their different origins.Macrophage responses to pathogens have been discussed previously(2),(7),(8),and therefore this Review focuses on the homeostatic mechanisms by which macrophages contribute to physiological and pathophysiological adaptations in mammals. Here we define the hallmarks of macrophages that perform particular

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functions, taking into account new insights into the diversity of their lineages, identity and regulation. This phenotypic diversity is essential to understand because macrophages are central to many disease states and have emerged as important therapeutic targets in many diseases.

2. Macrophage origins

Ontologically, the MPS has been proposed to arise from a rigid temporal succession of macrophage progenitors(9).In mice, these start to develop first at embryonic day 8 from the primitive ectoderm of the yolk sac and give rise to macrophages that do not have a monocytic progenitor. This primitive system is followed by definitive haematopoiesis in the fetal liver,which is initially seeded by haematopoietic progenitors fromthe yolk sac and subsequently from the hematogenic endothelium of the aortogonadal- mesonephros region of the embryo. After this point, the fetal liver is the source of definitive haematopoiesis that generates circulatingmonocytes during embryogenesis. Coincident with the postnatal formation of bone, fetal liver haematopoiesis declines and is replaced by bone marrow haematopoiesis. This definitive haematopoiesis is the source of circulatingmonocytes (resident, lymphocyte antigen 6c negative (Ly6c2) and inflammatory Ly6c1 in mice) and from which it has been considered that all resident macrophages in tissues are derived(4),However, this model for the formation of the MPS has been challenged. First, lineagetracing experiments have shown that microglia are primarily derived from the yolk-sac progenitors, whereas Langerhans cells have a mixed origin from yolk sac and fetal liver(10),(11).Second, experiments using ablationof c-Myb-dependent bone marrow haematopoiesis followed by transplantation with genetically dissimilar bone marrow together with lineage tracing showed that the major tissue-resident population of macrophages (defined as F4/80 bright) in skin, spleen, pancreas, liver, brain and lung arise from yolk sac progenitors. In a few tissues, such as kidney and lung, macrophages have a chimaeric origin being derived from yolk sac (F4/80high) and bone marrow (F4/80 low). In contrast to this yolk sac and fetal liver origin for most macrophages, classical dendritic cells and the F4/80low macrophages are continuously replaced by bone-marrow-derived progenitors(6).These data indicate that there are at least three lineages of macrophages in the mouse, which arise at different stages of development and persist to adulthood. The data also call into question the function of circulating monocytes because, at least in mice, these cells do not seed the majority of the adult tissues with macrophages. In fact, complete loss ofCD161monocytes in humans seemsto be of little consequence(12).Thus, the function of monocytes needs to be defined with the possibility that patrolling monocytes (Ly6c2) act to maintain vessel integrity and to detect pathogenswhile inflammatorymonocytes (Ly6c1) are recruited predominantly to sites of infection or injury, or to tissues that have continuous cyclical recruitment of macrophages, such as the uterus.Regardless of their origin, genetic and cell culture studies indicate that the major lineage regulator of almost all macrophages is macrophage colony-stimulating factor 1 receptor (CSF1R). This class III transmembrane tyrosine kinase receptor is expressed on most, if not all, mononuclear phagocytic cells, and a reporter mouse expressing green fluorescent protein (GFP) from the Csf1r locus illustrates their relative abundance (5–20% of cells) and tissue distribution(13).Csf1r expression and its requirement for differentiation distinguish macrophages frommany, but not all, dendritic-cell subtypes(14).Targeted ablation of the Csf1r causes severe depletion of macrophages inmany tissues, such as brain, skin, bone, testis and ovary. Moreover, an initial comparison of the Csf1r-

