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# Macrophage Activation

## Biology and Disease

*Edited by Khalid Hussain Bhat*

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# Meet the editor

Dr Khalid Hussain Bhat did his masters in biochemistry with a distinction from the University of Kashmir, India for which he received a university gold medal. After qualifying for a national fellowship for a research program, he moved to the Centre for DNA Fingerprinting and Diagnostics, Hyderabad, a premier basic research institute in India. Here he pursued his PhD in molecular immunology under the mentorship of Dr Sangita Mukhopadhyay. After completion of dissertation, he joined Prof. Amy L. Kenter at University of Illinois at Chicago, USA to investigate the mechanism of antibody repertoire generation. Dr Bhat has recently started his own laboratory at Sher-e-Kashmir University of Agricultural Sciences and Technology at Kashmir in India. Besides teaching undergraduate and graduate students, his research interests have expanded to plant microbes.

# Contents

# Preface

Macrophages are an essential player of the immune system and have been extensively studied and written about. Many aspects of macrophage biology have been discussed in recent works; however, the field is evolving at such a rapid pace that keeping up with the latest information is a challenging task. The current book is therefore intended to provide the reader with an account of the latest knowledge and developments in the field of macrophage biology. Ever since their discovery by lya Ilyich Mechnikov in 1882, these cells have intrigued the scientific community and provided fascinating insights in understanding general immunobiology as well as presented efficient therapeutic targets to translational research scientists. Macrophages represent an efficient homeostatic and protective system that evolved early in the evolution process. As a first line of defence they not only provide protection from pathogens but also play a role in the development and regulation of the immune system. They process and present the antigenic determinants to adaptive immune system to initiate a long-lasting memory against the encountered insult. In addition to this, macrophages possess a memory of their own which, though short lived, is included in their genetic makeup.

Macrophages are heterogenous in their function as well as phenotypes. Although they are endowed with a repertoire of receptors and efficient metabolic machinery, in response to environmental cues, macrophages adjust their expression patterns to efficiently clear the pathogenic microbes and dead materials as well as remodel the tissues to initiate the regeneration process. The phenomenon has been termed macrophage plasticity and is discussed in a few chapters in different contexts. Macrophage plasticity has been associated with many pathological conditions and is therefore an attractive target for drug development and therapeutic interventions. Macrophages play a critical role in development and dissemination of cancer. On one hand, they provide a suitable environment for angiogenesis and development of tumours and on the other hand they help in the dissemination of the cancerous cells to healthy parts of the body. Given our current knowledge about the macrophages associated with various pathological conditions like tumours, infectious diseases, autoimmune and metabolic disorders, we can develop efficient therapeutic interventions targeting macrophage activation and polarization. In addition to conventional pharmacological approaches, many traditional approaches like Chinese medicine are being used to modulate the macrophage plasticity for a favourable outcome. Some remarkably promising results have been reported, few of which have been discussed in this book.

This book covers a broad array of topics from reputed biologists from different parts of the World and from varied fields of research. Emphasis has been given to the current developments in the field and an in-depth analysis has been provided for each topic discussed. The scope of the topics covered encompass the basic as well as applied areas of macrophage research.

I am very thankful to all the authors who have done a commendable job in bringing this book to existence. I also thank the staff at IntechOpen without whose cooperation the publication would have been a daunting task.

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Chapter 1

# Macrophages: The Potent Immunoregulatory Innate Immune Cells

*Vijay Kumar*

## Abstract

Macrophages are ubiquitously present innate immune cells in humans and animals belonging to both invertebrates and vertebrates. These cells were first recognized by Elia Metchnikoff in 1882 in the larvae of starfish upon insertion of thorns of tangerine tree and later in *Daphnia magna* or common water flea infected with fungal spores as cells responsible for the process of phagocytosis of foreign particles. Elia Metchnikoff received the Noble prize (Physiology and Medicine) for his discovery and describing the process of phagocytosis in 1908. More than 130 years have passed and different subtypes and roles of macrophages as innate immune cells have been established by the researchers. In addition to their immunoregulatory role in immune homeostasis and pathogenic infection, they also play a crucial role in the pathogenesis of sterile inflammatory conditions including autoimmunity, obesity, and cancer. The present chapter describes the immunoregulatory role of macrophages in the homeostasis and inflammatory diseases varying from autoimmunity to metabolic diseases including obesity.

**Keywords:** macrophages, monocytes, innate immunity, inflammation, cytokines, pathogens

## 1. Introduction

The innate immune system evolved to protect the host from invading foreign pathogens, allergens, and different xenobiotics. The system comprises of both its cellular and humoral (circulating complement proteins, defensins, certain cytokines and chemokines secreted by innate immune cells) components. The innate immune cells comprise of epithelial cells, endothelial cells (ECs), the granulocytes (i.e. neutrophils, basophils, eosinophils, and mast cells (MCs), monocytes, macrophages, natural killer (NK) cells, dendritic cells (DCs), invariant NKT cells (iNKT cells), γδT cells, and newly described innate immune T cells called mucosal invariant T cells (MAIT) cells and innate lymphoid cells (ILCs) [1–9] (**Figure 1**). These innate immune cells are crucial to maintain the immune homeostasis and regulate adaptive immune system via acting as antigen presenting cells (APCs) along with providing other signaling molecules/factors required in the effective adaptive immune response in response to infection or other sterile chronic inflammatory diseases including, allergy, autoimmunity, cancer, and metabolic diseases including type 1 diabetes mellitus (T1DM), and obesity etc.

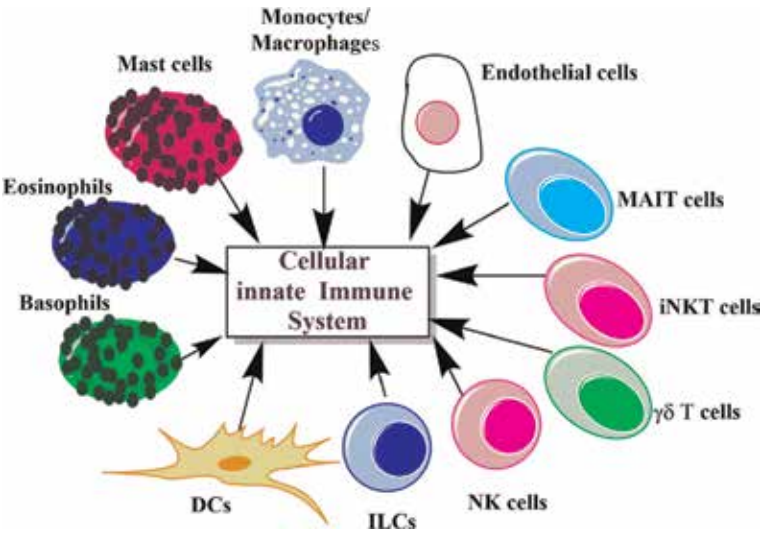


**Figure 1.**
*Schematic representation of cellular components of innate immune system. Macrophages also comprise a very important component of innate immune system along with other innate immune cells mentioned in the figure and text.*

Macrophages are type of innate immune cells that were first described by Elia Metchnikoff in 1882 in larvae of starfish upon the insertion of thorns of tangerine tree and later on in *Daphnia magna* or common water flea infected with fungal spores as cells responsible for the process of phagocytosis of foreign particles. Elia Metchnikoff received the Noble prize (Physiology and Medicine) for this discovery in the year 1908. Thus macrophages are first innate immune cells described almost 130 years ago. The continuous development in the field of immunology has established their role in various immunological and non-immunological processes including embryonic development. Along with acting as potential phagocytic cells involved in the phagocytosis of pathogens, xenobiotics, these cells also secrete various cytokines, chemokines, and growth factors including TNF-α, TGF-β, platelet-derived growth factor (PDGF), endothelial growth factor (EGF), and vascular endothelial growth factor (VEGF) [10–12]. Thus macrophages are very potent innate immune cells with diverse functions. The present chapter is intended to describe the immunoregulatory role of macrophages in the maintenance of immune homeostasis in the normal and disease stage.

## 2. Development of macrophages

Macrophages are the cells of the mononuclear phagocyte system (MPS) that was previously considered as reticuloendothelial system (RES), a system associated with the clearance and phagocytosis of dead cells [13]. They were included in the RES in 1924 to show their origin, residency, and renewal within RES. The RES was renamed to the MPS system in 1968 by Ralf van Furth, Zanvil Cohn and colleagues to distinguish them from polymorphonuclear leukocytes (PMNLs) or neutrophils and to show that all macrophages originate via terminal differentiation blood monocytes into different macrophages including pulmonary macrophages, liver macrophages (or Kupffer cells), and peritoneal macrophages etc. [14, 15]. The MPS comprises of monocytes, macrophages, and DCs involved in the maintenance of tissue and organismal homeostasis, the pathogenesis of inflammation, cancer,

autoimmune diseases, infection and the generation of immune response associated with the organ transplantation [16, 17].

Macrophages are developed during very early phase of embryogenesis called primitive hematopoiesis occurring at embryonic day 6.5 [E6.5]-E8.5 from precursor cells present in the extraembryonic yolk sac [18, 19]. The process of hematopoiesis in line with ontogeny progresses towards fetal liver at the beginning of E10.5 and finally to the bone marrow in the adult animal including humans [18, 19] (**Figure 2**). The primitive hematopoiesis occurring in the yolk sac of human embryos comprises of about 70% macrophages of the total nucleated blood cells at 4 weeks of gestational age, while in mice embryos macrophages predominate in the early stage of primitive hematopoiesis taking place in the yolk sac with the absence

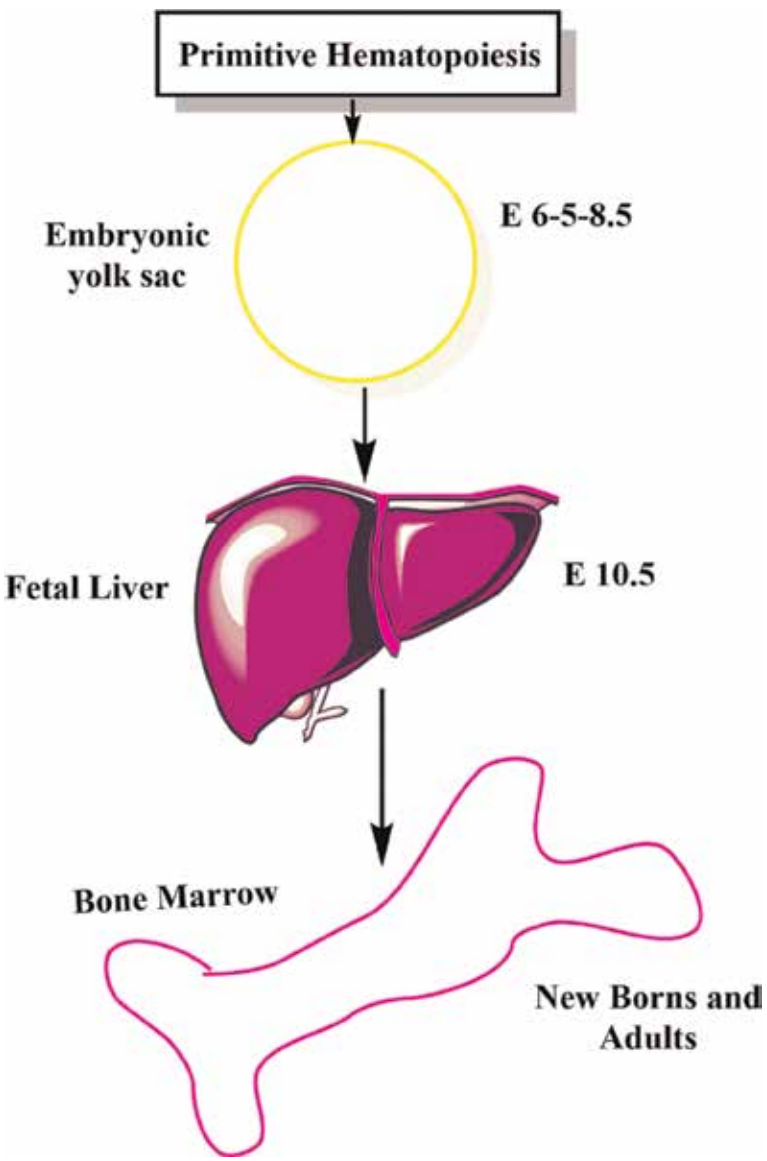


**Figure 2.**
*Schematic representation of production of macrophages in various organs throughout the mammalian (mouse and human) development. For example, at embryonic day 6.5 [E6.5]-E8.5 macrophages develop in the extraembryonic yolk sac from precursor cells, thereafter at E10.5 they develop in fetal liver, and in neonates and adults they develop in bone marrow as mentioned in the text.*

of monocytic cells [20, 21]. From embryonic day 8.5 [E8.5]-E10.5, the aorta-gonad-mesonephros give rise to hematopoietic stem cells (HSCs) giving birth to all immune lineages [19]. The cells committed to become macrophages within the mononuclear phagocyte lineage pass through morphologically-different but defined developmental stages including common myeloid progenitors (CMPs), shared with granulocytes giving rise to monoblasts, promonocytes and then monocytes that migrate to different tissues [22]. The differentiation of HSCs or hematopoietic progenitors (HPs) into different cell lineages including CMPs is governed by the activation of highly regulated gene expression programs integrated by different lineage-determining transcription factors (TFs) [23–25].

*Pu.1* serves as an essential factor to reconstitute the myeloid cell lineage and for the development of macrophages and monocytes in concentration-dependent manner [24, 26, 27]. A high concentration of the TF called PU.1 promotes the macrophage development whereas a low level of PU.1 supports the B cell development due to the presence of many low- and high-affinity PU.1 binding sites in the genome [28, 29]. PU.1 is regulated by Runt-related transcription factor 1 (RUNX1) or Acute myeloid leukemia 1 protein (AML1) or Core-binding factor subunit-alpha 2 (CBFA2) that are members of core-binding factor family of TFs [30]. The gene *Csf1r* encoding the receptor for the cytokine IL-34 and monocyte-colony stimulating factor (MCSF) is one of the major targets of PU.1 in macrophage development [31, 32] *Cebp-α*, *-β*, and *-ε* are important towards the development of different myeloid cell types primarily including granulocytes, macrophages, and monocytes [33, 34]. *Irf8* also serves as a crucial TF for monocyte lineage along with DC lineage by establishing monocyte- and DC-specific enhancers [35–38]. The TF called ZEB2 is essential for the maintenance of tissue-specific macrophages and its loss causes tissue-specific changes in different macrophage populations including KCs and their subsequent loss [39]. Thus these lineage-determining TFs, establish the central macrophage program during the pre-macrophage stage. This core macrophage program includes the expression of CX3CR1, pattern-recognition receptors (PRRs), phagocytic receptors (PRs), FcγRs including FcγR1 or CD64 and various other genes including *Sirpα*, *Iba1*, *Mertk* and *Adgre1* (F4/80) expressed by almost all types of macrophages [40, 41]. A bZip TF called MAFB (c-Maf) regulates the self-renewal of macrophages and its induction is a specific and crucial determinant of monocytic program in hematopoietic cells [42, 43].

There are two principal subtypes of monocytes in mice (**Figure 3**): (1) classical Ly6c$^{hi}$ monocytes (also called inflammatory monocytes expressing high levels of CC-chemokine receptor 2 (CCR2) but low levels of CX3C-chemokine receptor 1 (CX3CR1)) that descend directly from Ly6c$^{+}$ monocyte progenitors [44], and (2) Ly6c$^{low}$ non-classical monocytes expressing high levels of CX3CR1 and low levels of CCR2 that differentiate from Ly6c$^{hi}$ monocytes through an Nr4a1 (nuclear receptor subfamily 4 group A member 1 or Nur77)-dependent transcriptional program and are less prevalent in blood [44–47]. The Ly6c$^{hi}$ monocytes in mice represent approximately 2–5% population of the circulating white blood cells (WBCs) in a normal laboratory mouse without any infection and rapidly migrate towards the site of infection and inflammation [48]. However the deficiency of CCR2 significantly reduces the migration of Ly6c$^{hi}$ monocytes at the site of infection and inflammation indicating the importance of CCR2 in the trafficking of these monocytes [49–51]. These Ly6c$^{low}$ non-classical monocytes develop primarily to function within the vasculature and patrol the vasculature by crawling over the resting endothelium in an Lymphocyte function-associated antigen 1 (LFA-1) integrin and CXCR3-dependent manner [19, 52].