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null mice with those homozygous for a spontaneous (osteopetrotic (Csf1op)) null mutation in its cognate ligand (Csf1op/op mice) demonstrated that all phenotypes in the Csf1r-null mice were also found in the Csf1op/op mice,indicating that CSF1 has only a single receptor(15)..However, the phenotype of the Csf1r-null mice is more severe than that of the Csf1-null mice, including the complete loss of microglia and Langerhans cells(10),(16), in the Csf1r-null mice, which suggested the presence of another ligand. Indeed, IL-34, with a distinct but overlapping pattern of expression with Csf1, was recently identified as an additional ligand for the CSF1R(17). Targeted ablation of Il34 resulted in loss of microglia and Langerhans cells, but had little impact on bone marrow, liver or splenic macrophages(18). Despite the importance of the CSF1R in macrophage specification,Csf1r-null mutant mice still have some tissue macrophages, such as in the spleen, indicating the existence of other macrophage growth factors. Potential candidates include granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-3, which act as macrophage growth factors in tissue culture. However, mice lacking GM-CSF or IL-3 do not show notable defects in their tissue macrophages, except in alveolar macrophages, which indicates that they are regulated by GM-CSF(19).Vascular endothelium growth factor A (VEGFA) proteins are another candidate regulator of macrophages because they can compensate for the loss of Csf1 in osteoclast development in vivo(20).In contrast to CSF1 that is found in all tissues and serum, and is a basal regulator of macrophage number through a negative feedback loop(15).GM-CSF is not a steady-state ligand and seems to be synthesized in response to challenge(21). GM-CSF and FLT3L regulate the maturation of dendritic cell populations with the notable exception of Langerhans cells, whose development is dependent on Csf1r(22).Recent genomic profiling of Langerhans cells place them closer to macrophages than dendritic cells, and this data together with their lineage dependence on Csf1r may indicate that classification should be updated(14).In their basal state, resident tissue macrophages show great diversity in their morphologies, transcriptional profiles, anatomical locations and functional capabilities(23).This functional heterogeneity probably results from the dynamic crosstalk between resident tissue macrophages and the client cells that they support. To understand this macrophage diversitythere must be an understanding of transcriptional regulation. The most important of these transcription factors is SFPI1 (also known as PU.1), a member of the ETS family whose loss following targeted mutation results in complete depletion of CD11b1F4/801 macrophages,including those derived from the yolk sac(6).However, Sfpi1 action is not limited to macrophages as B cells are also severely depleted in these Sfpi1-null mutant mice. Similarly, other members of the ETS family are also involved in macrophage differentiation, including Ets2, which positively regulates the Csf1r promoter. In adults, Mafb (also known as v-Maf) is required for the local proliferation that maintains resident macrophages(4).In the differentiation of osteoclasts, Fos and Mitf are required(24),whereas Gata2 is required for monocyte development but not for resident macrophage populations(25).However, little is known about the transcriptional control of the differentiation of the diverse tissue macrophages, such as those in the liver and brain(13).Most research has focused on their functional activation in response to environmental challenges(23).as discussed below. Nevertheless, the recent transcriptional profiling of resident macrophages has identified many candidate transcription factors, including those that may regulate core macrophageassociated genes such as Mitf (micropthalmia) family members, Tcf3, Cebpa, Bach1, Creg1 and genes that are unique to subpopulations, including Gata6 and Spic, whose targeted gene ablation will undoubtedly define subsets of macrophages and their unique activities(1).

3. Macrophages in development

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Metchnikoff proposed that macrophages participate in the maintenance of tissue integrity and homoeostasis. To do so, macrophages would need to be able to discriminate self from non-self, sense tissue damage and recognize invading pathogens, an insight that led to the concept of innate immunity for which he was awarded the Nobel prize. The inherent properties of macrophages, which include sensing inside from out, motility throughout the organism, phagocytosis and degradation, were later sequestered to instruct the acquired immune system as it evolved to more efficiently deal with changing pathogenic challenges. Thisenhanced sophistication of the immune system probably resulted inthe evolution of dendritic cells as specialized mononuclear phagocytesto interface with the acquired immune system. Indeed, in mammals,dendritic cells seem to be focused on initiating tissue immune responses,whereas tissue macrophages seem to be focused on homeostasis andtissue integrity(9). Emphasis on the immunological and repair aspects of macrophage function has overshadowed their importance in the development of many tissues; for example, studies of Csf1op/op mice, which lack many macrophage populations, have revealed a cluster of developmental abnormalities(19).Most notable among these is the development of osteopetrosis, which is caused by the loss of bone-reabsorbing macrophages known as osteoclasts. This phenotype, which is also observed in Sfpi1-null mice, is axiomatic for the roles of macrophages in development, in that cell fate decisions are unchanged but the tissue remodelling and expression of growth factors is lost. Specifically, although bone formation is intact in Csf1- or Spi1-null mice, the bones are not sculpted to form the cavities in which haematopoiesis commences(19).Consequently, the functional integrity of the bones, in terms of load bearing and haematopoiesis, is compromised. Csf1op/op mice survive to adulthood because of extra-medullary haematopoiesis in the spleen and liver19, and as mice age,osteoclastogenesis is rescued by compensatory expression of VEGF and therefore bone marrow haematopoiesis commences(20). Remodelling deficiencies in the absence of macrophages have also been noted in several other tissues, including the mammary gland, kidney and pancreas, suggesting a general requirement for macrophages in tissue patterning and branching morphogenesis(19),(26).In the mammary gland, the best studied of these tissues, macrophages are recruited to the growing ductal structure and their loss results in a slower rate of outgrowth and limited branching, phenotypes that are reiterated during the mammary growth caused by pregnancy(19). This stems partly from the failure to remodel the extracellular matrix during the outgrowth of the ductal structures. However, recent studies have also implicated macrophages in maintaining the viability and function of mammary stem cells, which reside at the tip of the duct known as the terminal end bud and are responsible for the outgrowth of this structure(27).In stem cell biology similar roles for macrophages have been suggested in the maintenance of intestinal integrity and its regeneration after damage(28),whereas a subpopulation of macrophages in the haematopoietic niche regulates the dynamics of haematopoietic stem cell release and differentiation(29).Furthermore, in regenerating livers, macrophages specify hepatic progenitor fate through the expression of WNT ligands and antagonism of Notch signaling(30).Macrophage control of stem cell function is clearly an emerging and important research area. As 'professional' phagocytes (macrophages were originally defined by their exceptional phagocytic ability), macrophages perform critical functions in the remodelling of tissues, both during development and in the adult animal; for example, during erythropoiesis, maturing erythroblasts are surrounded by macrophages that ingest the extruded erythrocyte nuclei. Remarkably, this function of macrophages is critical because in its absence, erythropoiesis is blocked and lethality ensues(31).Macrophages also make decisions about haematopoietic egress from the bone marrow through engulfing cells that

do not express the CD47 ligand(32).They also maintain the haematopoietic steady state through engulfment of neutrophils and erythrocytes in the spleen and liver, and the failure of this activity results in neutropenia, splenomegaly and reduced body weight(33).Phagocytosis, particularly of apoptotic cells, is clearly central to macrophage function and this is emphasized by the build-up in macrophage-depleted mice of such cells during development; for example, during the resolution of the inter-digit areas during limb formation(34).However, there is no apparent consequence to this phenomenon, as less-efficient 'non-professional' phagocytes clear excess apoptoticcells. Despite this, macrophages have evolved to 'eat' cells, and to suppress inflammation and autoimmunity in response to self-antigens that may arise during homeostasis(35).Macrophages also regulate angiogenesis through a number of mechanisms. This has been most extensively studied in the eye during its development. Early in the postnatal period, during regression of the hyaloid vasculature, macrophages identify and instruct vascular endothelial cells to undergo apoptosis if these cells do not receive a counterbalancing signal from pericytes to survive. WNT7B that is synthesized by macrophages delivers this cell-death signal to the vascular endothelial cells, and in the absence of either WNT7B or macrophages there is vascular over-growth(36).WNT secretion is also required later in retinal vasculature development but in this case macrophage synthesized WNT5A and WNT11, a non-canonical WNT, induces expression of soluble VEGF receptor 1 (VEGFR1) through an autocrine mechanismthat titrates VEGF and thereby reduces vascular complexity so that the vascular systemis appropriately patterned(37).Furthermore, at other times of ocular development, macrophages regulate vascular complexity. In this circumstance, macrophage-synthesized VEGFC reinforces Notch signaling(38).In addition, during angiogenesis in the hindbrain, macrophages enhance the anastomosis of tip and stalk cells to give functional vessels(39).These macrophage functions are not restricted to the vascular arm of the circulatory system, as they also have roles in lymphangiogenesisduring development(40),and in adults they have a notable role in maintaining fluid balance through their synthesis of VEGFC(41).Brain development is also influenced by macrophages. These macrophages called microglia depend onCSF1Rsignalling for their presence.In the absence of this signalling there are no microglia, and the brains of these mice have substantial structural defects as they mature. Both CSF1 and IL-34 are expressed by neurons in a mutually restricted pattern of expression, and IL-34 is the major factor for microglial differentiation and viability(42).. The disruption of architecture in the brain of the Csf1rnull mouse, together with well-documented deficiencies in neuronal processing regulating olfaction and the reproductive axis in the hypothalamus in Csf1-null mice, strongly suggests that microglia are involved in the development of neuronal circuitry and the maintenance of brain structur. Indeed, microglia have been shown to promote neuron viability, modulate neuronal activity(43),and prune synapses during development(44), as well as express a range of neuronal growth and survival factors, including NGF. This conjecture is supported by the finding that hypomorphic mutation in CSF1R in humans is responsible for hereditary diffuse leukoencephalopathy with spheroids that results from loss of myelin sheaves and axonal destruction(45).These trophic activities of microglia are also consistent with macrophages having roles in neuroprotection after injury, as defined in a variety of models. These effects include the promotion of survival and proliferation of retinal progenitor cells, and the regeneration of adult sensory neurons(46),(47),(48).However, caution needs to be exercised in attributing all of the phenotypes observed in the brains of Csf1r-mutant mice or humans to the loss of microglia, as Csf1r expression has been reported on neuronal stem cells and their development in vivo is regulated by CSF1R (42). Nevertheless, it seems likely that microglia have important roles in the development of neuronal circuitry, though their effects on the