The non-classical monocytes patrol the vasculature to clear the damaged endothelial cells (ECs) for maintaining the integrity of endothelium, and thus the

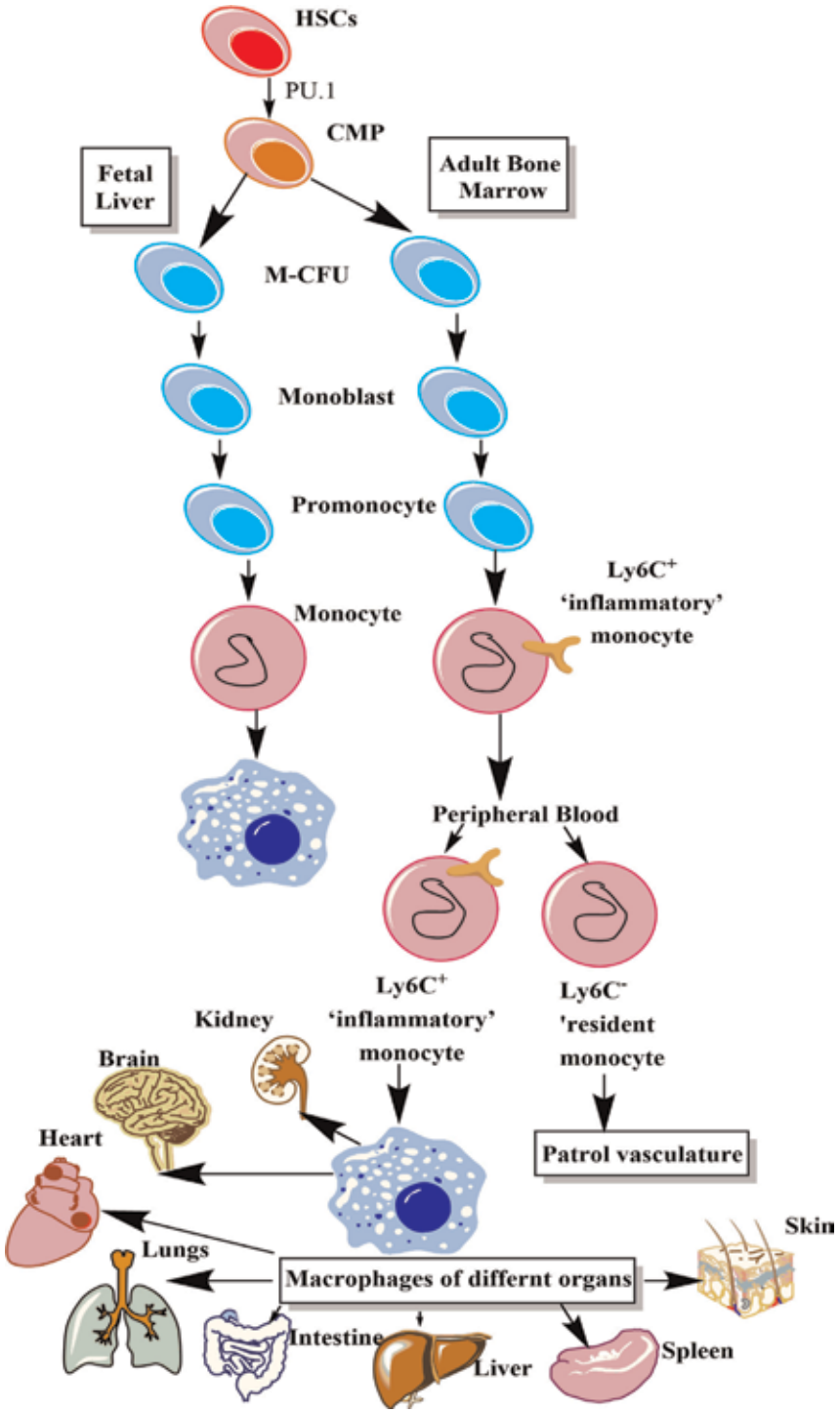


**Figure 3.**
*Schematic representation of developmental stages of macrophages. HSCs, in the presence of TFs including PU.1 develop into CMP that further differentiates into promonocytes by undergoing different developmental stages. The promonocytes in fetal liver develop into monocytes that further differentiate into macrophages. Whereas in bone marrow promonocytes develop into Ly6C$^+$ inflammatory monocytes also called classical monocytes. However, in peripheral blood circulation they are further differentiated into Ly6C$^+$ inflammatory monocytes and Ly6C$^-$ resident monocytes or non-classical monocytes residing in the blood and patrolling the vasculature. On the other hand Ly6C$^+$ inflammatory monocytes or classical monocytes migrate to different organs and develop into different tissue/organ specific macrophages as described in the figure.*

vasculature during homeostasis and inflammatory conditions [53, 54]. Thus, these Nr4a1-dependent non-classical monocytes serve as housekeepers for the endothelial vasculature and orchestrate the necrosis by neutrophils due to damaged ECs inducing the TLR7 signaling via *in situ* phagocytosis of cell debris derived from damaged ECs [53]. Hence these non-classical monocytes play a crucial role in the pathogenesis of various diseases associated with vasculature along with the process of wound healing and the resolution of the inflammation [54]. This patrolling nature of the monocytes distinguishes them from macrophages as macrophages have a very limited capacity to emigrate from their site of location. In humans monocytes are differentiated into two subsets on the basis of expression of surface expression of CD14 and CD16 [55]. In humans the $CD14^{++}CD16^{-}$ monocytes are known as classical monocytes and are most prevalent monocyte subset in the blood [56, 57]. Like mice $Ly6c^{hi}$ monocytes they also express CCR2 [58]. The $CD14^{+}CD16^{+}$ monocytes are considered as intermediate monocytes and $CD14^{low}CD16^{+}$ monocytes are called non-classical monocytes in humans [56].

The $CD14^{low}CD16^{+}$ monocytes in humans are similar to mice $Ly6c^{low}$ monocytes and patrol the vasculature or endothelium along with sensing the nucleic acids and virus via TLR7 and TLR8 receptors [59]. These monocytes have weak phagocytic potential and do not produce ROS and cytokines in response to cell-surface TLRs. However they produce TNF-α, IL-1β, and CCL3 in response to viruses and immune complexes containing nucleic acids due to the activation of TLR7 and TLR8 signaling pathways [59]. Thus it can be inference that mice and human monocytes do not precisely overlap in terms of their receptor expression including PPAR-γ (peroxisome proliferator-activated receptor-γ) that is signature for mouse monocytes but absent in humans, however, the process of their differentiation and the function in immune defense is apparently similar [60–62]. For example, approximately 270 genes in humans and 550 genes in mice monocytes (both types including classical or non-classical one) are expressed differentially and more than 130 of these gene expressions are conserved between mouse and human monocyte subsets [62]. Thus this difference between human and mouse monocytes should be kept in mind when developing and studying human diseases in mice.

The development of mononuclear phagocytes from monocyte/macrophage progenitor cells is directed by colony stimulating factors (CSFs) including M-CSF, granulocyte-monocyte colony-stimulating factor (GM-CSF), and fms-like tyrosine kinase 3 ligand (Flt3-ligand) [63–65]. The number of various tissue and organ monocytes/macrophages are regulated by M-CSF without any alteration in their activation stage [64]. However, GM-CSF is involved in the activation of both monocytes and macrophages along with its participation in the differentiation into DCs. The mature cells developed during fetal development and later in life are distributed accordingly as sinus-lining and interstitial resident macrophages in lymphohematopoietic and other organs including lungs, liver, spleen, gut, skin and brain. Major tissue-resident macrophages, including liver KCs, lung alveolar, splenic, and peritoneal macrophages, are established prior to birth and their maintenance starts subsequently by themselves independent of replenishment of blood monocytes during adulthood [47]. The macrophages present in endocrine and reproductive organs including testes, adipose, vascular, musculoskeletal and connective tissues are less well characterized.

## 3. Macrophage polarization

The polarization of macrophages gives a diverse heterogenic function and phenotypes to them depending on their activation in respect to their duration of

stimulation and spatial localization [66]. The macrophage polarization is not a fixed process due the plasticity of the macrophages to integrate multiple signals (different pathogens and their PAMPs, DMAPs, and normal tissue environment). Thus macrophage polarization occurs in response to cell-cell interaction and cell-molecule interaction within the host tissues or organs to maintain the homeostasis or during different pathological conditions [67, 68]. Thus macrophage polarization is regulated by at least three different mechanisms: (1) epigenetic and cell survival mechanisms, (2) external stimuli (pathogens, PAMPs, and allergens), and (3) tissue environment including DAMPs [66]. The inflammation and associated immune response is a good pathogenic condition to study the macrophage polarization as this process impacts the inflammation from its initiation to the resolution phase. The details of macrophage polarization are discussed elsewhere [66, 67, 69].

Depending on their polarization status the macrophages can be categorized in to M0, M1 (classically activated macrophages (CAMs) or pro-inflammatory), and M2 (alternatively activated macrophages (AAMs) or anti-inflammatory) macrophages (**Figure 4**). M0 macrophages can be considered as naïve macrophages that have not been exposed to any pro- or anti-inflammatory stimuli or environment. M1 or CAMs are developed when M0 macrophages are exposed to bacterial moieties including LPS and Th1 cytokines including IFN-γ, IL-2, IL-12, IL-18 and TNF-β (lymphotoxin β (LT-β)) etc., whereas M2 or AAMs are developed upon exposure to Th2 cytokines including IL-4, IL-5, IL-6, and IL-10 [70, 71]. The M2 macrophages can further be divided into M2a, M2b, and M2c depending on their stimulus for the activation. The M2 macrophages induced by IL-4 or IL-13 are called M2a (a stands for alternative), M2b macrophages are induced by poly I:C or TLR or IL-1R agonists, and M2c are induced by IL-10 and glucocorticoids [72]. M2 macrophages exhibit a higher phagocytic activity, higher expression of scavenging, mannose and galactose receptors, produce higher concentration of ornithine and polyamines due to high arginase pathway, secrete high amount of IL-10 and express higher levels of the IL-1 decoy receptor and IL-1RA [40]. Thus, M2 macrophages in general exert an anti-inflammatory action and play a crucial role in anti-parasitic immune response required for parasite clearance, promote tissue remodeling, vasculogenesis, tumor progression [70, 72, 73]. The M1 macrophages express Th1 cell-attracting chemokines including CCL5 or regulated upon activation, normal T cells expressed, and secreted (RANTES), CXCL9 and CXCL10, whereas M2 macrophages express the chemokines CCL17, CCL22 and CCL24 [74].

The M1 macrophages highly express cyclo-oxygenase 2 (COX 2) enzyme, inducible nitric oxide synthase (iNOS or NOS2) involved in nitric oxide ($NO^{\cdot}$) synthesis, whereas M2 macrophages express COX 1 and arginase is expressed in M2a and M2c required to synthesize ornithine and polyamines but not in M2b macrophages activated by Poly I:C and LPS [72, 74, 75]. The metabolic process of macrophages governing their pro-inflammatory and anti-inflammatory action also differs in M1 and M2 macrophages. M1 macrophages exhibit a shift from normal oxidative phosphorylation (OXPHOS) to increased glycolysis, increased release of lactate, a decreased oxygen consumption and glutaminolysis. On the other hand M2 macrophages are dependent on fatty acid oxidation (FAO) as a major source of energy along with the mitochondrial OXPHOS. The detailed description of macrophage (both M1 and M2) immunometabolism is beyond the scope of the chapter and described elsewhere [76, 77]. Succinate (a signaling metabolite) regulates the macrophage polarization via succinate receptor 1 (SUCNR1) and regulates the process of inflammation [78]. The myeloid-specific deficiency of SUCNR1 promotes a local pro-inflammatory or M1 phenotype among macrophages, disrupts glucose homeostasis in mice, exacerbates the metabolic effects of diet-induced obesity and impairs the browning of the adipose-tissue under cold conditions [78]. On the other

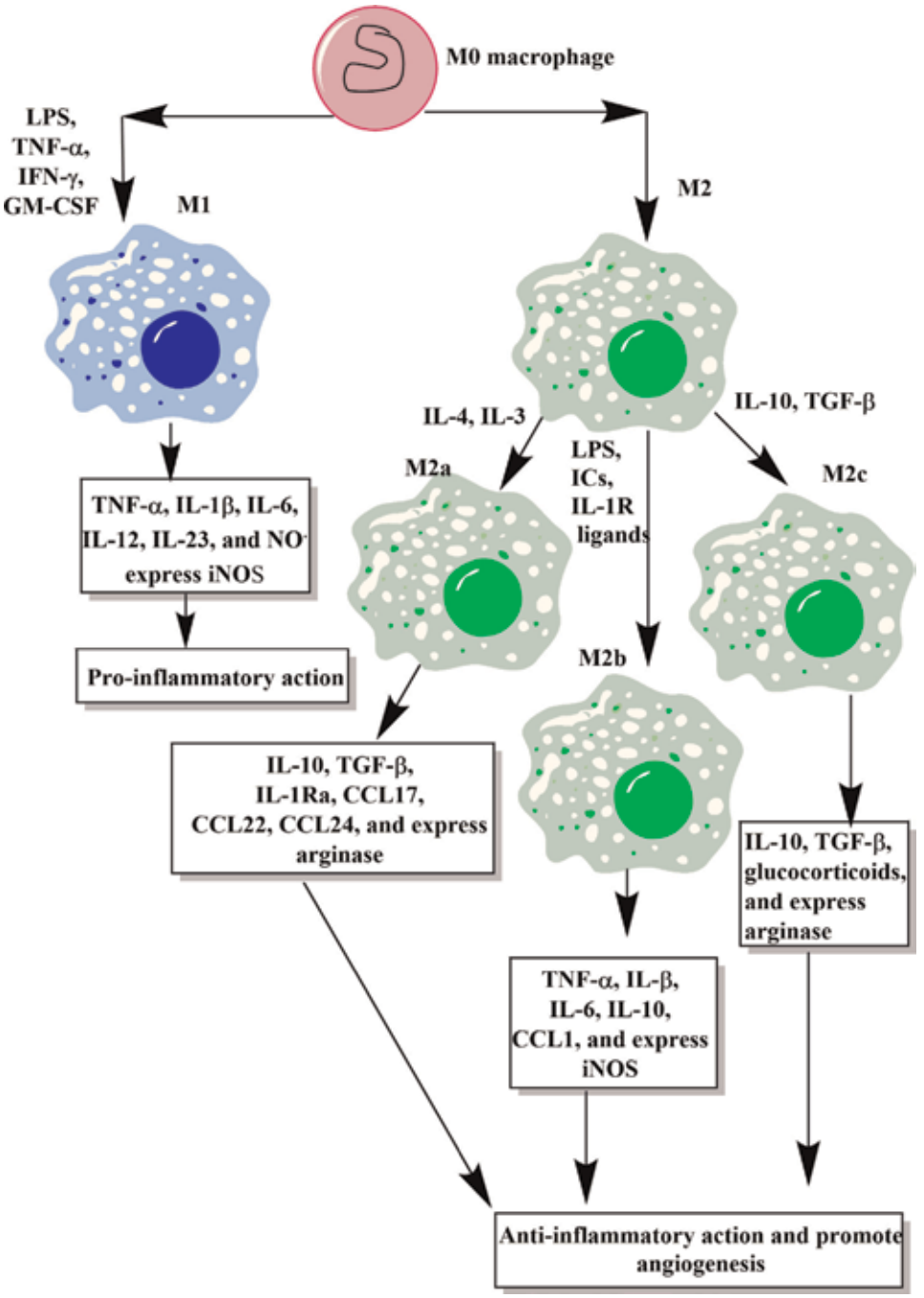


**Figure 4.**
*Schematic representation of macrophage polarization. Naïve or M0 macrophages upon different stimulation as describe in the figure and the text differentiate into pro-inflammatory M1 macrophages or classically activate macrophages (CAMs) and anti-inflammatory macrophages called alternatively activated macrophages (AAMs) or M2 macrophages. These M2 macrophages are further differentiated into M2a, M2b, and M2c macrophages depending on the stimulus as mentioned in the figure and the text.*

hand SUCNR1 via succinate binding stimulates the anti-inflammatory (M2) phenotype among macrophages as indicated by the release of type 2 or anti-inflammatory cytokines including IL-4. Thus succinate exerts the anti-inflammatory action via SUCNR1 on macrophages via controlling their polarization [78]. The macrophages

involved in the resolution of inflammation are called resolution-phase macrophages (rMs). The rMs differ from both M1 and M2 macrophages in terms that they have weak bactericidal properties and express an alternatively activated phenotype along with higher expression of markers of M1 macrophages (i.e. inducible cyclooxygenase (COX 2) and nitric oxide synthase (iNOS)) [79]. This phenotype of rMs is controlled by cyclic adenosine monophosphate (cAMP) as its inhibition converts rMs into M1 macrophages [79]. On the other hand the upregulation of cAMP in M1 macrophages converts them in rMs. Although rMs are nonessential to clear neutrophils during self-limiting inflammation but are required for the initiation of post resolution lymphocyte repopulation signaling event via COX 2 lipids. Thus, rMs are the hybrid of both M1 and M2 macrophages and play an important role in the post resolution innate-lymphocyte repopulation and the restoration of tissue/organ homeostasis. **Table 1** is showing the major differences between M1 and M2 macrophages. The detailed mechanism of macrophage polarization (M1 and M2), its

| | **M1 macrophages** | **M2 macrophages** |
|---|---|---|
| 1. Phenotype | Express high levels of MHC-II, CD68, and CD80 and CD86 costimulatory molecules | Express higher levels of CD206, CD200R, CD163 and transcription factor called CMAF (musculoaponeurotic fibrosarcoma) and response gene to complement 32 (RGC-32) |
| 2. Upregulated genes | Suppressor of cytokine signaling 3 (SOCS3), iNOS or NOS2, M*acrophage* receptor with collagenous structure (Marco), Il12B, Il23a (Il23p19) and Ptgs2 (Cox2) | Arg1, MMR (Mrc1), resistin-like molecule α (FIZZ1) or Relma or Retnla, Ym1, Irf4, Cxcl12, Cxcl13, Ccl24 and Klf4 |
| 3. Action | Pro-inflammatory | Anti-inflammatory |
| 4. Cytokines and chemokines produced | IFN-γ, IL-8, TNF-α, IL-1β, RANTES (CCL5), CXCL10 | IL-13, IL-10, CCL17, CCL18, CCL22 |
| 5. Metabolic pathway | Glycolysis and glutaminolysis | FAO and OXPHOS |
| 6. HIF-1α expression | High | Low |
| 7. Inducers or stimuli | IFN-γ, PAMPs (i.e. LPS), GM-CSF | Glucocorticoids, IL-10, IL-4, IL-13 and M-CSF |
| 8. ROS and RNS production | High ROS and $NO^{\cdot}$ production | Low ROS and $NO^{\cdot}$ production |
| 9. Rate of acidification | Low | High |
| 10. Antimicrobial action | High | Low |
| 11. Glucose uptake | Mainly depends on HIF-1α and Akt/mTORC1 activation | Mainly depends on Akt/mTORC1 activation |
| 12. Macrophage galactase-type C-type lectins | Low | High |
| 13. Autophagy | Induce autophagy during tuberculosis (TB) infection | Decrease autophagy during TB infection |

**Table 1.**
*Differences between M1 and M2 macrophages.*

regulation and impact on inflammatory process including in cancer are described somewhere else [66, 70–73, 75, 80, 81].

## 4. Role of monocytes and macrophages in host defense

Macrophages are present in almost every tissue or organ system including the barriers system comprising of respiratory tract (pulmonary alveolar and interstitial macrophages), skin, gastrointestinal tract (GIT), and reproductive tract [82–91]. Thus their presence in the every organ system along with the mucosal sites serving as potential sites for the entry of pathogens, toxins, allergens and xenobiotics makes them first line of defense.

Monocytes/macrophages are one of the major innate immune cells involved in the process of recognition of pathogens and the cell debris originated as a result of apoptosis and their engulfment by the process of phagocytosis. Thus along with other innate immune cells including neutrophils, dendritic cells (DCs), mast cells, monocytes, and macrophages are considered as 'professional' phagocytes. The professional phagocytes are differentiated from non-professional phagocytes on the basis of their effectiveness in mediating the phagocytosis [92]. The major factor contributing to the effectiveness of the phagocytosis and characteristic of professional phagocytes is the expression of various receptors on their cell surface involved in the recognition of molecules or ligands that are not normally expressed by normal and healthy cells [93]. For example, scavenger receptors (SRs) play important role in the recognition and binding of apoptotic and necrotic cells, opsonized pathogens (i.e. pathogens opsonized by complement protein C5a and C3a), and cell debris. The scavenger receptor-A1 (SR-A1)-mediated phagocytosis of low density lipids (LDLs) or oxidized lipids causes the formation of foam cells and this phenomenon is involved in the pathogenesis of atherosclerosis [94]. The absence of SR-A1 in macrophages increase their pro-inflammatory action due to the increased p42/44 mitogen-activated protein kinase (MAPK) phosphorylation, interferon regulatory factor-3 (IRF-3) and NF-κB nuclear translocation and increased production and secretion of TNFα, IL-6 and IFN-β due to the increased activation of TLR4 signaling pathway [95]. Thus SR-A1 antagonizes the TLR4-mediated phagocytosis and pro-inflammatory immune response of macrophages in the presence of LPS and gram-negative bacteria in a competitive manner [95].