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proliferation, survival and connectivity of neurons(43), through their effects on myelination, or by modulating angiogenesis and fluid balance in the brain. The examples given above indicate a few of the roles for macrophages in normal development and these are likely to expand with further study. Phenotypically in mice, macrophages are CD11b1, CD681 CSF1R1 F4/801 and phagocytic and their activities are through the temporal and spatial delivery of developmentally important molecules such VEGFs and WNTs as well as proteases. These developmental roles of macrophages are re-capitulated in repair as described below but are also intimately involved in chronic conditions that lead to pathologies aswell as the development and progression of malignancies.

4. Biology of Macrophages

Macrophages are mononuclear cells capable of phagocytosis. They are distributed throughout mammalian organs and their morphology varies depending on their state of activity. Peritoneal macrophages (in Giemsa-stained or May-Grunwald-Giesma stained preparations) measure 10 to 30 um in diameter. The cytoplasm contains vacuoles and is slightly basophilic. The nucleus is ovoid and measures 6 to 12 um in diameter. By phase contrast microscopy, peritoneal macrophages contain light gray diffuse cytoplasm with dark gray rod-shaped mitochondria. Granules and vacuoles are seen depending on the physiological state of the cell.' North and Mackaness(49),found that normal peritoneal macrophages from nonimmunized mice had a cytoplasm enclosed by a three-layered membrane 80 A thick with many protuberances and invaginations due to a high degree of activity. The periphery of the cytoplasm was finely granular and lacked structures such as endoplasmic reticula, with and without attached ribosomes, and cylindrical mitochondria. These structures were found in the rest of the cytoplasm rather than at the periphery.Three types of cytoplasmic vesicles were seen ranging from 300 A to 0.5 ,um in diameter and enclosed by a unit membrane. The three types of vesicles were small pinocytic vesicles, various sized organelles containing a fine granular material and a larger, denser vacuole. Ribosomes were attached to the external portion of the nuclear membrane which was continuous with the endoplasmic reticula.Various types of stimuli may cause "macrophage activation."Examples are stimuli which accompany phagocytosis of bacteria. Activated macrophages are metabolically highly active and contain organelles such as lysosomes rich in hydrolytic enzymes. North and Mackaness(49),observed that followingingestion of Listeria monocytogenes by mouse peritoneal macrophages, the bacteria within phagocytic vacuoles were surrounded at first by a clear area bounded by a unit membrane and later the clear area was filled with an amorphous material which apparently had been transferred from cytoplasmic vesicles (lysosomes) into the phagosomes to form phago-lysosomes.In other studies it was observed that there is a limit to the volume of particles taken up, and that this either imposes or is associated with a limit to the increased rate of respiration. As glycolysis is considered to be responsible for the energy needed for particle uptake in the macrophage, it is unlikely that a limit to the rate of oxygen consumption sets the limit to the number of particles taken Up(50).Peritoneal macrophages of mice that survived an initial infection with L monocytogenes were regarded as "immune" because they could kill the specific organism rather than support its intracellular growth. Macrophages from mice infected with Mycobacterium bovis, strain BCG, were observed to be refractory to infection by unrelated pathogens in vitro(51).In addition,such macrophages were found to have greater in vitro chemotactic response than the response of those from uninfected mice(52).North and