Additionally, alveolar macrophages expressing SR-A1 and class A scavenger receptors (SRAs) called macrophage receptor with collagenous structure (MARCO) protect the host from inhaled toxicant and pathogens by phagocytosing the oxidized lipids and decreasing the inflammatory damage [96]. The detailed information of scavenger receptors is beyond the scope of the chapter and is described elsewhere [97–101]. In addition, professional phagocytes including monocytes and macrophages express various Toll-like receptors (TLRs) [93]. However the interplay between phagocytic receptors (which initiate and assist in the mechanics of phagocytosis) and pattern recognition receptors (PRRs, such as TLRs, which detect PAMPs or DAMPs) is complex. The interplay between these receptors may involve both synergistic and antagonistic interactions, including downstream signaling mechanisms within the phagocytic cell that remain largely unknown [102, 103].

During and following phagocytosis, PRRs (including TLRs, C-type lectin receptors (CLRs), scavenger receptors, retinoic acid-inducible gene 1 (RIG1)-like helicase receptors (RLRs) and NOD-like receptors (NLRs)) recognize different PAMPs and DAMPs along with different xenobiotics including silica or asbestos [104, 105]. Some PRRs including mannose receptor, DC-specific ICAM3-grabbing non-integrin (DC-SIGN) and MARCO are also involved in the process of pathogen

recognition and phagocytosis, whereas signaling PRRs (which include the TLRs, NLRs and RLRs) sense microbial products and aberrant self-molecules on the cell surface or in the cytoplasm of cells and activate transcriptional mechanisms that lead to phagocytosis, cellular activation and the release of cytokines, chemokines and growth factors [106–109]. During phagocytosis of the pathogens, the TLR2 recruits into the phagosome and discriminates between pathogens along with initiating the pro-inflammatory immune response [110]. The TLR-induced phagocytosis of bacteria is reliant on MyD-88-dependent signaling via interleukin-1 receptor-associated kinase-4 (IRAK-4) and p38 MAP kinase causing an up-regulation of SRs [111]. TLR9 is the strongest inducer of phagocytosis among all the TLRs, whereas TLR3 is the weakest inducer of the process [111]. However, TLR4-stimulated phagocytosis also requires the activation of MyD-88-independent actin-Cdc42/Rac pathway [112, 113].

Macrophages also express various complement receptors (CRIg, C1qR, CR3, C5aR, C5L2 or C5bR, etc.) and Fc receptors on their cell surface that bind and phagocytose the opsonized pathogens or other molecules and activate the complement system (CS)-mediated immune response for increasing the process of phagocytosis [114–116]. CRIg is a member of complement receptor of the immunoglobulin superfamily that binds to complement fragments C3b and iC3b opsonizing the pathogens to initiate their phagocytosis [115]. The expression of CRIg on macrophages increases in the presence of dexamethasone and IL-10, but decreases in the presence of IFN-γ, IL-4, TGF-β1, arachidonic acid (AA) [117]. AA decreases the expression of CRIg on macrophages by activating the protein kinase C (PKC) independent of its metabolism via cyclooxygenase and lipoxygenase pathway [117]. The CR3-mediated phagocytosis of the pathogens is mediated by the activation of Syk-kinase that becomes tyrosine-phosphorylated and accumulates around the nascent phagosomes [114]. However, it also negatively regulates the phagocytosis of degenerated myelin sheath by activating Syk-kinase and cofilin (an actin-depolymerizing protein controlling F-actin remodeling) in microglia and macrophages [118]. C1q component of the CS plays a crucial role in the process of phagocytosis by triggering the rapid enhancement of the phagocytosis independent of its role in direct activation of the classical complement pathway [119]. The engulfment of the membrane attack complex (MAC) deposited on pathogens by the macrophages during the process of phagocytosis activates the NALP3 (NACHT, LRR and PYD domains-containing protein 3 or cryopyrin) inflammasome via inducing $K^+$ efflux and ROS generation [120]. The NALP3 activation activates caspase 1 (CASP1) to cause the maturation and release of IL-1β and IL-18 [120]. This also induces the differentiation of T cells into Th17 cells when these macrophages are used as antigen presenting cells (APCs). Thus, macrophages use various surface receptors and secreted molecules to monitor and respond to changes in the vicinity of their tissue environment.

## 5. Role of macrophages in homeostasis (angiogenesis, wound repair, and regeneration) and diverse inflammatory conditions (metabolic diseases and autoimmunity)

### 5.1 Macrophages in angiogenesis

Macrophages play a crucial role in the immune homeostasis via regulating the process of inflammation under both sterile and infectious inflammatory conditions. In addition to this they also play a crucial role in the process of angiogenesis (**Figure 5**), metabolism, and salt and water balance [121]. For example, myeloid

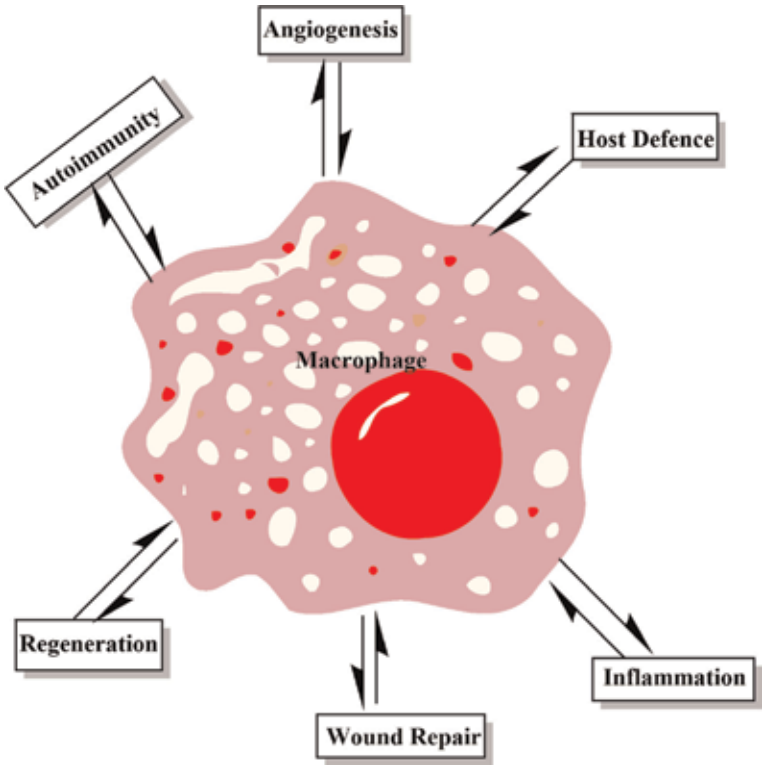


**Figure 5.**
*Macrophages play important role in host defense, immune homeostasis, regeneration, and inflammation. The detailed mechanisms of macrophages impact on the processes mentioned in the figure are described in the text.*

cells including monocytes and neutrophils are the first innate immune cells migrating through post capillary venules (PEVs) at the site of inflammation and tissue injury or tissues requiring microvascular growth and remodeling including several tumors due to the expression of CCR2 that binds to the chemokine called CCL2 [122, 123]. Furthermore an inhibition in the chemo-attraction and migration of monocytes at the site of tissue ischemia causes a flap necrosis due to impaired neovascularization [124]. Macrophages also synthesize, release, and respond (or reprogram themselves) to various pro- and anti-angiogenic factors including vascular endothelial growth factor-A (VEGF-A), and several angiopoietins including angiopoietin (ANG) 1 and ANG 2 [125–127]. Thus these recruited monocytes or tissue macrophages reprogram themselves in the presence of theses angiogenic factors to serve as angiogenic and arteriogenic professional cells (APCs) [125]. For example, ANG1 exerts its angiogenic action on macrophages via repressing the expression of prolyl hydroxylase domain protein 2 (PHD2) through angiopoietin (ANG)-TIE2 (angiopoietin-1 receptor or CD202B) signaling that supports their reprograming into angiogenic and APCs [127, 128]. ANG2-dependent TIE2-signaling in macrophages plays a crucial role in the induction of angiogenesis during inflammation and tumor growth as both condition are associated with increased hypoxia causing an induction of hypoxia inducible factors (HIFs) including HIF-1α and HIF-2α enhancing the generation of tumor and angiogenesis promoting molecules and cytokines (CXCR4, GLUT1 (glucose transporter 1), VEGF A, IL-1β, IL-8, adrenomedullin, and ANG 2) [129–131].

These angiogenesis supportive macrophages exhibit the similarity with M2 macrophages and in tumor environment they are called tumor-associated macrophages

(TAMs) with higher levels of IL-6, iNOS, and TIE2 [132]. These M2 macrophages and TAMs support the growth, proliferation, and migration of endothelial cells (ECs) and blood vessel formation or sprouting by releasing VEGF-A as well as promoting the synthesis and release of VEGF-A and fibroblast growth factor-2 (FGF-2) or basic-FGF (b-FGF) from the tissue or tumor microenvironment cells [133]. The TAM-mediated support of angiogenesis and tumor growth is determined by TIMP-1 (tissue-inhibitor of matrix metalloproteinase-1) levels free of or complexed with pro-MMP-9 (matrix metalloproteinase-9) [134]. For example, MMP-9 null macrophages are non-angiogenic. In addition to secreting the angiogenic factors, macrophages also interact with cells including pericytes, ECs, and vascular smooth muscle cells for regulating angiogenesis observed during embryonic development, adult responses to injury, and in tumor microenvironment [135]. Furthermore the depletion of macrophages disrupts the process of vascular patterning in response to insufficient vascular pruning due to decreased phagocytosis of endothelial cells and pericytes during both embryonic and postnatal development of organs [135–137].

### 5.2 Macrophages in wound repair

Macrophages also serve as crucial immune cells involved in the process of wound repair in response to stimuli generated in the local tissue milieu [138, 139]. The phenomenon of wound repair is mainly regulated by AAMs or M2 macrophages due to their anti-inflammatory action, induction of angiogenesis, and decreased apoptosis that induces the extracellular matrix remodeling and the process of wound repair and regeneration [138, 139]. These wound repair macrophages are characterized by the higher production of various growth factors including platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-α (TGF-α), TGF-β, and VEGF-A causing angiogenesis and supporting cell proliferation to alleviate the hypoxia caused by the inflammatory tissue insult [140]. The TGF-β stimulates the differentiation of the local and recruited tissue fibroblasts into myofibroblasts facilitating the contraction and closure of the wound area along with the synthesis of the extracellular matrix (ECM) components [141]. Additionally macrophages also release amphiregulin (AREG) that serves as an epidermal growth factor receptor ligand (EGFRL) to play a role in the restoration of tissue homeostasis after injury or wound healing [142, 143]. The wound healing or repair mechanism by AREG involves the release of TGF-β from latent complexes via integrin-$\alpha_V$ activation that induces the differentiation of mesenchymal stromal cells (pericytes) into myofibroblasts to restore the vascular barrier function within injured tissue during the process of wound healing [142].

These wound repair macrophages also augment the proliferation and expansion of many neighboring parenchymal and stromal cells along with activating stem cells and local progenitor cells to participate actively in tissue repair response during chronic or severe injury [144]. Hence, the disruption of monocyte recruitment and the inhibition of local macrophages and their conversion into M2 or AAMs may dampen the process of wound repair. For example, in some cases the disruption in the process of wound repair may lead to the development of tissue or organ fibrosis or scarring due to the overactivation of wound repair macrophages that can further impair organ's normal function causing ultimate organ failure and death of the patient [145, 146]. For example, idiopathic pulmonary fibrosis (IPF), hepatic fibrosis and systemic sclerosis, are tightly regulated by 'pro-fibrotic' macrophages producing PDGF, IGF-1, TGF-β1 (induces myofibroblast transdifferentiation and promotes matrix accumulation), and directly activating fibroblasts [93, 147–150]. These pro-fibrotic macrophages also secrete pro-inflammatory cytokines including

IL-1β that stimulates Th17 cells to secrete IL-17 involved in the bleomycin pulmonary fibrosis, MMPs and TIMPs that regulate the inflammatory cell recruitment and the ECM turnover [146, 151–154]. Hence macrophages are involved in the process of wound repair and the impairment in their function may lead to the poor wound healing and the development of fibrosis causing organ failure and the death of the patient. Therefore targeting of the pulmonary macrophages and their mediators play a crucial role in the process of pulmonary fibrosis [155].

### 5.3 Macrophages in regeneration

Macrophages also play a crucial role in the process of tissue and organ regeneration that refers to the process of proliferation of cells and tissues to replace the damaged and lost structures [156]. The organs and tissues including skeletal muscles and liver exhibit a higher degree of regenerative capacity through the regeneration of parenchymal cells involving monocytes and hepatocytes [157]. In most tissues the complete regeneration of intact tissues is not achieved and results in the formation of scar [158]. Macrophages play a very important role in the regeneration process of skeletal muscle by coordinating the inflammation and regeneration [157]. They act as essential immune cells for the recovery of tissue integrity and function following the injury [150]. The macrophages involved in the process of regeneration of skeletal muscle are located in the interstitial space between myofibers, specifically in the perimysium (the connective tissue surrounding muscle fascicles), epimysium, (the connective tissue surrounding the whole muscle), and perivascular space that recruit circulating neutrophils and monocytes following the muscle injury to initiate the process of inflammation [157]. The monocytes infiltrated into the damaged skeletal muscle undergo the process of *in situ* transition to develop into $Ly6C^{hi}$ (inflammatory) and $Ly6C^{low}$ (regenerative or repair) macrophages that is independent of NR4A1 (nuclear receptor subfamily 4 group A member 1) or NUR77 or nerve growth factor IB (NGFIB) [159]. The NUR77 belongs to the family of the *Nur* nuclear receptors acting as intracellular transcription factors and plays a crucial role in the macrophage-mediated inflammatory immune response generation [160]. The transition of monocytes into $Ly6C^{hi}$ (inflammatory) and $Ly6C^{low}$ (regenerative or repair) macrophages plays a crucial role in the process of muscle regeneration [161]. The $Ly6C^{low}$ macrophages in the skeletal muscle exhibit a distinct pro-resolving signature [specialized pro-resolving lipid mediators (SPMs), including resolvins (for example, RvD1, RvD2, RvE1)] that helps in the functional improvement in the process of muscle regeneration [162]. On the other hand $Ly6C^{hi}$ inflammatory monocytes further differentiate into skeletal tissue macrophages (both M1 and M2) and secrete pro-inflammatory cytokines (i.e. FN-γ, TNF-α, IL-1β, and IL-6) that are also integral component of myogenic precursor cells (MPCs) or myoblasts. The M2 macrophages on the other hand promote the differentiation and maturation of MPCs [157, 163, 164]. In addition macrophages are also shown to involve in the process of regeneration of heart/cardiomyocytes in different animals (Zebra fish, Salamander, and the laboratory mouse) [165–167]. Even studies have also shown the involvement of macrophages in the regeneration of spinal cord and tail fin of Zebra fish [168, 169]. Wnt signaling in macrophages plays a critical role in driving parenchymal regeneration in animal models of liver injury [170]. After the death of hepatocytes phagocytic uptake of the cell debris by macrophages synthesizes Wnt3a that in nearby hepatic progenitor cells (HPCs) induces the canonical Wnt signaling cascade facilitating their specification to hepatocytes [171]. Even the regeneration of hair follicles also involves the macrophage-mediated key signals to local stem cells facilitating the regeneration of hair follicles upon plucking of hairs [172]. The plucking of hairs causes the local generation of CCL2 that promotes

pro-inflammatory TNF-α generating macrophages and initiates the process of hair-follicle regeneration [172]. Thus the fine tuning of macrophages is essential for their protective function during would healing or repair, regeneration or the induction of fibrosis due to the loss of this fine tuning leading to the organ damage and failure.

### 5.4 Macrophages in autoinflammation and autoimmunity

The uncontrolled activation of macrophages in response to DAMPs recognized by various PRRs and apoptotic cells (uncontrolled phagocytosis) may lead to chronic and uncontrolled inflammation that may induce autoinflammation and autoimmune diseases including severe autoimmune anemia, systemic lupus erythematosus (SLE), and chronic arthritis [173–176]. The increased infiltration of macrophages into the brain (i.e., in meninges surrounding the CNS, the perivascular space, and the choroid plexus) is also reported in experimental autoimmune encephalitis (EAE), an animal model for multiple sclerosis (MS) [177, 178]. The chronic up-regulation of CCR2, CCL2, CCL3, CCL4, and CCL22 stimulates the process of macrophage accumulation at the sites of the brain affected during EAE [179, 180]. Both M1 and M2 macrophages play a crucial role in the pathogenesis of EAE or MS [180, 181]. Macrophages also play a very important role in the pathogenesis of rheumatoid arthritis (RA) by secreting various pro-inflammatory cytokines, controlling the generation and function of regulatory T cells (Tregs) via binding and release of transforming growth factor-β (TGF-β), and their therapeutic targeting proves beneficial to the patients [182–185]. Sjogren's syndrome (SS), a chronic autoimmune disease of exocrine glands specifically salivary glands and lacrimal glands causing also systemic autoimmune lesions also shows the accumulation of monocytes and macrophages in the inflamed lesions [185–187]. In addition to these autoimmune diseases, both M1 and M2 macrophages also play a crucial role in the pathogenesis of type 1 diabetes mellitus by contributing to the destruction of β cells of the pancreas through controlling the generation of Th1 cells and acting as antigen presenting cells (APCs) to stimulate cytotoxic $CD8^+$ T cells (T1DM) [188–190].

### 5.5 Macrophages in metabolic diseases

Obesity is an altered stage of metabolism originating due to the increased availability of nutrients (except in the genetically impaired conditions causing the deposition of the white adipose tissue (WAT)) [191]. However, both obesity caused by the genetic factors or due to the increased food intake and sedentary life style cause a low-grade systemic chronic inflammation that may lead to the development of type 2 diabetes mellitus (T2DM) and atherosclerosis [192–194]. The death of adipocyte serves as a major trigger for the recruitment of inflammatory $LY6C^{hi}CCR2^+$ monocytes and the accumulation of macrophages in the WAT as more than 90% of the macrophages in WAT are localized to the dead adipocytes [195, 196]. These macrophages then fuse to form syncytia sequestering and scavenging the residual "free" adipocyte lipid droplets and ultimately forming the multinucleate giant cells that serve as a hallmark of chronic inflammation. Furthermore, these macrophages recognize fatty acids (FAs) as potential inflammogens and reprogram themselves into classical macrophages (M1 macrophages) during obesity [104, 197, 198]. For example, saturated but not unsaturated fatty acids promote the inflammatory activation of macrophages via the activation of TLR4 as TLR4 is essential for high-fat diet-induced insulin resistance in adipose tissue and liver [199–203]. Additionally, Fetuin A (FetA or AHSG, a secreted glycoprotein) serves as an endogenous ligand for TLR4 for promoting the lipid-induced insulin resistance, lipotoxicity in β cells of the pancreas, and T2DM [204, 205]. However, M2 macrophages generated in the

environment promote the health of the WAT and the insulin sensitivity by an unknown mechanism in a lean state [206]. It can be hypothesized that the M2 macrophages via maintaining the health of adipocytes in WAT prevent the generation of signals including the death of adipose tissue that chemo-attract the pro-inflammatory monocytes reprogramming later into classical M1 macrophages. The genetic depletion of the M2 gene or M2 macrophages cause the induction of metabolic diseases upon high-fat-diet [206]. IL-6 promotes the generation of AAMs or M2 macrophages in adipose tissue environment during obesity [207]. The depletion of CD11b also increases the number of AAMs in adipose tissue during obesity and prevents the development of obesity-induced insulin resistance [208]. Thus targeting CD11b during obesity may prevent obesity-induced insulin resistance. Recently, a population of sympathetic neuron-associated macrophages (SAMs) has been identified controlling the obesity by engulfing and clearing norepinephrine (NE) [209].

## 6. Conclusion and future perspective

Macrophages are innate immune cells that serve as a first line of defense against invading pathogens almost in every organ system including lungs, liver, intestine, kidneys, and brain. Along with acting as first line of defense against pathogens, PAMPs, DAMPs, and other xenobiotics they act as antigen presenting cells (APCs) and provide processed antigens to activate the adaptive immune response comprising of B and T cells. Thus macrophages are sentinel innate immune cells taking part in the generation of both acute and chronic inflammation induced during both sterile and infectious tissue damaging conditions via controlling the migration and activation of other innate immune cells including neutrophils and dendritic cells (DCs) as well as cells of the adaptive immune system. In addition to their role in controlling the process of inflammation they are also involved in the process of wound repair and regeneration, autoimmunity, obesity and associated insulin tolerance, angiogenesis and embryonic development of the fetus. Thus macrophage are the potent immunoregulatory cells of the innate immune system involved in host defense against infections and other inflammatory diseases including cancer and autoimmunity along with the maintenance of immune homeostasis involving the process of resolution phase during inflammation [210–212]. Hence macrophages are very important innate immune cells with immune regulatory function depending on their fine tuning or polarization during diverse inflammatory conditions as described here in the chapter.

Although macrophages have been discovered a century ago and revolutionized the immunology research and opened the road to the branch of immunology called innate immunity but much more is still remaining to explore in macrophage biology and their role in the regulation of development, homeostasis, immune homeostasis, inflammation, and disease pathogenesis. For example, macrophage immunometabolism and epigenetic mechanisms regulating their polarization and pro-and anti-inflammatory phenotype and action have started to answer the several previously unknown questions that may influence the future immunotherapeutics and immunomodulatory approaches to target several immune-based diseases varying from autoimmune diseases to several cancers to metabolic diseases.

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## References

[1] Kumar V, Ahmad A. Role of MAIT cells in the immunopathogenesis of inflammatory diseases: New players in old game. International Reviews of Immunology. 2018;**37**:90-110

[2] Kumar V. Innate lymphoid cells: New paradigm in immunology of inflammation. Immunology Letters. 2014;**157**:23-37

[3] Kumar V. Innate lymphoid cells: Immunoregulatory cells of mucosal inflammation. European Journal of Inflammation. 2014;**12**:11-20

[4] Kumar V, Sharma A. Mast cells: Emerging sentinel innate immune cells with diverse role in immunity. Molecular Immunology. 2010;**48**:14-25

[5] Kumar V, Sharma A. Neutrophils: Cinderella of innate immune system. International Immunopharmacology. 2010;**10**:1325-1334

[6] Van Kaer L, Parekh VV, Wu L. Invariant natural killer T cells: Bridging innate and adaptive immunity. Cell and Tissue Research. 2011;**343**:43-55

[7] Brennan PJ, Brigl M, Brenner MB. Invariant natural killer T cells: An innate activation scheme linked to diverse effector functions. Nature Reviews. Immunology. 2013;**13**:101-117

[8] Konigshofer Y, Chien Y-h. γδ T cells —Innate immune lymphocytes? Current Opinion in Immunology. 2006;**18**: 527-533

[9] Ferreira LM. Gammadelta T cells: Innately adaptive immune cells? International Reviews of Immunology. 2013;**32**:223-248

[10] Bhat A, Wooten RM, Jayasuriya AC. Secretion of growth factors from macrophages when cultured with microparticles. Journal of Biomedical Materials Research. Part A. 2013;**101**: 3170-3180

[11] Shimokado K, Raines EW, Madtes DK, Barrett TB, Benditt EP, Ross R. A significant part of macrophage-derived growth factor consists of at least two forms of PDGF. Cell. 1985;**43**:277-286

[12] Nathan CF. Secretory products of macrophages. The Journal of Clinical Investigation. 1987;**79**:319-326

[13] Martinez FO, Gordon S. The evolution of our understanding of macrophages and translation of findings toward the clinic. Expert Review of Clinical Immunology. 2015;**11**:5-13

[14] Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. Nature Immunology. 2013;**14**:986-995

[15] van Furth RCZ, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: A new classification of macrophages, monocytes, and their precursor cells. Bulletin of the World Health Organization. 1972;**46**:845-852

[16] Chow A, Brown BD, Merad M. Studying the mononuclear phagocyte system in the molecular age. Nature Reviews Immunology. 2011;**11**:788

[17] Hume DA, Irvine KM, Pridans C. The mononuclear phagocyte system: The relationship between monocytes and macrophages. Trends in Immunology. 2019;**40**(2):98-112

[18] Samokhvalov IM. Deconvoluting the ontogeny of hematopoietic stem cells. Cellular and Molecular Life Sciences. 2014;**71**:957-978

[19] Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. Immunity. 2014;**41**:21-35

[20] Takahashi K, Yamamura F, Naito M. Differentiation, maturation, and proliferation of macrophages in the mouse yolk sac: A light-microscopic, enzyme-cytochemical, immunohistochemical, and ultrastructural study. Journal of Leukocyte Biology. 1989;**45**:87-96

[21] Takahashi K. Development and differentiation of macrophages and related cells historical review and current concepts. Journal of Clinical and Experimental Hematopathology. 2001; **41**:1-31

[22] Hume DA. Probability in transcriptional regulation and its implications for leukocyte differentiation and inducible gene expression. Blood. 2000;**96**:2323-2328

[23] Moignard V, Macaulay IC, Swiers G, Buettner F, Schutte J, Calero-Nieto FJ, et al. Characterization of transcriptional networks in blood stem and progenitor cells using high-throughput single-cell gene expression analysis. Nature Cell Biology. 2013;**15**:363-372

[24] Graf T, Enver T. Forcing cells to change lineages. Nature. 2009;**462**: 587-594

[25] Orkin SH, Zon LI. Hematopoiesis: An evolving paradigm for stem cell biology. Cell. 2008;**132**:631-644

[26] Kurotaki D, Sasaki H, Tamura T. Transcriptional control of monocyte and macrophage development. International Immunology. 2017;**29**:97-107

[27] Pang SHM, de Graaf CA, Hilton DJ, Huntington ND, Carotta S, Wu L, et al. PU.1 is required for the developmental progression of multipotent progenitors to common lymphoid progenitors. Frontiers in Immunology. 2018;**9**:1264

[28] DeKoter RP, Singh H. Regulation of B lymphocyte and macrophage development by graded expression of PU.1. Science. 2000;**288**:1439-1441

[29] Pham T-H, Minderjahn J, Schmidl C, Hoffmeister H, Schmidhofer S, Chen W, et al. Mechanisms of in vivo binding site selection of the hematopoietic master transcription factor PU.1. Nucleic Acids Research. 2013;**41**: 6391-6402

[30] Imperato MR, Cauchy P, Obier N, Bonifer C. The RUNX1–PU.1 axis in the control of hematopoiesis. International Journal of Hematology. 2015;**101**: 319-329

[31] T'Jonck W, Guilliams M, Bonnardel J. Niche signals and transcription factors involved in tissue-resident macrophage development. Cellular Immunology. 2018;**330**:43-53

[32] Zhang DE, Hetherington CJ, Chen HM, Tenen DG. The macrophage transcription factor PU.1 directs tissue-specific expression of the macrophage colony-stimulating factor receptor. Molecular and Cellular Biology. 1994;**14**: 373-381

[33] Liu H, Shi B, Huang CC, Eksarko P, Pope RM. Transcriptional diversity during monocyte to macrophage differentiation. Immunology Letters. 2008;**117**:70-80

[34] Laiosa CV, Stadtfeld M, Xie H, de Andres-Aguayo L, Graf T. Reprogramming of committed T cell progenitors to macrophages and dendritic cells by C/EBP alpha and PU.1 transcription factors. Immunity. 2006; **25**:731-744

[35] Paul F, Ya A, Giladi A, Jaitin Diego A, Kenigsberg E, Keren-Shaul H, et al. Transcriptional heterogeneity and lineage commitment in myeloid progenitors. Cell. 2015;**163**:1663-1677

[36] Becker AM, Michael DG, Satpathy AT, Sciammas R, Singh H, Bhattacharya

D. IRF-8 extinguishes neutrophil production and promotes dendritic cell lineage commitment in both myeloid and lymphoid mouse progenitors. Blood. 2012;**119**:2003-2012

[37] Kurotaki D, Yamamoto M, Nishiyama A, Uno K, Ban T, Ichino M, et al. IRF8 inhibits C/EBPα activity to restrain mononuclear phagocyte progenitors from differentiating into neutrophils. Nature Communications. 2014;**5**:4978

[38] Kurotaki D, Nakabayashi J, Nishiyama A, Sasaki H, Kawase W, Kaneko N, et al. Transcription factor IRF8 governs enhancer landscape dynamics in mononuclear phagocyte progenitors. Cell Reports. 2018;**22**: 2628-2641

[39] Scott CL, T'Jonck W, Martens L, Todorov H, Sichien D, Soen B, et al. The transcription factor ZEB2 is required to maintain the tissue-specific identities of macrophages. Immunity. 2018;**49**: 312-325.e5

[40] Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science. 2010;**327**:656-661

[41] Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nature Immunology. 2012;**13**:1118-1128

[42] Aziz A, Soucie E, Sarrazin S, Sieweke MH. MafB/c-Maf deficiency enables self-renewal of differentiated functional macrophages. Science. 2009; **326**:867-871

[43] Kelly LM, Englmeier U, Lafon I, Sieweke MH, Graf T. MafB is an inducer of monocytic differentiation. The EMBO Journal. 2000;**19**:1987-1997

[44] Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. Nature Reviews Immunology. 2011;**11**:762

[45] Hanna RN, Carlin LM, Hubbeling HG, Nackiewicz D, Green AM, Punt JA, et al. The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C− monocytes. Nature Immunology. 2011;**12**:778

[46] Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. Nature Immunology. 2013; **14**:821-830

[47] Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity. 2013;**38**: 79-91

[48] Serbina NV, Jia T, Hohl TM, Pamer EG. Monocyte-mediated defense against microbial pathogens. Annual Review of Immunology. 2008;**26**:421-452

[49] Kurihara T, Warr G, Loy J, Bravo R. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. The Journal of Experimental Medicine. 1997;**186**: 1757-1762

[50] Kuziel WA, Morgan SJ, Dawson TC, Griffin S, Smithies O, Ley K, et al. Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**:12053-12058

[51] Maus U, von Grote K, Kuziel WA, Mack M, Miller EJ, Cihak J, et al. The role of CC chemokine receptor 2 in alveolar monocyte and neutrophil immigration in intact mice. American

Journal of Respiratory and Critical Care Medicine. 2002;**166**:268-273

[52] Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science. 2007;**317**: 666-670

[53] Carlin LM, Stamatiades EG, Auffray C, Hanna RN, Glover L, Vizcay-Barrena G, et al. Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal. Cell. 2013;**153**: 362-375

[54] Thomas G, Tacke R, Hedrick CC, Hanna RN. Nonclassical patrolling monocyte function in the vasculature. Arteriosclerosis, Thrombosis, and Vascular Biology. 2015;**35**:1306-1316

[55] Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: Their role in infection and inflammation. Journal of Leukocyte Biology. 2007;**81**:584-592

[56] Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. Blood. 2010;**116**: e74-e80

[57] Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: A unified nomenclature based on ontogeny. Nature Reviews. Immunology. 2014;**14**:571-578

[58] Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity. 2003;**19**:71-82

[59] Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. Immunity. 2010; **33**:375-386

[60] Grage-Griebenow E, Flad HD, Ernst M. Heterogeneity of human peripheral blood monocyte subsets. Journal of Leukocyte Biology. 2001;**69**:11-20

[61] Yona S, Jung S. Monocytes: Subsets, origins, fates and functions. Current Opinion in Hematology. 2010;**17**:53-59

[62] Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. Blood. 2010; **115**:e10-e19

[63] Hamilton JA, Achuthan A. Colony stimulating factors and myeloid cell biology in health and disease. Trends in Immunology. 2013;**34**:81-89

[64] Ushach I, Zlotnik A. Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage. Journal of Leukocyte Biology. 2016;**100**: 481-489

[65] Hamilton TA, Zhao C, Pavicic PG Jr, Datta S. Myeloid colony-stimulating factors as regulators of macrophage polarization. Frontiers in Immunology. 2014;**5**:554-554

[66] Murray PJ. Macrophage polarization. Annual Review of Physiology. 2017;**79**:541-566

[67] Parisi L, Gini E, Baci D, Tremolati M, Fanuli M, Bassani B, et al. Macrophage polarization in chronic inflammatory diseases: Killers or builders? Journal of Immunology Research. 2018;**2018**:25

[68] Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, et al. Macrophage plasticity, polarization, and function in health and disease. Journal of Cellular Physiology. 2018;**233**: 6425-6440

[69] Atri C, Guerfali FZ, Laouini D. Role of human macrophage polarization in inflammation during infectious diseases. International Journal of Molecular Sciences. 19 Jun 2018;**19**(6):E1801. DOI: 10.3390/ijms19061801

[70] Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: Cancer as a paradigm. Nature Immunology. 2010; **11**:889-896

[71] Locati M, Mantovani A, Sica A. Macrophage activation and polarization as an adaptive component of innate immunity. Advances in Immunology. 2013;**120**:163-184

[72] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends in Immunology. 2004;**25**: 677-686

[73] Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. The Journal of Pathology. 2013;**229**:176-185

[74] Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: New molecules and patterns of gene expression. Journal of Immunology. 2006;**177**:7303-7311

[75] Mosser DM. The many faces of macrophage activation. Journal of Leukocyte Biology. 2003;**73**:209-212

[76] Kumar V. Targeting macrophage immunometabolism: Dawn in the darkness of sepsis. International Immunopharmacology. 2018;**58**:173-185

[77] Van den Bossche J, Saraber DL. Metabolic regulation of macrophages in tissues. Cellular Immunology. 2018;**330**: 54-59

[78] Keiran N, Ceperuelo-Mallafré V, Calvo E, Hernández-Alvarez MI, Ejarque M, Núñez-Roa C, et al. SUCNR1 controls an anti-inflammatory program in macrophages to regulate the metabolic response to obesity. Nature Immunology. 2019;**20**(5):581-592

[79] Bystrom J, Evans I, Newson J, Stables M, Toor I, van Rooijen N, et al. Resolution-phase macrophages possess a unique inflammatory phenotype that is controlled by cAMP. Blood. 2008;**112**: 4117-4127

[80] Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: Time for reassessment. F1000prime Reports. 2014;**6**:13

[81] Chávez-Galán L, Olleros ML, Vesin D, Garcia I. Much more than M1 and M2 macrophages, there are also CD169+ and TCR+ macrophages. Frontiers in Immunology. 2015;**6**:263. DOI: 10.3389/ fimmu.2015.00263

[82] Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. Immunity. 2013;**39**:925-938

[83] Yanez DA, Lacher RK, Vidyarthi A, Colegio OR. The role of macrophages in skin homeostasis. Pflugers Archiv: European Journal of Physiology. 2017; **469**:455-463

[84] Pepe G, Locati M, Della Torre S, Mornata F, Cignarella A, Maggi A, et al. The estrogen-macrophage interplay in the homeostasis of the female reproductive tract. Human Reproduction Update. 2018;**24**:652-672

[85] Lee SK, Kim CJ, Kim D-J, Kang J-H. Immune cells in the female reproductive tract. Immune Network. 2015;**15**:16-26

[86] Cipriani G, Gibbons SJ, Kashyap PC, Farrugia G. Intrinsic gastrointestinal

macrophages: Their phenotype and role in gastrointestinal motility. Cellular and Molecular Gastroenterology and Hepatology. 2016;**2**:120-130.e1

[87] Grainger JR, Konkel JE, Zangerle-Murray T, Shaw TN. Macrophages in gastrointestinal homeostasis and inflammation. Pflugers Archiv: European Journal of Physiology. 2017; **469**:527-539

[88] De Schepper S, Stakenborg N, Matteoli G, Verheijden S, Boeckxstaens GE. Muscularis macrophages: Key players in intestinal homeostasis and disease. Cellular Immunology. 2018;**330**: 142-150

[89] Mossadegh-Keller N, Sieweke MH. Testicular macrophages: Guardians of fertility. Cellular Immunology. 2018; **330**:120-125

[90] Joshi N, Walter JM, Misharin AV. Alveolar macrophages. Cellular Immunology. 2018;**330**:86-90

[91] Liegeois M, Legrand C, Desmet CJ, Marichal T, Bureau F. The interstitial macrophage: A long-neglected piece in the puzzle of lung immunity. Cellular Immunology. 2018;**330**:91-96

[92] Mantovani B, Rabinovitch M, Nussenzweig V. Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG). The Journal of Experimental Medicine. 1972;**135**: 780-792

[93] Murray PJW, T.A. Protective and pathogenic functions of macrophage subsets. Nature Reviews. Immunology. 2011;**11**:723-737

[94] Ricci R, Sumara G, Sumara I, Rozenberg I, Kurrer M, Akhmedov A, et al. Requirement of JNK2 for scavenger receptor A-mediated foam cell formation in atherogenesis. Science. 2004;**306**:1558-1561

[95] Ohnishi K, Komohara Y, Fujiwara Y, Takemura K, Lei X, Nakagawa T, et al. Suppression of TLR4-mediated inflammatory response by macrophage class A scavenger receptor (CD204). Biochemical and Biophysical Research Communications. 2011;**411**:516-522

[96] Dahl M, Bauer AK, Arredouani M, Soininen R, Tryggvason K, Kleeberger SR, et al. Protection against inhaled oxidants through scavenging of oxidized lipids by macrophage receptors MARCO and SR-AI/II. The Journal of Clinical Investigation. 2007;**117**:757-764

[97] Peiser L, Gordon S. The function of scavenger receptors expressed by macrophages and their role in the regulation of inflammation. Microbes and Infection. 2001;**3**:149-159

[98] Peiser L, Mukhopadhyay S, Gordon S. Scavenger receptors in innate immunity. Current Opinion in Immunology. 2002;**14**:123-128

[99] Zani IA, Stephen SL, Mughal NA, Russell D, Homer-Vanniasinkam S, Wheatcroft SB, et al. Scavenger receptor structure and function in health and disease. Cell. 2015;**4**:178-201

[100] Pluddemann A, Neyen C, Gordon S. Macrophage scavenger receptors and host-derived ligands. Methods. 2007;**43**: 207-217

[101] PrabhuDas MR, Baldwin CL, Bollyky PL, Bowdish DME, Drickamer K, Febbraio M, et al. A consensus definitive classification of scavenger receptors and their roles in health and disease. Journal of Immunology. 2017; **198**:3775-3789

[102] Erwig LP, Henson PM. Clearance of apoptotic cells by phagocytes. Cell Death and Differentiation. 2008;**15**: 243-250