Mackaness(49),compared the ultrastructure of peritoneal macrophages from mice immunized with L monocytogenes with that of peritoneal macrophages from nonimmunized mice. They observed that the cytoplasmic membrane of immunemacrophages was smoother, and had fewer protuberances and invaginationsthan the membrane of nonimmunized macrophages. Immune macrophages containedmany free ribosomes but very few profiles of endoplasmic reticula.The mitochondria were smaller, more numerous and contained more cristae. The cytoplasm of immune macrophages was less dense and appeared to be highly dehydrated. It contained fewer vesicles and many Golgi bodies. Dumontand Sheldon(53),found similar results using peritoneal macrophages from nonimmunized hamsters. The morphology of mouse and hamster peritoneal macrophageswas significantly different from that of other mammalian and avian peritoneal macrophages. Alveolar macrophages were morphologically distinct from peritonealmacrophages. Leake and Heise8 observed these differences with light and electron microscopy. They found that the nuclei of alveolar macrophages were round or slightly ovoid and nucleoli were seen more often than in peritoneal macrophages. Peritoneal macrophages had elongated, deeply indented nuclei. Rough endoplasmicreticulum was frequently seen in peritoneal macrophages, but rarely in alveolar macrophages. Peritoneal macrophages had more Golgi bodies than did alveolar macrophages. Alveolar macrophages had dense cytoplasmic granules not observedin peritoneal macrophages. Mitochondria from peritoneal macrophages were larger and more elongated than mitochondria from alveolar macrophages and the overall diameter of alveolar macrophage was approximately 1.5 times that of the peritoneal macrophage.Kajitaet a19 studied the alveolar macrophages of soot-exposed mice. These workers found that the mice that inhaled soot particles had clear membrane-bound vacuoles. The cells contained eccentric rounded nuclei in an abundant lowdensity cytoplasm. The mitochondria were elongated with regularly arranged cristae and the cell membrane showed invaginations. Karrer"" l" reported similar observations on the morphology of normal mouse alveolar macrophages and those that had phagocytized Indiaink. Splenic macrophage morphology was similar to peritoneal macrophages.

5. Phenotype of M1 and M2 macrophage subsets and their functions

Macrophages derived from monocyte precursors undergo specific differentiation depending on the local tissue environment. They respond to environmental cues within tissues such as damaged cells, activated lymphocytes, or microbial products, to differentiate into distinct functional phenotypes. The M1 macrophage phenotype is characterized by the production of high levels of pro-inflammatory cytokines, an ability to mediate resistance to pathogens, strong microbicidal properties, high production of reactive nitrogen and oxygen intermediates, and promotion of Th1 responses. In contrast, M2 macrophages are characterized by their involvement in parasite control, tissue remodeling, immune regulation, tumor promotion and efficient phagocytic activity. LPS, IFN-gamma and granulocyte-macrophage colony stimulating factor (GM-CSF) polarize macrophages towards the M1 phenotype, which induces secretion of large amounts of cytokines such as IL-1-beta, tumor necrosis factor (TNF), IL-12, IL-18 and IL-23. This helps to drive antigen specific Th1 and Th17 cell inflammatory responses.

Phenotypically, M1 macrophages express high levels of major histocompatibility complex class II (MHC II), the CD68 marker, and co-stimulatory molecules CD80 and CD86. M1 macrophages have also been shown to up-regulate the expression of the intracellular protein suppressor of cytokine signaling 3 (SOCS3), as well as activate inducible nitric oxide synthase (*NOS2* or iNOS) to produce NO from L-arginine(54),(55).In disease contexts, M1 macrophages are implicated in initiating and sustaining inflammation, and can therefore be detrimental to health. In contrast, M2 macrophage activation is induced by fungal cells, immune complexes, helminth infections, complement components, apoptotic cells, macrophage colony stimulating factor (MCSF), IL-4, IL-13, IL-10 and TGF-beta. This activation leads to the secretion of high amounts of IL-10 and low levels of IL-12. Phenotypically M2 macrophages have been characterized as IL-12lowIL-10highIL-1decoyRhighIL-1RAhigh. They are also defined as IL-2low, IL-23low, IL-1betalow and caspase-1low. In addition, they express high levels of scavenger mannose and galactose E-type and C-type receptors, and repurpose arginine metabolism to express ornithine and polyamine, which promotes growth. It is now appreciated that the M2 terminology encompasses a functionally diverse group of macrophages rather than a unique activation state. Accordingly, M2 macrophages can be further divided into subsets, specifically M2a, M2b, M2c and M2d based on their distinct gene expression profiles(56),(57).The M2a subtype is elicited by IL-4, IL-13 or fungal and helminth infections. M2b is elicited by IL-1 receptor ligands, immune complexes and LPS whereas M2c is elicited by IL-10, TGF-beta and glucocorticoids. The fourth type, M2d, is elicited by IL-6 and adenosine. M2d macrophages have phenotypic and functional attributes similar to ovarian TAMs but are distinct from M2a-c(58).M1 and M2 macrophages have distinct chemokine and chemokine receptor profiles, with M1 secreting the Th1 cell attracting chemokines CXCL9 and CXCL10 and M2 secreting CCL17, CCL22 and CCL24. It has recently been demonstrated that *in vitro*, macrophages are capable of complete repolarization from M2 to M1, and can reverse their polarization depending on the chemokine environment(59).The change in polarization is rapid and involves rewiring of signaling networks at both the transcriptional and translational levels.