[103] Hajishengallis G, Lambris JD. Microbial manipulation of receptor crosstalk in innate immunity. Nature Reviews Immunology. 2011;**11**:187-220

[104] Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nature Reviews. Immunology. 2005;**5**:953-964

[105] Iborra S, Sancho D. Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors. Immunobiology. 2015;**220**: 175-184

[106] Elinav E, Strowig T, Henao-Mejia J, Flavell RA. Regulation of the antimicrobial response by NLR proteins. Immunity. 2011;**34**:665-679

[107] Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011;**34**:637-650

[108] Osorio F, Reis e Sousa C. Myeloid C-type lectin receptors in pathogen recognition and host defense. Immunity. 2011;**34**:651-664

[109] Blander JM, Medzhitov R. Regulation of phagosome maturation by signals from toll-like receptors. Science. 2004;**304**:1014-1018

[110] Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, et al. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature. 1999;**401**:811-815

[111] Doyle SE, O'Connell RM, Miranda GA, Vaidya SA, Chow EK, Liu PT, et al. Toll-like receptors induce a phagocytic gene program through p38. The Journal of Experimental Medicine. 2004;**199**: 81-90

[112] Kong L, Ge BX. MyD88-independent activation of a novel actin-Cdc42/Rac pathway is required for Toll-like receptor-stimulated phagocytosis. Cell Research. 2008;**18**:745-755

[113] Tricker E, Cheng G. With a little help from my friends: Modulation of phagocytosis through TLR activation. Cell Research. 2008;**18**:711-712

[114] Tohyama Y, Yamamura H. Complement-mediated phagocytosis—The role of Syk. IUBMB Life. 2006;**58**: 304-308

[115] Helmy KY, Katschke KJ Jr, Gorgani NN, Kljavin NM, Elliott JM, Diehl L, et al. CRIg: A macrophage complement receptor required for phagocytosis of circulating pathogens. Cell. 2006;**124**: 915-927

[116] Le Cabec V, Carréno S, Moisand A, Bordier C, Maridonneau-Parini I. Complement receptor 3 (CD11b/CD18) mediates type I and type II phagocytosis during nonopsonic and opsonic phagocytosis, respectively. The Journal of Immunology. 2002;**169**:2003-2009

[117] Gorgani NN, Thathaisong U, Mukaro VRS, Poungpair O, Tirimacco A, Hii CST, et al. Regulation of CRIg expression and phagocytosis in human macrophages by arachidonate, dexamethasone, and cytokines. The American Journal of Pathology. 2011; **179**:1310-1318

[118] Hadas S, Spira M, Hanisch UK, Reichert F, Rotshenker S. Complement receptor-3 negatively regulates the phagocytosis of degenerated myelin through tyrosine kinase Syk and cofilin. Journal of Neuroinflammation. 2012;**9**: 166

[119] Galvan MD, Greenlee-Wacker MC, Bohlson SS. C1q and phagocytosis: The perfect complement to a good meal. Journal of Leukocyte Biology. 2012;**92**: 489-497

[120] Suresh R, Sutterwala F, Mosser D. Complement mediated phagocytosis

induces the activation of the NALP3 inflammasome (INC5P.331). The Journal of Immunology. 2014;**192**:-120.111

[121] Jantsch J, Binger KJ, Muller DN, Titze J. Macrophages in homeostatic immune function. Frontiers in Physiology. 2014;**5**:146

[122] Low-Marchelli JM, Ardi VC, Vizcarra EA, van Rooijen N, Quigley JP, Yang J. Twist1 induces CCL2 and recruits macrophages to promote angiogenesis. Cancer Research. 2013;**73**: 662-671

[123] Bruce AC, Kelly-Goss MR, Heuslein JL, Meisner JK, Price RJ, Peirce SM. Monocytes are recruited from venules during arteriogenesis in the murine spinotrapezius ligation model. Arteriosclerosis, Thrombosis, and Vascular Biology. 2014;**34**:2012-2022

[124] Khan B, Rangasamy S, McGuire PG, Howdieshell TR. The role of monocyte subsets in myocutaneous revascularization. The Journal of Surgical Research. 2013;**183**:963-975

[125] Avraham-Davidi I, Yona S, Grunewald M, Landsman L, Cochain C, Silvestre JS, et al. On-site education of VEGF-recruited monocytes improves their performance as angiogenic and arteriogenic accessory cells. The Journal of Experimental Medicine. 2013;**210**: 2611-2625

[126] Favre J, Terborg N, Horrevoets AJ. The diverse identity of angiogenic monocytes. European Journal of Clinical Investigation. 2013;**43**:100-107

[127] Hamm A, Veschini L, Takeda Y, Costa S, Delamarre E, Squadrito ML, et al. PHD2 regulates arteriogenic macrophages through TIE2 signalling. EMBO Molecular Medicine. 2013;**5**: 843-857

[128] Meneses AM, Wielockx B. PHD2: From hypoxia regulation to disease progression. Hypoxia (Auckl). 2016;**4**: 53-67

[129] Fang HY, Hughes R, Murdoch C, Coffelt SB, Biswas SK, Harris AL, et al. Hypoxia-inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. Blood. 2009;**114**:844-859

[130] Krausz S, Garcia S, Ambarus CA, de Launay D, Foster M, Naiman B, et al. Angiopoietin-2 promotes inflammatory activation of human macrophages and is essential for murine experimental arthritis. Annals of the Rheumatic Diseases. 2012;**71**:1402-1410

[131] Krock BL, Skuli N, Simon MC. Hypoxia-induced angiogenesis: Good and evil. Genes & Cancer. 2011;**2**: 1117-1133

[132] Willenborg S, Lucas T, van Loo G, Knipper JA, Krieg T, Haase I, et al. CCR2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair. Blood. 2012;**120**:613-625

[133] Sunderkotter C, Goebeler M, Schulze-Osthoff K, Bhardwaj R, Sorg C. Macrophage-derived angiogenesis factors. Pharmacology & Therapeutics. 1991;**51**:195-216

[134] Zajac E, Schweighofer B, Kupriyanova TA, Juncker-Jensen A, Minder P, Quigley JP, et al. Angiogenic capacity of M1- and M2-polarized macrophages is determined by the levels of TIMP-1 complexed with their secreted proMMP-9. Blood. 2013;**122**: 4054-4067

[135] Corliss BA, Azimi MS, Munson JM, Peirce SM, Murfee WL. Macrophages: An inflammatory link between angiogenesis and lymphangiogenesis. Microcirculation (New York, N.Y.: 1994). 2016;**23**:95-121

[136] DeFalco T, Bhattacharya I, Williams AV, Sams DM, Capel B.

Yolk-sac-derived macrophages regulate fetal testis vascularization and morphogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**: E2384-E2393

[137] Poche RA, Hsu CW, McElwee ML, Burns AR, Dickinson ME. Macrophages engulf endothelial cell membrane particles preceding pupillary membrane capillary regression. Developmental Biology. 2015;**403**:30-42

[138] Ricardo SD, van Goor H, Eddy AA. Macrophage diversity in renal injury and repair. The Journal of Clinical Investigation. 2008;**118**:3522-3530

[139] Alikhan MA, Ricardo SD. Mononuclear phagocyte system in kidney disease and repair. Nephrology (Carlton). 2013;**18**:81-91

[140] Rappolee DA, Mark D, Banda MJ, Werb Z. Wound macrophages express TGF-alpha and other growth factors in vivo: Analysis by mRNA phenotyping. Science. 1988;**241**:708-712

[141] Akhurst RJ, Hata A. Targeting the TGFbeta signalling pathway in disease. Nature Reviews. Drug Discovery. 2012; **11**:790-811

[142] Minutti CM, Modak RV, Macdonald F, Li F, Smyth DJ, Dorward DA, et al. A macrophage-pericyte axis directs tissue restoration via amphiregulin-induced transforming growth factor beta activation. Immunity. 2019;**50**:645-654.e6

[143] Zaiss DMW, Gause WC, Osborne LC, Artis D. Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. Immunity. 2015;**42**:216-226

[144] Stappenbeck TS, Miyoshi H. The role of stromal stem cells in tissue regeneration and wound repair. Science. 2009;**324**:1666-1669

[145] Wynn TA, Ramalingam TR. Mechanisms of fibrosis: Therapeutic translation for fibrotic disease. Nature Medicine. 2012;**18**:1028-1040

[146] Duffield JS, Lupher M, Thannickal VJ, Wynn TA. Host responses in tissue repair and fibrosis. Annual Review of Pathology. 2013;**8**:241-276

[147] Vernon MA, Mylonas KJ, Hughes J. Macrophages and renal fibrosis. Seminars in Nephrology. 2010;**30**: 302-317

[148] Wynn TA, Barron L. Macrophages: Master regulators of inflammation and fibrosis. Seminars in Liver Disease. 2010;**30**:245-257

[149] Pardali E, Sanchez-Duffhues G, Gomez-Puerto MC, Ten Dijke P. TGF-beta-induced endothelial-mesenchymal transition in fibrotic diseases. International Journal of Molecular Sciences. 2017;**18**(10):E2157. DOI: 10.3390/ijms18102157

[150] Vannella KM, Wynn TA. Mechanisms of organ injury and repair by macrophages. Annual Review of Physiology. 2017;**79**:593-617

[151] Lekkerkerker AN, Aarbiou J, van Es T, Janssen RA. Cellular players in lung fibrosis. Current Pharmaceutical Design. 2012;**18**:4093-4102

[152] Wynn TA. Cellular and molecular mechanisms of fibrosis. The Journal of Pathology. 2008;**214**:199-210

[153] Kolb M, Margetts PJ, Anthony DC, Pitossi F, Gauldie J. Transient expression of IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. The Journal of Clinical Investigation. 2001;**107**: 1529-1536

[154] Wilson MS, Madala SK, Ramalingam TR, Gochuico BR, Rosas IO, Cheever AW, et al. Bleomycin and

IL-1beta-mediated pulmonary fibrosis is IL-17A dependent. The Journal of Experimental Medicine. 2010;**207**: 535-552

[155] Byrne AJ, Maher TM, Lloyd CM. Pulmonary macrophages: A new therapeutic pathway in fibrosing lung disease? Trends in Molecular Medicine. 2016;**22**:303-316

[156] Das A, Sinha M, Datta S, Abas M, Chaffee S, Sen CK, et al. Monocyte and macrophage plasticity in tissue repair and regeneration. The American Journal of Pathology. 2015;**185**:2596-2606

[157] Oishi Y, Manabe I. Macrophages in inflammation, repair and regeneration. International Immunology. 2018;**30**: 511-528

[158] Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008;**453**:314-321

[159] Varga T, Mounier R, Gogolak P, Poliska S, Chazaud B, Nagy L. Tissue LyC6-macrophages are generated in the absence of circulating LyC6-monocytes and Nur77 in a model of muscle regeneration. Journal of Immunology. 2013;**191**:5695-5701

[160] Pei L, Castrillo A, Tontonoz P. Regulation of macrophage inflammatory gene expression by the orphan nuclear receptor Nur77. Molecular Endocrinology. 2006;**20**: 786-794

[161] Wang H, Melton DW, Porter L, Sarwar ZU, McManus LM, Shireman PK. Altered macrophage phenotype transition impairs skeletal muscle regeneration. The American Journal of Pathology. 2014;**184**:1167-1184

[162] Giannakis N, Sansbury BE, Patsalos A, Hays TT, Riley CO, Han X, et al. Dynamic changes to lipid mediators support transitions among macrophage subtypes during muscle regeneration. Nature Immunology. 2019;**20**(5):626-636

[163] Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. The Journal of Experimental Medicine. 2007;**204**: 1057-1069

[164] Saclier M, Cuvellier S, Magnan M, Mounier R, Chazaud B. Monocyte/ macrophage interactions with myogenic precursor cells during skeletal muscle regeneration. The FEBS Journal. 2013; **280**:4118-4130

[165] Leor J, Palevski D, Amit U, Konfino T. Macrophages and regeneration: Lessons from the heart. Seminars in Cell & Developmental Biology. 2016;**58**:26-33

[166] Godwin JW, Debuque R, Salimova E, Rosenthal NA. Heart regeneration in the salamander relies on macrophage-mediated control of fibroblast activation and the extracellular landscape. npj Regenerative Medicine. 2017;**2**:22

[167] Aurora AB, Porrello ER, Tan W, Mahmoud AI, Hill JA, Bassel-Duby R, et al. Macrophages are required for neonatal heart regeneration. The Journal of Clinical Investigation. 2014;**124**: 1382-1392

[168] Petrie TA, Strand NS, Tsung-Yang C, Rabinowitz JS, Moon RT. Macrophages modulate adult zebrafish tail fin regeneration. Development. 2014;**141**:2581-2591

[169] Tsarouchas TM, Wehner D, Cavone L, Munir T, Keatinge M, Lambertus M, et al. Dynamic control of proinflammatory cytokines Il-1β and Tnf-α by macrophages is necessary for functional spinal cord regeneration in zebrafish. bioRxiv 2018:332197

[170] Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity. 2016;**44**:450-462

[171] Boulter L, Govaere O, Bird TG, Radulescu S, Ramachandran P, Pellicoro A, et al. Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. Nature Medicine. 2012;**18**: 572-579

[172] Chen CC, Wang L, Plikus MV, Jiang TX, Murray PJ, Ramos R, et al. Organ-level quorum sensing directs regeneration in hair stem cell populations. Cell. 2015;**161**:277-290

[173] Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. Cell. 2010;**140**:619-630

[174] Nagata S. Autoimmune diseases caused by defects in clearing dead cells and nuclei expelled from erythroid precursors. Immunological Reviews. 2007;**220**:237-250

[175] Janko C, Schorn C, Grossmayer GE, Frey B, Herrmann M, Gaipl US, et al. Inflammatory clearance of apoptotic remnants in systemic lupus erythematosus (SLE). Autoimmunity Reviews. 2008;**8**:9-12

[176] Kawane K, Ohtani M, Miwa K, Kizawa T, Kanbara Y, Yoshioka Y, et al. Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. Nature. 2006;**443**:998-1002

[177] Rawji KS, Yong VW. The benefits and detriments of macrophages/ microglia in models of multiple sclerosis. Clinical & Developmental Immunology. 2013;**2013**:948976

[178] Abourbeh G, Theze B, Maroy R, Dubois A, Brulon V, Fontyn Y, et al. Imaging microglial/macrophage activation in spinal cords of experimental autoimmune encephalomyelitis rats by positron emission tomography using the mitochondrial 18 kDa translocator protein radioligand [(1)(8)F]DPA-714. The Journal of Neuroscience. 2012;**32**: 5728-5736

[179] Dogan RN, Long N, Forde E, Dennis K, Kohm AP, Miller SD, et al. CCL22 regulates experimental autoimmune encephalomyelitis by controlling inflammatory macrophage accumulation and effector function. Journal of Leukocyte Biology. 2011;**89**: 93-104

[180] Jiang Z, Jiang JX, Zhang GX. Macrophages: A double-edged sword in experimental autoimmune encephalomyelitis. Immunology Letters. 2014;**160**:17-22

[181] Yin J, Valin KL, Dixon ML, Leavenworth JW. The role of microglia and macrophages in CNS homeostasis, autoimmunity, and cancer. Journal of Immunology Research. 2017;**2017**:12

[182] Wallet MA, Wallet SM, Guiulfo G, Sleasman JW, Goodenow MM. IFNgamma primes macrophages for inflammatory activation by high molecular weight hyaluronan. Cellular Immunology. 2010;**262**:84-88

[183] Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, et al. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. Nature Immunology. 2011; **12**:231-238

[184] Schmidt A, Zhang XM, Joshi RN, Iqbal S, Wahlund C, Gabrielsson S, et al. Human macrophages induce CD4(+) Foxp3(+) regulatory T cells via binding and re-release of TGF-beta. Immunology and Cell Biology. 2016;**94**:747-762

[185] Laria A, Lurati A, Marrazza M, Mazzocchi D, Re KA, Scarpellini M. The macrophages in rheumatic diseases. Journal of Inflammation Research. 2016; **9**:1-11

[186] Greenwell-Wild T, Moutsopoulos NM, Gliozzi M, Kapsogeorgou E, Rangel

Z, Munson PJ, et al. Chitinases in the salivary glands and circulation of patients with Sjogren's syndrome: Macrophage harbingers of disease severity. Arthritis and Rheumatism. 2011;**63**:3103-3115

[187] Mustafa W, Zhu J, Deng G, Diab A, Link H, Frithiof L, et al. Augmented levels of macrophage and Th1 cell-related cytokine mRNA in submandibular glands of MRL/lpr mice with autoimmune sialoadenitis. Clinical and Experimental Immunology. 1998; **112**:389-396

[188] Jun HS, Yoon CS, Zbytnuik L, van Rooijen N, Yoon JW. The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. The Journal of Experimental Medicine. 1999;**189**: 347-358

[189] Lee KU, Amano K, Yoon JW. Evidence for initial involvement of macrophage in development of insulitis in NOD mice. Diabetes. 1988;**37**:989-991

[190] Navegantes KC, de Souza Gomes R, Pereira PAT, Czaikoski PG, Azevedo CHM, Monteiro MC. Immune modulation of some autoimmune diseases: The critical role of macrophages and neutrophils in the innate and adaptive immunity. Journal of Translational Medicine. 2017;**15**:36-36

[191] Kopelman PG. Obesity as a medical problem. Nature. 2000;**404**:635-643

[192] Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. Nature Reviews. Immunology. 2008;**8**:923-934

[193] Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. The Journal of Clinical Investigation. 2003;**112**: 1821-1830

[194] Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. Nature. 2017;**542**:177-185

[195] Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. Journal of Lipid Research. 2005;**46**: 2347-2355

[196] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of Clinical Investigation. 2003;**112**:1796-1808

[197] Castoldi A, Naffah de Souza C, Câmara NOS, Moraes-Vieira PM. The macrophage switch in obesity development. Frontiers in Immunology. 2016;**6**:637-637

[198] Lauterbach MAR, Wunderlich FT. Macrophage function in obesity-induced inflammation and insulin resistance. Pflugers Archiv: European Journal of Physiology. 2017;**469**:385-396

[199] Chawla A, Nguyen KD, Goh YPS. Macrophage-mediated inflammation in metabolic disease. Nature Reviews Immunology. 2011;**11**:738

[200] Konner AC, Bruning JC. Toll-like receptors: Linking inflammation to metabolism. Trends in Endocrinology and Metabolism. 2011;**22**:16-23

[201] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. The Journal of Clinical Investigation. 2006;**116**: 3015-3025

[202] Rocha DM, Caldas AP, Oliveira LL, Bressan J, Hermsdorff HH. Saturated fatty acids trigger TLR4-mediated inflammatory response. Atherosclerosis. 2016;**244**:211-215

[203] Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, et al. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. Cell Metabolism. 2009;**10**:419-429

[204] Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nature Medicine. 2012;**18**:1279-1285

[205] Shen X, Yang L, Yan S, Zheng H, Liang L, Cai X, et al. Fetuin A promotes lipotoxicity in beta cells through the TLR4 signaling pathway and the role of pioglitazone in anti-lipotoxicity. Molecular and Cellular Endocrinology. 2015;**412**:1-11

[206] Fujisaka S, Usui I, Nawaz A, Takikawa A, Kado T, Igarashi Y, et al. M2 macrophages in metabolism. Diabetology International. 2016;**7**: 342-351

[207] Braune J, Weyer U, Hobusch C, Mauer J, Brüning JC, Bechmann I, et al. IL-6 regulates M2 polarization and local proliferation of adipose tissue macrophages in obesity. The Journal of Immunology. 2017;**198**:2927-2934

[208] Zheng C, Yang Q, Xu C, Shou P, Cao J, Jiang M, et al. CD11b regulates obesity-induced insulin resistance via limiting alternative activation and proliferation of adipose tissue macrophages. Proceedings of the National Academy of Sciences. 2015;**112**: E7239-E7248

[209] Pirzgalska RM, Domingos AI. Macrophages in obesity. Cellular Immunology. 2018;**330**:183-187

[210] Herold S, Mayer K, Lohmeyer J. Acute lung injury: How macrophages orchestrate resolution of inflammation and tissue repair. Frontiers in Immunology. 2011;**2**:65

[211] Aggarwal NR, King LS, D'Alessio FR. Diverse macrophage populations mediate acute lung inflammation and resolution. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2014;**306**:L709-L725

[212] Ariel A, Maridonneau-Parini I, Rovere-Querini P, Levine JS, Mühl H. Macrophages in inflammation and its resolution. Frontiers in Immunology. 2012;**3**:324-324

Chapter 2

# The Pivotal Role of Macrophages in Metabolic Distress

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## Abstract

Obesity is a prevalent condition with several associated co-morbidities including the development of metabolic diseases. In obesity there is immune cell infiltration into the white adipose tissue and this is associated with the generation of inflammation and insulin resistance (IR). A large majority of the infiltrating leukocytes in obese adipose tissue are pro-inflammatory macrophages, which upon activation induce a switch in metabolism from oxidative phosphorylation, as is utilised by macrophages in lean adipose tissue, towards aerobic glycolysis. The signalling pathways evoked in the recruited macrophages induce the release of pro-inflammatory cytokines, in signalling pathways which directly interfere with insulin signalling and thus induce a state of IR. As macrophages appear to play such a pivotal role in the generation of IR and are the largest leukocyte population in the adipose tissue, they provide a promising therapeutic target. Indeed, there are several strategies currently being studied to induce a 'switch' in macrophages associated with obese adipose tissue, towards the phenotype of those associated with lean adipose tissue, with arguably the most promising being those strategies designed to target the metabolic pathways within the macrophages. This chapter will discuss the polarisation and activation of macrophages within lean and obese adipose tissue and how these cells can be targeted therapeutically.

**Keywords:** macrophage, obesity, metabolism, inflammation

## 1. Introduction

Obesity is defined as abnormal or excessive fat accumulation and is linked with increased risk of development of multiple co-morbidities, including cardiovascular disease, type 2 diabetes, musculoskeletal disorders and certain cancers. Obesity and its associated co-morbidities are a significant health concern facing the global population. Worldwide obesity has tripled since 1975, with 39% of adults considered overweight and 13% considered obese [1]. This situation is prominent in childhood, with 41 million of the global under five population overweight or obese [1].

Obesity induces a state of low-grade systemic inflammation, characterized by increased serum levels of pro-inflammatory mediators, including C Reactive Protein (CRP), Tumour Necrosis Factor (TNF)-α, Interleukin (IL)-1β and IL-6, which contributes to metabolic dysfunction and insulin resistance (IR) [2]. Although the mechanisms underlying this inflammatory response are not fully understood, activation of adipose tissue macrophages (ATM) contributes to this inflammatory state, and therefore to the development of insulin resistance (IR) [3, 4]. Conversely, in lean

individuals the immune repertoire constitutes a more anti-inflammatory phenotype, with ATM alongside regulatory T cells (Tregs) releasing cytokines such as IL-10 and transforming growth factor (TGF)-β, which increase insulin sensitivity [5]. Therefore, the role of ATM in metabolic function is clearly an area of interest, indeed transcriptional profiling has identified how quickly macrophages can respond and adapt to alterations in their microenvironment [6]. This chapter will focus on the role macrophages play in the pathogenesis of metabolic disorders and explore if re-education of these cells provides a target for therapeutic intervention in obesity and its related co-morbidities.

## 2. The microenvironment of the adipose tissue in lean and obese individuals

Obesity historically was believed to be due to a combination of genetic predisposition and environmental factors, however, more recently it has been recognised that immunological factors can also contribute to the pathogenesis of obesity. Indeed, while over 30 gene loci combinations have been associated with the development of obesity and metabolic disease, these loci are only associated with 2–3% of the incidence of these conditions [7]. Further the energy-dense modern Western diet combined with a sedentary lifestyle undoubtedly adds to the obesity epidemic. Recent work has identified the links between dysbiosis in the intestinal microbiome and immune cell activation, linked to the ingestion of high-fat, low-fibre diets, and the development of obesity [8].

### 2.1 The role of the microbiome

The intestinal microbiome is essential for processing dietary polysaccharides and has been identified as a key regulator of systemic inflammation in obesity [9]. Mouse studies are routinely used to study the mechanisms underlying obesity and metabolic disease. Due to the nature of obesity being largely related to diet, diet-induced models are often favoured over genetic models (for example, leptin deficient ob/ob mice). Indeed, studies using a high-fat diet (HFD; equivalent to 60% animal-derived fats in the diet) have been used to study the potential implications of alteration in the microbiome related to diet as well as other obesity-related pathogenesis. It has been shown that the microbiome in obese mice has an increased capacity to harvest energy from the diet compared to the microbiome from lean mice [10]. Microbiome transfer studies, in which intestinal microbiota from mice raised in conventional housing was transferred into germ-free mice, induced a 60% increase in body fat and IR within 14 days, despite a reduction in food consumption [11]. The transfer of microbiota-derived products such as lipopolysaccharides and peptidoglycans, have shown to promote metabolic endotoxemia, which induces pro-inflammation in adipose tissue [12]. In contrast, the microbiota of the gut bacterial fermentation of dietary fibre was shown to have anti-inflammatory effects [12]. Indeed, it was shown that the transfer of intestinal microbiota from lean donors increased the insulin sensitivity in individuals with metabolic syndrome [13].

### 2.2 Adipose tissue

Adipose tissue (AT) is an important metabolic organ, which helps orchestrates metabolic and endocrine functions as well as immune responses [12]. AT functions to store excess nutrients as triacylglycerides and releases fatty acids in the fasted

state, provide cold insulation and protection of vital organs. In the AT of obese individuals, there is significant adipocyte hyperplasia and adipose tissue hypertrophy [14]. AT consists of mature adipocytes, pre-adipocytes, fibroblasts, endothelial cells, histocytes and populations of immune cells including monocytes, macrophages, natural killer (NK) cells, innate lymphoid cells (ILCs) and lymphocytes. AT is classified into three categories, namely white (WAT), beige or 'brite' (beige/brite) and brown (BAT). WAT accounts for approximately 50% of body mass and can release free fatty acids (FFA) into circulation when glucose levels are low. Whilst BAT plays an important role in thermogenesis and the production of heat [15].

The AT of obese individuals is in a state of chronic low-grade inflammation with marked infiltration various pro-inflammatory immune cells such as CD8 cells, NK cells, ILC1, Th1 cells, neutrophils and pro-inflammatory macrophages [16]. Conversely, the immune repertoire of AT from lean individuals comprises anti-inflammatory cell populations, including eosinophils, ILC2, Tregs, $Th_2$ cells and anti-inflammatory macrophages [16] (**Figure 1**). In lean mice, ATM constitutes approximately 5% of cells, conversely in obese mice ATM can account for up to 50% of the cells [3]. Whilst in lean human AT, ATM comprises 4% of cells compared to 12% in excess adiposity [17]. In addition to macrophages, lymphocytes and ILCs also play roles in the regulation of AT inflammation, the roles of which seem to largely involve supporting the polarisation state of the ATM populations. For example, eosinophils provide a source of IL-4 promoting an M2 phenotype. In obese mice adipose eosinophils are decreased, whilst depletion of eosinophils results in increased M1 ATM, weight gain and systemic IR [18]. Furthermore, ILC2

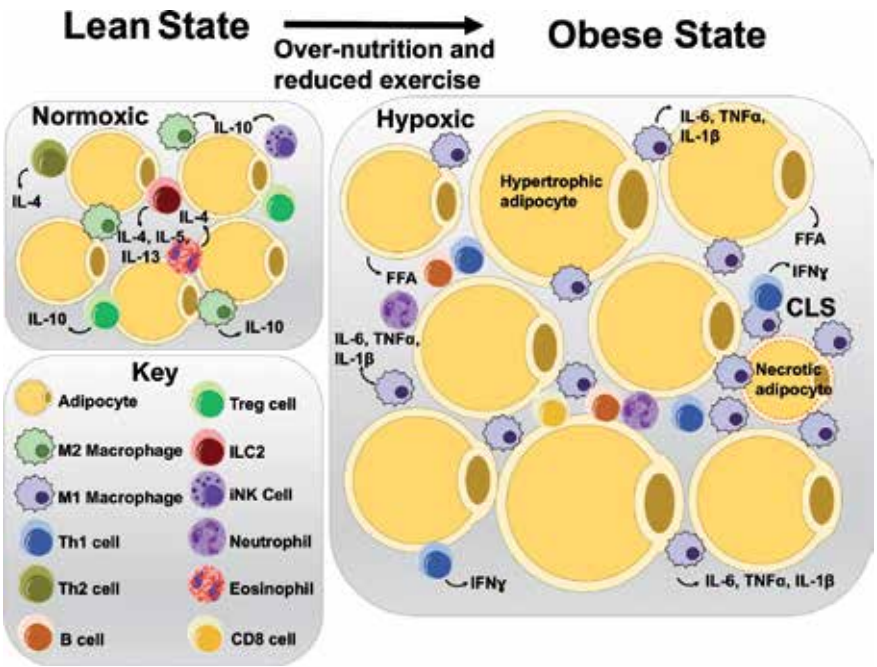


**Figure 1.**
*Immune cell composition of adipose tissue in a lean and obese state. In the lean state, eosinophils and type 2 innate lymphoid cells (ILC2s) produce $Th_2$ cytokines (IL-4, IL-5 and IL-13), which promotes eosinophil recruitment and anti-inflammatory polarisation of macrophages towards an M2 phenotype, which is supported in the normoxic state of lean adipose tissue. In turn, M2 macrophages secrete anti-inflammatory cytokines such as IL-10. In the obese state, adipocyte hypertrophy, hyperplasia and hypoxia cause necrotic adipocytes, resulting in pro-inflammatory state and macrophage recruitment, forming crown like structures (CLS) surrounding the adipocytes. These macrophages are polarised towards an M1 phenotype and secrete the pro-inflammatory cytokines IL-6, IL-1β and TNF-α.*

have been identified as a source of IL-5, a key cytokine in eosinophil recruitment. Thus, accumulation of eosinophils and maintenance of M2 ATM relies on ILC2 [19].

### 2.3 The role of the adaptive immune system in obesity

The adaptive immune system also plays an important role in obesity and metabolic disease. B cells have been shown to be involved in obesity induced inflammation and IR [20]. In obese mice, there was an identified increased in $IgG^+$B cells and IgG production, associated with activation of M1 ATM, increased Th1 cells and conversely, attrition of Treg cells [21]. In addition, transferring B cells into B cell deficient mice induced IR [20]. Furthermore, in obese mice, there is increased $CD8^+$ effector T cell recruitment in epididymal AT. Interestingly, it is reported that $CD8^+$ T cells precede macrophage infiltration and deletion of $CD8^+$ T cells resulted in reduced macrophage infiltration and AT inflammation whilst improving IR [22]. Conversely, both Treg cells and iNKT cells are negatively associated with obesity-induced inflammation and are enriched in lean AT. Indeed, both these immune cells are known to secrete IL-10 which promotes M2 macrophage polarisation [23, 24].

### 2.4 Adipose tissue macrophages

ATMs appear to play a major role in the regulation of obesity-related inflammation, with different macrophage phenotypes associated with divergent roles in the AT. In lean animals, ATM function to maintain the homeostatic micro-environment in AT by taking up excess lipids and phagocytosing dead adipocytes. Broadly speaking macrophages present in lean AT are of an M2 phenotype, which have been shown to suppress inflammation in AT [25]. Furthermore, M2 macrophages in lean AT have been associated with brown fat activation and 'beiging' of WAT in mouse models of obesity, via expression of tyrosine hydroxylase, which induces thermogenesis [26, 27]. However, this process has recently been queried, with IL-4-stimulated macrophages failing to generate sufficient levels of catecholamines to contribute to adipose tissue adaptive thermogenesis [28]. Conversely, excess lipid uptake in obese AT, induces M1 polarisation and along with excess lipid droplets, immune cells and necrotic adipocytes this forms a component called 'crown-like' structures (CLS) [29, 30]. Indeed, it has been shown that more than 90% of all macrophages in WAT of obese mice and humans are localized to dead adipocytes [31]. This metabolic activation of M1 macrophages in obese AT is associated with increased pro-inflammatory cytokines in the AT and recruitment and activation of M1 macrophages in the AT [32].

ATM in lean AT is considered a resident macrophage population, which originates from yolk-sac progenitors and self-renews via proliferation under homeostatic conditions. Over time into adulthood resident ATMs are replaced with circulating monocytes derived from bone marrow [33]. Using mouse bone marrow chimera experiments, following transplanting donor $CD45.1^+$ bone marrow into recipient $CD45.2^+$ mice, and maintenance on obesity-inducing HFD, 85% of the ATM were donor-derived compared to 15% that were recipient-derived [3]. Interestingly, the polarization of macrophages in obesity from an M2 to an M1 phenotype has been mainly attributed to the recruitment of monocytes to AT, rather than the conversion of tissue resident M2 macrophages [34]. Murine monocytes can be classified through the expression of Ly6C, with $Ly6C^{hi}$ monocytes considered inflammatory. In the steady state $Ly6C^{hi}$ monocytes differentiate into $Ly6C^{lo}$ monocytes in the circulation, which are believed to differentiate into M2 macrophages in the tissue. However, in obese AT in response to inflammatory stimuli such as the monocyte chemoattractant CCL2, $Ly6C^{hi}$ macrophages are recruited to the AT where they

differentiate to M1-like ATM [25]. Indeed, in absence of $Ccl_2$ expression macrophages expressed an M2 gene profile [35].

ATM represents the largest population of leukocytes within the AT and plays many vital homeostatic roles including tissue remodelling and insulin sensitivity. However, with progressive obesity ATM are the key mediators of inflammation, IR and the impairment of adipocyte function.

## 3. Polarization of ATM and the link to IR

Macrophages are extremely heterogenic in function and phenotype, and have historically been characterized into two phenotypes; M1 and M2. M1 macrophages are often defined as 'classically activated' and are generally pro-inflammatory in function, with a vital role in eliminating pathogens and virus-infected cells. Whereas, M2 macrophages and termed 'alternatively activated' are anti-inflammatory in function and promote tissue repair and wound healing. This is very simplified and dated model however, evidence now suggests that ATMs include highly plastic cell populations, with their phenotype largely dependent on the microenvironment of the AT. Whilst the exact number and function of ATM in the AT is evolving, it is clear there are distinct populations in the lean and obese AT, with unique tissue distribution, marker expression, transcriptional profiles and functions. Indeed, obese ATM display markers that are largely induced by their metabolic state rather than cytokine stimuli that classically polarise M1 and M2 cells [32].

### 3.1 M1 and M2 macrophage phenotypes

M1 macrophages are activated by signals associated with infection such as IFN-ɣ as well as bacterial-derived products such lipopolysaccharide (LPS) and free fatty acids (FFA). M1 macrophages are loosely identified by surface expression of $F4/80^+CD11C^+$ with high levels of MHC-II, CD68, CD80 and CD86 costimulatory molecules in addition to release of TNF-α and inducible nitric oxide synthase (iNOS) [36]. M2 macrophages are activated via $Th_2$ cytokines, IL-4 and IL-13 as well as by parasitic products. M2 macrophages are loosely identified as $F4/80^+CD206^+CD301^+CD11C^-$ and express genes encoding anti-inflammatory proteins such as *Chil3*, *Arg1* and *Il10* in mice [34]. A crucial transcription factor in M2 macrophage polarisation is peroxisome proliferator-activated receptor (PPAR-γ/δ), which can be driven by adipocyte derived IL-4 and IL-13 [37–39] (**Figure 2**). Some markers vary between mice and human macrophages. For example, there are no human homologues of the M2-associated genes *Chil3*, *Arg1 and Fizz1,* with human M2 identified based on expression of tranglutaminase-2 (TGM2) and CD68 [40].

### 3.2 MMe and Mox macrophage phenotypes

Macrophages with a phenotype associated with obesity are induced by several metabolic stimuli such as FFA, high insulin and glucose, oxidised phospholipids and low-density lipoproteins. These macrophages display surface markers that are neither representative of typical M1 or M2 macrophages and give rise to a population of metabolic activated (MMe) and oxidised (Mox) macrophages [41, 42] (**Figure 2**). Both these macrophage phenotypes are associated with a state of IR. MMe macrophages cell-surface markers express ABCA1, CD36 and PLIN2 and are involved in the clearance of dead adipocytes through lysosomal exocytosis as well as potentiating inflammation. NADPH-oxidase-2 (NOX2) has been identified as a key driver of the functions of MMe macrophages, with *Nox2*-deficient mice displaying attenuated ATM inflammation

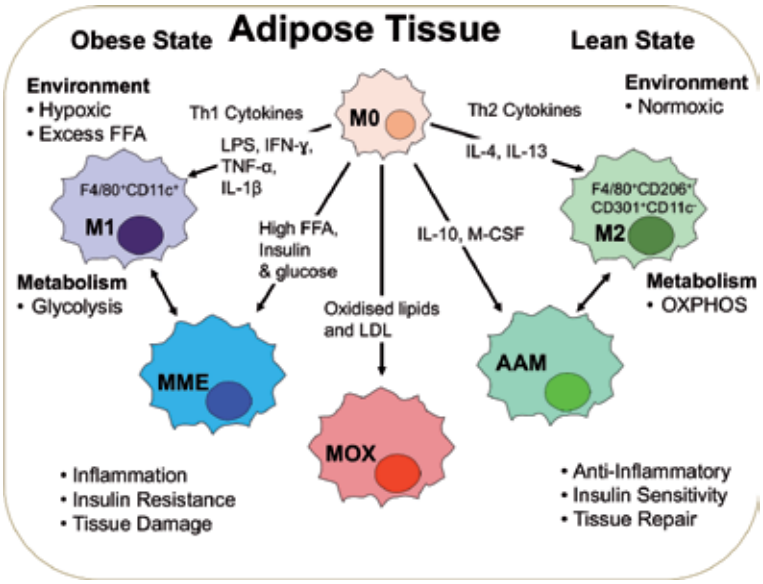


**Figure 2.**
*Adipose tissue macrophage (ATM) polarisation in lean and obese state. ATM originate from polarisation of 'M0' macrophages. Depending on the stimuli and local environment facilitates the macrophage polarisation. Th1 cytokines, LPS, IFN-γ, TNF-α and IL-1β promote a M1 macrophage that is characterised by F4/80$^{+}$CD11c$^{+}$. In contrast, Th$_2$ cytokines, IL-4 and IL-13 promote an M2 macrophage that is characterised by F4/80$^{+}$CD206$^{+}$CD301$^{+}$CD11c$^{-}$. Interestingly, in an obese state, the hypoxic environment and metabolic cues such as excess free fatty acids, high insulin and glucose, oxidised phospholipids and low-density lipoprotein, which promotes a metabolic activated (MME) or oxidised (MOX) macrophage. In the obese state, macrophages promote inflammation, IR and tissue damage. In contrast, in the lean state, macrophages promote ant inflammation, insulin sensitivity and tissue repair.*

and improved glucose sensitivity in a model of diet-induced obesity, when compared to WT animals [41]. Mox macrophages are driven by oxidised phospholipids derived from oxidised low density lipoproteins (LDL) [43] and express surface markers Srnx-1 and Txnrd-1 [42]. Mox macrophages have been studied primarily in the context of atherosclerosis, where the oxidation of accumulated LDL leads to enrichment of the tissue with oxidised lipids, causing the polarisation of macrophages towards a phenotype dependent on the transcription factor Nrf2 [43]. However, a recent paper identified that ATM with a Mox phenotype (CX3CR1$^{neg}$F4/80$^{low}$Txnrd1$^{+}$HO1$^{+}$) are the predominant phenotype present the AT of lean mice, driven by individual oxidised phospholipids in the AT [44]. It will be an interesting further area study to appraise the role of these novel macrophage phenotypes within the context of obesity and IR.