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6. Signaling molecules involved in M1/M2 polarization

A network of transcription factors and post-transcriptional regulators are involved in M1/M2 polarization(3).Interferon regulatory factor (IRF), signal transducers and activators of transcription (STAT) and suppressor of cytokine signaling (SOCS) proteins all play a role in skewing macrophage function towards either the M1 or M2 phenotype. The IRF/STAT pathways, activated by IFNs and toll-like receptor (TLR) signaling, polarize macrophages to the M1 activation state via STAT1. On the other hand, IL-4 and IL-13 skew macrophages toward the M2 activation state via STAT 6(60).M1 macrophages have been shown to upregulate IRF5, which is critical for M1 polarization and the induction of IL-12, IL-23 and TNF(61),as well as Th1 and Th17 responses. The LPS/TLR4 pathway also plays a role in M1 polarization by activating STAT1-alpha/beta in a MyD88 independent fashion(62).A role for Bruton's tyrosine Kinase (Btk) has also been implicated in macrophage polarization in response to LPS stimulation. Absence of Btk was shown to skew macrophages towards an M2 phenotype,

indicating its critical role in M1 polarization(63).Other molecules implicated in the induction of the M1 phenotype state are the G-protein coupled receptor, P2Y(2)R, which plays a role in inducing NO via *NOS2*(64),SOCS3, which activates NF-κB/ PI-3 kinase pathways to produce NO(55),and the growth and differentiation factor Activin A, which promotes M1 markers and down-regulates IL-10(65).Arginase 1 production is a distinct hallmark of M2 macrophages and is transcribed by STAT6, which is downstream of IL-4/IL-13 receptor signaling. Krüppel-like factor 4 (KLF-4) coordinates with STAT6 to induce M2 genes such as *Arg-1*, *Mrc1*, *Fizz1* and *PPARγ*, and inhibit M1 genes such as *TNFalpha*, *Cox-2*, *CCL5 and NOS2* (Figure 2). This is mediated through sequestration of co-activators necessary for NF-κB activation(66)Accordingly, the NF-κB p50 subunit (as homodimers) has been shown to be essential for M2 polarization *in vitro* and *in vivo* (67).In addition, the nuclear receptor, peroxisome proliferator-activated receptor γ (PPARγ), has been shown to regulate genes involved in oxidative metabolism and activation of the M2 phenotype(68),(69).The hypoxia inducible factors HIF-1α and HIF-2α also play a role in regulating M1/M2 polarization with HIF-1α regulating *NOS2* expression and the M1 state and HIF-2α arginase 1 expression and the M2 state(70).Other signaling molecules shown to play a role specifically in M2 polarization include the cytokine IL-21, which mediates M2 polarization by decreasing *NOS2* expression and increasing STAT3 phosphorylation(71),IRF4 which negatively regulates TLR signaling in a MyD88 independent manner to drive M2 activation(72),and BMP-7 which induces M2 polarization *in vitro* via activation of the SMAD-PI3K-Akt-mTOR pathway (73).

7. Macrophages in metabolic homeostasis

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Mammalian metabolic organs, such as the liver, pancreas and adipose tissue, are composed of parenchymal and stromal cells, including macrophages, which function together to maintain metabolic homeostasis(74). By regulating this interaction, mammals are able to make marked adaptations to changes in their environment and in nutrient availability. For example, during bacterial infection, innate activation of macrophages results in secretion of pro-inflammatory cytokines, such as TNF-a, IL-6 and IL-1b, which collectively promote peripheral insulin resistance to decrease nutrient storage(75),(76). This metabolic adaptation is necessary for mounting an effective defence against bacterial and viral pathogens because nearly all activated immune cells preferentially use glycolysis to fuel their functions in host defence. However, this adaptive strategy of nutrient re-allocation becomes maladaptive in the setting of diet-induced obesity, a state the sections below, we provide a general framework for understanding the pleiotropic functions carried out by macrophages to maintain metabolic homeostasis. Although our current knowledge in this area is primarily derived from studies in obese insulin-resistant mice, it is likely that tissue-resident macrophages also participate in facilitating metabolic adaptations in healthy animals.

8. Macrophage and Immunity

Studies have shown that mouse macrophages obtained after intraperitonealstimulation with thioglycolate medium accumulated and secreted high levels of plasminogen activator in culture whereas macrophages obtained from unstimulatedmice did not (49),(77).Hibbs'4 reported that activated macrophages from mice with chronic toxoplasma infection are capable of destroying tumorigenic target cells in vitro. Blood monocytes can mature into tissue and exudative

macrophages. Volkman and Gowans, conducted radioautographicstudies and found evidence which suggested that blood monocytes accounted for many macrophages in local inflammatory sites. Gough et al(49),'demonstrated that pure preparations of blood lymphocytes did not transform into macrophages during culture in vitro, but when neutrophils wereadded in small aliquots, lymphocyte macrophage transformation occurred. This suggested that the lymphocyte to macrophage transformation occurred in vivo depending on the homeostatic control by neutrophils or their degradation products. Jones(53),produced evidence that neutrophils stimulate macrophage differentiation. This was demonstrated by mixing cultures with genetically similar blood leukocytes. Many macrophages were present at three days and numerous blast cells were seen at six to nine days. When neutrophils were removed before the mixed cultures were prepared, small numbers of macrophages were found. Jones'reported that the structure of macrophages varies with the length of time in culture.The phases in its culture were described as adherence, spreading and phagocytosis of debris, mitosis, and extended culture. The shape of peritoneal macrophages was observed during the process of settling on a glass surface using scanning electron microscopy. During prolonged culture the shape was flattened and approximatelycircular, elongated, or fully extended measuring 15 to 18 gm(78).Carr' stated that macrophages had to be able to move in order to carry out their biological functions, and that the process of extension onto glass was regarded as a form of movement. Once this was over, they pushed pseudopodia out in various directions and pulled them back again. It was reported that actual change in position was scanty or absent and the rate of movement of macrophages in culture de-pended on the origin and cultural technique but did not give an indication of macrophage movement in the body. ' Chemotaxis is a reaction whereby the direction of locomotion (of cells) is determined by chemical substances in the environment. It is often described as being positive or negative according to whether the movement is towards or away from the substance in question. Keller and Storkin(79),showed that the chemotactic effect of many substances, including antigen-antibody complexes, endotoxins, and bacteria, were due to the formation of mediators which act directly on cells (cytotaxins). Many substances react with normal serum to release cytotaxins. Distinct specific cytotaxins exist for polymorphs and mononuclear cells. It was demonstrated that factors in normal rabbit serum caused directional migration of both neutrophils and mononuclear cells. ' The movement of neutrophils was increased by the addition of antigen-antibody complexes to the serum, while that of monocytes was reduced(80).George and Vaughan2' showed that migration of macrophages was inhibited both in tuberculin hypersensitivity and hyper-sensitivity induced by immunization with a protein in Freund's adjuvant(81),(82).Inhibition of migration was specific and due to an effect exerted by a relatively small number of sensitized cells. Bennett'8 stated that it was likely that the sensitized cells were lymphocytes and that they released a substance, probably a protein, into the medium; this inhibited the migration of macrophages. There was evidence that a migration inhibition factor might be produced by polymorphs(80).Boyden and Sorkin(83),were the first to coin the term "cytophilic" to designate affinity for cells. These authors described cytophilic antibodies formed from rabbits in response to human serum albumin. In the Free State these antibodies had an affinity for certain cells in a mixed population of cells from the spleen of normal rabbits. It was also reported that the sites on antibodies responsible for their affinity for cells were independent of the sites which bound antigen.(83).Boyden(84),reported that phagocytes that dispose of foreign material must have a recognition mechanism for distinguishing between these materials and the viable cells of itself. In addition, humoral recognition factors (antibodies) fulfilled this role, either by coating the particle to be phagocytized or by first attaching to the phagocyte. It was shown that humoral