### 3.3 Regulators of macrophage polarization

Macrophage polarization has been well studied over the past decade leading to the discovery of several key regulators which orchestrate macrophage polarization, such as the Signal Transducer and Activator of Transcription (STAT) family, interferons, regulators of lipid metabolism, transcription factor families, microRNAs (miRNAs) and long non-coding RNAs [45] (**Figure 3**).

#### *3.3.1 STAT family members*

The Janus Kinase (JAK)/STAT signalling pathway transmits signals from extracellular cytokines into the nucleus. Indeed, JAK/STATs are arguably the most widely studied pathway within the context of macrophage polarisation, with IFNɣ binding

to its receptor triggering activation of JAK1/2-mediated tyrosine phosphorylation and subsequent dimerization of STAT1 one of the first pathways to M1 polarisation identified [46]. In addition, LPS binding to TLR4 induces autocrine production of IFN-ß that activates the type 1 IFN receptor triggering STAT1 and STAT2 phosphorylation and heterodimerisation [47]. STAT3 has a dichotomous role in macrophage polarisation, it is the key transcriptional regulator in the production of the anti-inflammatory cytokine IL-10, which can drive an anti-inflammatory macrophage phenotype, however, STAT3 can also be activated by IL-6 and IFN-ß, inducing a pro-inflammatory phenotype [48]. Conversely, IL-4 and IL-13 induces M2 macrophage polarisation largely through induction of STAT6 and KLF4 via the dual catalytic activities of MCP-1-induced protein and inducing PPAR-ɣ [49–51].

### *3.3.2 Interferon regulatory factors*

Interferon regulatory factors (IRFs) are intracellular proteins that regulate immune cell maturation and play a pivotal role in macrophage polarization. Two key and opposing IRFs in macrophage polarisation are IRF4 and IRF5, which directly compete for binding to MyD88 and subsequent transcription factors such as NFκB. Interestingly, IRF5 was shown to promote M1 macrophages, while IRF5 expression was upregulated in obese individuals compared to lean individuals at both the mRNA and protein levels and is negatively associated with insulin sensitivity [52–54]. It was also shown that IRF5 promotes inflammatory macrophage polarization by activating the transcription of IL-12 and repressing IL-10 [55]. Conversely, IRF4 acts as an antagonist of M1 macrophage polarisation, promoting M2 macrophages [53, 54, 56]. In the context of obesity, macrophage-specific knockout of IRF4 resulted in significant IR and an increase in expression of pro-inflammatory genes [57]. Furthermore, IRF6 has also been implicated in

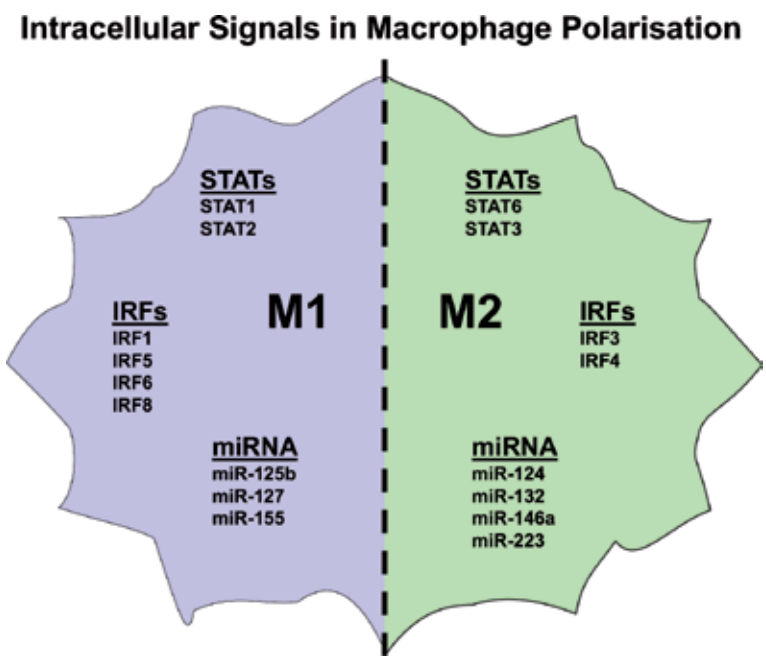


**Figure 3.**
*Intracellular signalling of M1 and M2 macrophage polarisation. Respective intracellular key regulators of M1 and M2 macrophage polarisation. Signal transducer and activator of transcription (STAT) family, interferons (IRFs) and microRNA (miRNAs).*

macrophage polarisation, promoting M1 macrophages due to suppression of PPARɣ expression, a critical regulator of M2 macrophages. Overexpression of IRF6 reduced M2 activation, whilst IRF6 knockdown enhanced M2 macrophage activation [58]. The impact of IRFs on macrophage polarisation and plasticity is clearly quite complex and further studies will hopefully provide information on how IRFs function in different microenvironments, for example, in lean versus obese AT.

*3.3.3 MicroRNAs*

miRNA are short inhibitory non-coding RNAs (~22 nucleotides) that degrade specific mRNA targets or block RNA translation, and have also been implicated in driving macrophage polarisation. Indeed, miR-125b expression was shown to be upregulated in murine macrophages following IFN-ɣ stimulation and was identified to promote M1 macrophage polarisation, while suppressing IRF4, an important M2 transcription factor [59]. Additionally, miR-155 was also shown to promote M1 polarisation with expression in murine macrophages increased upon TLR activation or stimulation with pro-inflammatory cytokines (TNF-α, IFN-β or IFN-ɣ) [45]. Whilst in human macrophages, miR-155 was shown to target IL-13Rα1 and inhibit STAT6 activation, thus inhibiting M2 macrophage polarisation [60]. Additionally, miR-9 enhances M1 macrophage polarisation by suppressing PPARδ [61]. Whilst miR-127 suppresses B-Cell lymphoma protein (Bc6), which promotes M1 polarisation [62]. Conversely, miR-124 promotes M2 polarisation via LPS-induced cytokine production by targeting STAT3 to decrease IL-6 production and reduce TNF-α [63]. Furthermore, miR-132, miR-146a and miR-223 induce M2 macrophages by inhibiting NF-κB [64–66].

### 3.4 Hypoxia and macrophage polarisation

Of interest in the context of obesity is the potential for hypoxia to influence macrophage polarisation of macrophages (**Figure 2**). Indeed, hypoxic areas in adipose tissue occur in obese individuals when rapid tissue expansion occurs without sufficient accompanying blood flow to these areas. M1 macrophages display high expression levels of Hypoxia-related genes including *Hif1α*, which has been shown to induce a pro-inflammatory phenotype in macrophages via TLR4 activation, involving the PI3K/Akt signalling pathway [67, 68]. TLR4 expression in macrophages was shown to increase in a hypoxic environment [69]. *Hif1α* enhances the transcriptional activity of NF-κB, driving production of pro-inflammatory cytokines and decreasing the induction of immune regulatory mediators [70]. In contrast, M2-like macrophages in obese individuals express *Hif2α* [71]. Indeed, HIF-2α is also upregulated under low oxygen levels [5]. It was shown that HIF-2α overexpressing macrophages suppressed pro-inflammatory responses and improved IR. Whilst knockdown of HIF-2α in macrophages induced pro-inflammatory gene expression in adipocytes [71]. Thus, it was suggested that HIF-2α counteracts the pro-inflammatory responses to relieve obesity induced IR in AT [71].

### 3.5 IR and macrophage polarisation

Insulin acts in the adipose tissue to promote uptake and storage of fatty acids, stored as triglycerides, and inhibits the lipolysis of stored triglycerides. IR is a reduced response to insulin in the liver, muscle and AT, due to an impairment in the insulin-signalling pathway, leading to hyperglycaemia [72]. In obesity, the increased recruitment of macrophages to AT positively correlates with IR [4]. Indeed, obese mice show increased macrophage-mediated inflammation that resulted in

long-term IR [73], with elevated levels of FFA which activated CD36 expressing macrophages and adipocytes, inducing inflammation and impairing insulin signalling [74]. In AT from lean states, the presence of M2 macrophages maintains insulin sensitivity via anti-inflammatory actions of IL-10 and STAT3 [25]. In obese individuals, there is a change in adipocyte metabolism and gene expression. Consequently, there is increased lipolysis and release of FFA which can lead to TLR4 activation of M1 macrophages [75]. Indeed, TLR4 expression is increased on macrophages in obese individuals. In co-culture studies, it was shown that macrophage activation through TLR4 signalling increased secretion of pro-inflammatory cytokines, blocking the insulin signalling cascade [76, 77]. In obese individuals, the IκB kinase (IKK) complex is activated in macrophages resulting in phosphorylation of IκBα on Ser32 and 36, degrading IκBα and allowing NF-κB to translocate to the nucleus and upregulated target genes such as pro-inflammatory cytokines including TNF-α, IL-1β and IL-6 [78].

Increased expression of pro-inflammatory cytokines negatively affects insulin signalling pathways [79]. The effect of these cytokines on IR are seen locally in AT, but also systemically as they are released into circulation [76]. TNF-α phosphorylates insulin receptor substrates (IRS), consequently preventing downstream signalling via inhibiting IKK, c-Jun N-terminal kinase (JNK) and atypical protein kinase C (aPKC) [80]. Interestingly, males have been shown to have increased TNF-α plasma concentration compared to females, leading to the suggestion that obese males are more susceptible to develop IR [81]. IL-6 is increased in the serum of obese individuals and mice, with weight loss reducing circulating IL-6, which improves insulin sensitivity [82]. Systemic IR has also observed during pregnancy, puberty and during infection such as sepsis driven by TNF-α and IL-6 [83]. It is clear from such studies that the presence of pro-inflammatory cells and the release of pro-inflammatory cytokines lead to a loss of sensitivity to insulin and a state of IR. This is possibly the biggest incentive to therapeutically target the macrophages in cases of metabolic disease.

## 4. The metabolic signature of macrophages

Metabolism is a series of highly interconnected pathways that generate metabolic products such as energy and macromolecules from nutrients in the microenvironment. Whilst the metabolic pathways are plastic, cells, in particular macrophages, tend to utilise a pathway that suits their immediate energy requirements. M1 macrophages have huge metabolic demands, and rely largely on glycolysis, conversely, M2 macrophages meet their energy requirements using oxidative phosphorylation (OXPHOS) pathways. During glycolysis, extracellular glucose is taken up by the cell and converted to two molecules of pyruvate and ATP; $NAD^+$ is converted to $NADH^+H^+$ regenerated through the breakdown of pyruvate to lactate. Glycolysis also provides the first molecule in the pentose-phosphate pathway, glucose-6-phosphate, which provides NADPH to maintain the cellular redox balance and the production of fatty acids. In M1 macrophages, the increased glucose consumption is associated with the capacity for rapid cytokine production and antimicrobial activity through ROS generation [84, 85]. Conversely, in the presence of oxygen cells produce ATP via the electron transport chain (ETC), which is linked to the tricarboxylic acid (TCA) cycle. The TCA cycle uses carbon sources, such as Acetyl CoA, glutamine or fatty acids to fuel a cycle which generates the reducing agents NADH and FADH2 that serve as electron carriers for the ETC for OXPHOS. Glycolysis is a poor producer of energy, with only two molecules of ATP per glucose molecule, compared to 36 molecules produced by OXPHOS. However,

the use of each pathway will depend on the environment and functional requirements of the cells, as glycolysis provides energy rapidly.

The TCA cycle is truncated in M1 macrophages, resulting in a reduced production of alpha-ketoglutarate (α-KG) and accumulation of citrate and succinate metabolites [86]. The accumulation of citrate leads to the production of the macrophage specific metabolite itaconic acid, which is a major feature of LPS stimulated macrophages [86]. The build-up of itaconic acid has been identified as a driver for succinate accumulation, through its ability to inhibit succinate dehydrogenase [87, 88]. The excess succinate leads to the induction of IL-1β through the stabilization of HIF-1a further enhancing inflammation in the macrophages [89]. Indeed, blocking glycolysis reduces release of CCL2 from TNF-a or LPS stimulated adipocytes, providing further evidence for a link between metabolism and inflammation [90] (**Figure 4**).

Macrophage polarization is also influenced by the metabolism of arginine. M1 macrophages upregulated nitric oxide synthase (iNOS), which catabolize arginine to citrulline and nitric oxide (NO). This NO is important for intracellular killing of pathogens. In addition, M1 macrophages use the pentose phosphate pathway, which generates NADPH for the NADPH oxidase, which produces ROS and NO. Consequently, these metabolic pathways provide M1 macrophages with rapid energy. Conversely, in M2 macrophages, arginase-1 (Arg1) is induced which produces urea, ornithine and polyamines which are key in tissue repair [36, 91].

The metabolic profile of ATM alters dependent on the microenvironment of the AT. In mice, transcriptome and extracellular flux analysis have shown that in lean AT fatty acid oxidation, glycolysis and glutaminolysis all participate in cytokine release by ATM [92]. In obese AT, both glycolysis and OXPHOS are utilised, however glycolysis takes precedence, potentially due to the hypoxic environment in the AT [92].

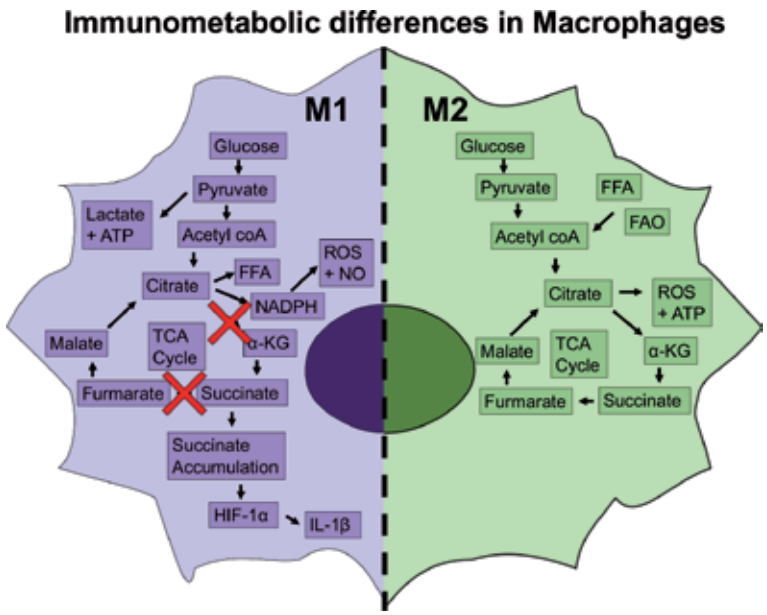


**Figure 4.**
*Immunometabolic differences in M1 and M2 macrophages. M2 macrophages use OXPHOS and the TCA cycle to produce energy. They have increased ability to uptake free fatty acid (FFA) and fatty acid oxidation (FAO) to facilitate the TCA cycle. In contrast, M1 macrophages increase energy production oxidative glycolysis. M1 macrophages also have a 'broken' TCA cycle, resulting in accumulate of citrate and succinate. Increased succinate results in secretion of pro-inflammatory cytokine IL-1β via HIF-1α. TCA, tricarboxylic acid; HIF-1a, hypoxia-inducible factor-1a; a-KG, alpha-ketoglutarate.*

## 5. Could targeting macrophages provide a therapeutic strategy in metabolic disorders?

As described above, as macrophages play a significant role in obesity and other metabolic disorders they are an attractive therapeutic target. The therapeutic strategies that target macrophages look to re-educate polarized macrophages, depletion of polarized macrophages or silencing macrophages. Additionally, the link between macrophage polarization and cellular metabolism suggests a potential therapeutic strategy by modulating the macrophage metabolic state. There are several therapeutic strategies commonly used to target macrophages such as depletion, proliferation, inflammation and gene silencing.

### 5.1 Macrophage depletion

It was shown that macrophages could be depleted *in vivo* by inducing apoptosis following accumulation of toxic particles [93]. Interesting, it was shown that by depletion of pro-inflammatory macrophages resulted in normalizing insulin sensitivity in IR obese mice [94]. Furthermore, in obese mice, the depletion of visceral adipose tissue macrophages (VATMs) by Intraperitoneal injection of clodronate liposomes, results in improved systemic insulin sensitivity, glucose homeostasis and further blocked high-fat diet-induced weight gain [95, 96]. Consequently, depletion of VATMs also resulted in prevention of CLS in WAT and a low level of blood TNF-α [96]. However, liposomes treatment as a therapy is prone to degradation and significant risks of potential off-target effects. An alternative approach is altering macrophage proliferation, such as using a nanoparticle-based delivery of simvastatin, which may provide therapeutic benefit for atherosclerosis [97]. However, as previously mentioned, in obesity, macrophages are recruited from circulating monocytes, so reducing proliferation may not provide therapeutic benefit for IR [98].

### 5.2 Biological therapeutics

There are several orally active synthetic ligands for PPARɣ which are used to treat IR in patients with T2D. It has been shown *in vivo* that pioglitazone, belonging to the chemical class thiazolidinediones, reduces LPS induced TLR2 and TLR4 expression on peritoneal macrophages. Whilst *in vitro*, pioglitazone reduces the synthesis and gene expression of TLR2, TLR4, IL-1β, TNF-α, IL-6 and MCP-1 in human blood monocytes [99]. However, the use of thiazolidinediones like pioglitazone in clinical studies to treat T2D has resulted in increased cardiovascular events and death [100].