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antibodies of certain classes could be absorbed by membranes of macrophages before combining specifically with antigen. Thus, the macrophages probably contained surface specific recognition factors in the form of cytophilic antibodies which promoted the adherence and phagocytosis of antigen bearing particles. Benacerraf(85),postulated that under proper conditions all cytophilic antibodies might act as opsonins to promote phagocytosis. Boyden(86),produced antibodies cytophilic for macrophages by administering sheep red blood cells (SRBCs) in Freund's complete adjuvant to guinea pigs. The resulting antibodies combined with receptors on macrophages and were then able to bind SRBCs to give the appearance of a rosette, this binding was considered a prerequisite to efficient phagocytosis.Carr1 reported that macrophage cytophilic antibodies vary in characteristics depending on factors such as species of origin and the route and schedule of antigen administration and that within a species, macrophage cytophilic receptors for IgG and IgM antibodies might differ.Nelson and Mildenhall(87),used SRBCs in Freund's complete adjuvant to produce both delayed hypersensitivity and cytophilic antibody in guinea pigs. When the antigen emulsion was administered intraperitoneally, intradermally, or into the foot pads, high titers of macrophage cytophilic antibodies were obtained in two weeks; the intradermally injected animals also exhibited pronounced delayed reactions. Carr' reported that macrophages engulfed a volume greater than their starting volume and as this occurred, the cytoplasm of the macrophage became more voluminous, thus increasing their size and capacity. The process of phagocytosis was described as an energyconsuming process and part of consumed energy was utilized in the manufacturing of phospholipid which was necessary for making new cell surface membranes and phagocytic vacuoles. Carr1 also reported that phagocytosis might involve ingestion, digestion, sequestration, and ejection. These processes were outlined as follows: phagocytosis first involved contact of the cell's plasma membrane with the material to be ingested. The material attached to the macrophage surface and was brought in.This material first lay within an indented plasma membrane and the membrane continued to invaginate further, forming a deep recess at the cell surface. The material became continuous with the plasma membrane by a narrow neck and then detached from the inside surface of the cell membrane, becoming freein the cytoplasm as a phagocytic vacuole or phagosome which moved centrally. Lysosomes containing hydrolytic enzymes moved to the phagosome and the lysosomal and phagocytic membranes fused and disintegrated causing the phagocytic vacuole and lysosome to coalesce and form a heterolysosome. As a result, the lysosomal enzymes were mixed with the engulfed material and were set to digest it.' Digested material was eventually reduced to dense bodies of varied composition. These bodies fused into large irregular masses and placed in a peripheral part of the cell to be pinched off and thus separated from the cell. Macrophages further digested their phagocytic load and tended to isolate it in the cytoplasm. This was termed sequestration apparatus. The cell might then eject this material. In addition to the presence of hydrolytic enzymes in lysosomes, Weiss2" stated that macrophages had other enzymes also found in lysosomes and among these were acid phosphotase, cathepsin, /3-glucoronidase, acid ribonuclease, acid deoxyribonuclease, aryl sulphatase, lipase, and as mentioned previously, lysozymes. Some hydrolytic enzymes were found to appear through the cytoplasm, were not membrane bound, and included nonspecific esterase and lipase (80).Weiss2" reported that enzymes were not necessarily confined within the vital macrophage because the fluid surrounding the macrophages was enzyme rich and that the macrophage itself secreted enzymes. Additional enzymes associated with macrophages included cholesterolesterase utilized for breaking down cholesterol and that the macrophages manufacture phospholipids in relationship to cholesterol breakdown and the synthesis of new membranes(80).

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9. Conclusion

Macrophages are involved in almost every disease and represent attractive therapeutic targets because their function can be augmented or inhibited to alter disease outcome. it is now understood that they have diverse origins, transcriptional complexity and lability, and are capable of phenotypic switching in accordance with homeostatic demands and in response to insult. However, for these therapies to be effective it is necessary to understand macrophage diversity and define their phenotypes according to anatomical location and function, and according to the regulation of the particular set-points that define the recognizable macrophages, such as microglia, osteoclasts and Kuppffer cells. Their different origins may in fact provide unique opportunities to target the recruited monocytes and macrophages selectively in the context of the chronic diseases, thereby inhibiting the pathology without disturbing resident macrophages and thereby maintaining normal homeostasis.The field of genomic analysis is advancing rapidly and will provide unique insights and novel methods to define macrophage types. Therapeutic targeting of macrophages is now in progress. Most of the therapies are targeted at pan-macrophage markers such as CSF1R. In the case of CSF1R reagents, including small molecules and monoclonal antibodies that inhibit the ligand, ligand binding or tyrosine kinase activity of the receptor are at various stages of clinical trials for the treatment of cancer.

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