Clinical studies have shown that anti-inflammatories are efficacious in patients with systemic IR. Members of the interferon family have been used to suppress the release of pro-inflammatory cytokines, however, the use of type 1 interferons, as well as other anti-inflammatory strategies, is associated with cell toxicity in long-term use. Recently studies using interferon tau (IFNT), an alternative member of the type 1 interferon family, in mice with diet-induced obesity show enhanced insulin sensitivity when compared to untreated mice. There was also a significant decrease in secretion of pro-inflammatory cytokines and increased M2 macrophages in AT, suggesting IFNT as a novel bio-therapeutic agent for treating obesity-associated disorders [101].

Interestingly, yeast-derived β-glucans (Y-BGs) have been shown to be beneficial in models for obesity. In obese humans, Y-GBs administered orally increased AT

expression of anti-inflammatory cytokine IL-10 and serum IL-10 [102]. In addition, macrophages uptake of Y-GBs increased reactive oxygen species (ROS) formation, phagosomal maturation and induction of autophagy [103].

### 5.3 RNA interference

Another attractive therapeutic approach in targeting macrophage polarisation would be to use RNA interference (RNAi), which reduces gene expression. This approach could target the inflammatory mediators such as TNF-α, IL-6 and IL-1β [98]. Indeed, it was shown that intraperitoneal (i.p.) administration of small interfering RNA (siRNA) selectively silenced genes such as TNF-α in epididymal ATM of obese mice and improved glucose tolerance [7]. Additionally, it was shown that i.p. administration of a rabies virus glycoprotein-derived acetylcholine receptor-binding peptide delivers siRNA into ATM and peritoneal macrophages in HFD mice. This resulted in inhibition of ATM infiltration and reduced pro-inflammatory cytokines, thus improving glucose tolerance and insulin sensitivity [104].

### 5.4 Metabolic reprogramming

As stated previously, macrophage phenotypes have distinct metabolism pathways. Therefore, altering the metabolic state of macrophages provides a potential therapeutic approach to metabolic disorders. Indeed, the strong link between macrophage polarization and cellular metabolism makes altering the metabolic state of the cells an attractive therapeutic prospect. To prove this principle, inducing oxidative metabolism in M1 macrophages has been shown to shift the phenotype to an M2 profile [105], while blocking oxidative metabolism in macrophages inhibits the M2 phenotype and drives the M1 macrophage phenotype. Furthermore, it was shown that by driving macrophage metabolism with glucose, insulin and fatty acids resulted in an increased pro-inflammatory ATM phenotype in obese mice [32].

Modulation of the metabolic pathways in macrophages has been studied extensively in recent years to assess the extent to which inflammatory status can be influenced by the metabolic profile of the cells. Glucose transporter (GLUT)-1 is upregulated in macrophages localised to the CLS in inflamed obese AT. *In vitro* studies show that overexpression of GLUT1 increases glucose uptake in the cells and induces release of pro-inflammatory cytokines, linking the metabolic phenotype with the inflammatory function of the cells [106]. Furthermore, knockout of fatty acid transporter protein (FATP)-1 expression, which is elevated in M2 macrophages, is associated with priming of macrophages towards an M1 phenotype, upregulating expression of NOS1 [107]. Further pathways of current interest to therapeutically target include Notch, carbohydrate kinase-like protein (CARKL), mammalian target of rapamycin (mTOR), IL-4 and IL-10, all of which show intricate links between the metabolic and inflammatory pathways in macrophages [108–112].

## 6. Conclusions

Obesity has long been considered a low-grade systemic inflammatory condition, which appears to be mediated largely through the prominent populations of ATM. Alterations in environmental cues, including changes in metabolites, the microbiota and inflammatory stimuli act to influence the ATM, coordinating the recruitment of pro-inflammatory monocytes and altering the metabolic state of ATM. While in lean individuals the resident ATM function to clear dead adipocytes and sequester excess lipids from the AT to maintain homeostasis within the AT,

the recruited inflammatory ATM release pro-inflammatory cytokines to induce inflammation within the AT and are involved in the pathogenic remodelling of the AT. This state of inflammation within the ATM is largely associated with IR and metabolic dysfunction through interference with insulin signalling pathways. Macrophages are heterogenous and extremely plastic and as such it has historically been difficult to define subsets. With the use of transcriptional and metabolic profiling it is now becoming possible to appraise the full role of ATM in obesity. This knowledge will aid the search for novel therapeutics targeting the metabolic capacity and inflammatory potential of ATM, restoring the homeostatic functions of resident lean ATM, to modulate obesity.

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## Conflict of interest

The authors declare no conflict of interest.

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## References

[1] WHO. WHO—Obesity and Overweight. 2018. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight [Accessed: 19 April 2019]

[2] Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annual Review of Immunology. 2011;**29**:415-445

[3] Weisberg SP et al. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of Clinical Investigation. 2003;**112**(12):1796-1808

[4] Xu H et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. The Journal of Clinical Investigation. 2003;**112**(12):1821-1830

[5] Fang HY et al. Hypoxia-inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. Blood. 2009;**114**(4):844-859

[6] Xue J et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity. 2014;**40**(2):274-288

[7] Aouadi M et al. Gene silencing in adipose tissue macrophages regulates whole-body metabolism in obese mice. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(20):8278-8283

[8] Sittipo P et al. Intestinal microbiota and the immune system in metabolic diseases. Journal of Microbiology. 2018;**56**(3):154-162

[9] Cox LM, Blaser MJ. Pathways in microbe-induced obesity. Cell Metabolism. 2013;**17**(6):883-894

[10] Turnbaugh PJ et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;**444**(7122):1027-1031

[11] Backhed F et al. The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(44):15718-15723

[12] Mraz M, Haluzik M. The role of adipose tissue immune cells in obesity and low-grade inflammation. The Journal of Endocrinology. 2014;**222**(3):R113-R127

[13] Vrieze A et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology. 2012;**143**(4):913-6.e7

[14] Jo J et al. Hypertrophy and/or hyperplasia: Dynamics of adipose tissue growth. PLoS Computational Biology. 2009;**5**(3):e1000324

[15] Rosen ED, Spiegelman BM. What we talk about when we talk about fat. Cell. 2014;**156**(1-2):20-44

[16] Guzik TJ et al. The role of infiltrating immune cells in dysfunctional adipose tissue. Cardiovascular Research. 2017;**113**(9):1009-1023

[17] Harman-Boehm I et al. Macrophage infiltration into omental versus subcutaneous fat across different populations: Effect of regional adiposity and the comorbidities of obesity. The Journal of Clinical Endocrinology and Metabolism. 2007;**92**(6):2240-2247

[18] Wu D et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science. 2011;**332**(6026):243-247

[19] Molofsky AB et al. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. The Journal of Experimental Medicine. 2013;**210**(3):535-549

[20] Winer DA et al. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nature Medicine. 2011;**17**(5):610-617

[21] DeFuria J et al. B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(13):5133-5138

[22] Nishimura S et al. $CD8^+$ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nature Medicine. 2009;**15**(8):914-920

[23] Exley MA et al. Interplay between the immune system and adipose tissue in obesity. The Journal of Endocrinology. 2014;**223**(2):R41-R48

[24] Feuerer M et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nature Medicine. 2009;**15**(8):930-939

[25] Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of Clinical Investigation. 2007;**117**(1):175-184

[26] Nguyen KD et al. Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature. 2011;**480**(7375):104-108

[27] Qiu Y et al. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell. 2014;**157**(6):1292-1308

[28] Fischer K et al. Alternatively activated macrophages do not synthesize catecholamines or contribute to adipose tissue adaptive thermogenesis. Nature Medicine. 2017;**23**(5):623-630

[29] Prieur X et al. Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. Diabetes. 2011;**60**(3):797-809

[30] Murano I et al. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. Journal of Lipid Research. 2008;**49**(7):1562-1568

[31] Cinti S et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. Journal of Lipid Research. 2005;**46**(11):2347-2355

[32] Kratz M et al. Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. Cell Metabolism. 2014;**20**(4):614-625

[33] Ginhoux F, Guilliams M. Tissue-resident macrophage ontogeny and homeostasis. Immunity. 2016;**44**(3):439-449

[34] Lumeng CN et al. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. Diabetes. 2008;**57**(12):3239-3246

[35] Oh DY et al. Increased macrophage migration into adipose tissue in obese mice. Diabetes. 2012;**61**(2):346-354

[36] Galvan-Pena S, O'Neill LAJ. Metabolic reprograming in

macrophage polarization. Frontiers in Immunology. 2014;**5**:420

[37] Van Dyken SJ, Locksley RM. Interleukin-4- and interleukin-13-mediated alternatively activated macrophages: Roles in homeostasis and disease. Annual Review of Immunology. 2013;**31**:317-343

[38] Ying W et al. MicroRNA-223 is a crucial mediator of PPARgamma-regulated alternative macrophage activation. The Journal of Clinical Investigation. 2015;**125**(11):4149-4159

[39] Stienstra R et al. Peroxisome proliferator-activated receptor gamma activation promotes infiltration of alternatively activated macrophages into adipose tissue. The Journal of Biological Chemistry. 2008;**283**(33):22620-22627

[40] Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: Time for reassessment. F1000Prime Reports. 2014;**6**:13

[41] Coats BR et al. Metabolically activated adipose tissue macrophages perform detrimental and beneficial functions during diet-induced obesity. Cell Reports. 2017;**20**(13):3149-3161

[42] Russo L, Lumeng CN. Properties and functions of adipose tissue macrophages in obesity. Immunology. 2018;**155**(4):407-417

[43] Kadl A et al. Identification of a novel macrophage phenotype that develops in response to atherogenic phospholipids via Nrf2. Circulation Research. 2010;**107**(6):737-746

[44] Serbulea V et al. Macrophage phenotype and bioenergetics are controlled by oxidized phospholipids identified in lean and obese adipose tissue. Proceedings of the National Academy of Sciences of the United States of America. 2018;**115**(27):E6254-E6263

[45] Li C et al. Macrophage polarization and meta-inflammation. Translational Research. 2018;**191**:29-44

[46] Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science. 1994;**264**(5164):1415-1421

[47] Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: Enabling diversity with identity. Nature Reviews. Immunology. 2011;**11**(11):750-761

[48] Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: Cancer as a paradigm. Nature Immunology. 2010;**11**(10):889-896

[49] Kapoor N et al. Transcription factors STAT6 and KLF4 implement macrophage polarization via the dual catalytic powers of MCPIP. Journal of Immunology. 2015;**194**(12):6011-6023

[50] Malyshev I, Malyshev Y. Current concept and update of the macrophage plasticity concept: Intracellular mechanisms of reprogramming and M3 macrophage "switch" phenotype. BioMed Research International. 2015;**2015**:341308

[51] Odegaard JI et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature. 2007;**447**(7148):1116-1120

[52] Thomas RS et al. Increased expression of IRF-5 in the adipose tissue in obesity: Implication in metabolic inflammation. The Journal of Immunology. 2016;**196**(1 Supplement): 124.51

[53] Gunthner R, Anders HJ. Interferon-regulatory factors determine macrophage phenotype polarization.

Mediators of Inflammation. 2013;**2013**:731023

[54] Dalmas E et al. Irf5 deficiency in macrophages promotes beneficial adipose tissue expansion and insulin sensitivity during obesity. Nature Medicine. 2015;**21**(6):610-618

[55] Krausgruber T et al. IRF5 promotes inflammatory macrophage polarization and TH1–TH17 responses. Nature Immunology. 2011;**12**(3):231-238

[56] El Chartouni C, Schwarzfischer L, Rehli M. Interleukin-4 induced interferon regulatory factor (Irf) 4 participates in the regulation of alternative macrophage priming. Immunobiology. 2010;**215**(9-10):821-825

[57] Eguchi J et al. Interferon regulatory factor 4 regulates obesity-induced inflammation through regulation of adipose tissue macrophage polarization. Diabetes. 2013;**62**(10):3394-3403

[58] Li C et al. IRF6 regulates alternative activation by suppressing PPARgamma in male murine macrophages. Endocrinology. 2017;**158**(9):2837-2847

[59] Chaudhuri AA et al. MicroRNA-125b potentiates macrophage activation. Journal of Immunology. 2011;**187**(10):5062-5068

[60] Martinez-Nunez RT, Louafi F, Sanchez-Elsner T. The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 via direct targeting of interleukin 13 receptor alpha1 (IL13Ralpha1). The Journal of Biological Chemistry. 2011;**286**(3):1786-1794

[61] Thulin P et al. MicroRNA-9 regulates the expression of peroxisome proliferator-activated receptor delta in human monocytes during the inflammatory response. International Journal of Molecular Medicine. 2013;**31**(5):1003-1010

[62] Ying H et al. MiR-127 modulates macrophage polarization and promotes lung inflammation and injury by activating the JNK pathway. Journal of Immunology. 2015;**194**(3):1239-1251

[63] Sun Y et al. MicroRNA-124 mediates the cholinergic anti-inflammatory action through inhibiting the production of pro-inflammatory cytokines. Cell Research. 2013;**23**(11):1270-1283

[64] Liu F et al. MiR-132 inhibits lipopolysaccharide-induced inflammation in alveolar macrophages by the cholinergic anti-inflammatory pathway. Experimental Lung Research. 2015;**41**(5):261-269

[65] Taganov KD et al. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(33):12481-12486

[66] Zhuang G et al. A novel regulator of macrophage activation: miR-223 in obesity-associated adipose tissue inflammation. Circulation. 2012;**125**(23):2892-2903

[67] Fujisaka S et al. Adipose tissue hypoxia induces inflammatory M1 polarity of macrophages in an HIF-1alpha-dependent and HIF-1alpha-independent manner in obese mice. Diabetologia. 2013;**56**(6):1403-1412

[68] Kim SY et al. PI3K/Akt contributes to increased expression of Toll-like receptor 4 in macrophages exposed to hypoxic stress. Biochemical and Biophysical Research Communications. 2012;**419**(3):466-471

[69] Kim SY et al. Hypoxic stress up-regulates the expression of Toll-like receptor 4 in macrophages via hypoxia-inducible factor. Immunology. 2010;**129**(4):516-524

[70] Liu W et al. Targeted genes and interacting proteins of hypoxia inducible factor-1. International Journal of Biochemistry and Molecular Biology. 2012;**3**(2):165-178

[71] Choe SS et al. Macrophage HIF-2alpha ameliorates adipose tissue inflammation and insulin resistance in obesity. Diabetes. 2014;**63**(10):3359-3371

[72] Castoldi A et al. The macrophage switch in obesity development. Frontiers in Immunology. 2015;**6**:637

[73] Lee YS et al. Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. Diabetes. 2011;**60**(10):2474-2483

[74] Kennedy DJ et al. A CD36-dependent pathway enhances macrophage and adipose tissue inflammation and impairs insulin signalling. Cardiovascular Research. 2011;**89**(3):604-613

[75] Nguyen MT et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. The Journal of Biological Chemistry. 2007;**282**(48):35279-35292

[76] Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annual Review of Physiology. 2010;**72**:219-246

[77] Norseen J et al. Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism. Molecular and Cellular Biology. 2012;**32**(10):2010-2019

[78] Chen L et al. Mechanisms linking inflammation to insulin resistance. International Journal of Endocrinology. 2015;**2015**:508409

[79] Hotamisligil GS. Inflammatory pathways and insulin action. International Journal of Obesity and Related Metabolic Disorders. 2003;**27**(Suppl 3):S53-S55

[80] Nieto-Vazquez I et al. Insulin resistance associated to obesity: The link TNF-alpha. Archives of Physiology and Biochemistry. 2008;**114**(3):183-194

[81] El-Haggar SM, Mostafa TM. Adipokines and biochemical changes in Egyptian obese subjects: Possible variation with sex and degree of obesity. Endocrine. 2015;**48**(3):878-885

[82] Bastard JP et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. The Journal of Clinical Endocrinology and Metabolism. 2000;**85**(9):3338-3342

[83] Lauterbach MA, Wunderlich FT. Macrophage function in obesity-induced inflammation and insulin resistance. Pflügers Archiv. 2017;**469**(3-4):385-396

[84] West AP et al. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature. 2011;**472**(7344):476-480

[85] Weichhart T, Hengstschlager M, Linke M. Regulation of innate immune cell function by mTOR. Nature Reviews Immunology. 2015;**15**(10):599-614

[86] Jha AK et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity. 2015;**42**(3):419-430

[87] Cordes T et al. Immunoresponsive gene 1 and itaconate inhibit succinate dehydrogenase to modulate intracellular succinate levels. The

Journal of Biological Chemistry. 2016;**291**(27):14274-14284

[88] Lampropoulou V et al. Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. Cell Metabolism. 2016;**24**(1):158-166

[89] Tannahill GM et al. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature. 2013;**496**(7444):238-242

[90] Grant RW, Boudreaux JI, Stephens JM. 2-deoxyglucose inhibits induction of chemokine expression in 3T3-L1 adipocytes and adipose tissue explants. Obesity (Silver Spring). 2017;**25**(1):76-84

[91] Geelhaar-Karsch A et al. Evaluation of arginine metabolism for the analysis of M1/M2 macrophage activation in human clinical specimens. Inflammation Research. 2013;**62**(9):865-869

[92] Boutens L et al. Unique metabolic activation of adipose tissue macrophages in obesity promotes inflammatory responses. Diabetologia. 2018;**61**(4):942-953

[93] van Rooijen N, van Kesteren-Hendrikx E. "In vivo" depletion of macrophages by liposome-mediated "suicide". Methods in Enzymology. 2003;**373**:3-16

[94] Patsouris D et al. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metabolism. 2008;**8**(4):301-309

[95] Feng B et al. Clodronate liposomes improve metabolic profile and reduce visceral adipose macrophage content in diet-induced obese mice. PLoS One. 2011;**6**(9):e24358

[96] Bu L et al. Intraperitoneal injection of clodronate liposomes eliminates visceral adipose macrophages and blocks high-fat diet-induced weight gain and development of insulin resistance. The AAPS Journal. 2013;**15**(4):1001-1011

[97] Tang J et al. Inhibiting macrophage proliferation suppresses atherosclerotic plaque inflammation. Science Advances. 2015;**1**(3)

[98] Peterson KR et al. Macrophage-targeted therapeutics for metabolic disease. Trends in Pharmacological Sciences. 2018;**39**(6):536-546

[99] Dasu MR et al. Pioglitazone inhibits Toll-like receptor expression and activity in human monocytes and db/db mice. Endocrinology. 2009;**150**(8):3457-3464

[100] Stafylas PC, Sarafidis PA, Lasaridis AN. The controversial effects of thiazolidinediones on cardiovascular morbidity and mortality. International Journal of Cardiology. 2009;**131**(3):298-304

[101] Ying W et al. Interferon tau alleviates obesity-induced adipose tissue inflammation and insulin resistance by regulating macrophage polarization. PLoS One. 2014;**9**(6):e98835

[102] Kohl A et al. Increased interleukin-10 but unchanged insulin sensitivity after 4 weeks of (1, 3)(1, 6)-beta-glycan consumption in overweight humans. Nutrition Research. 2009;**29**(4):248-254

[103] Fatima N et al. Particulate beta-glucan induces early and late phagosomal maturation in murine macrophages. Frontiers in Bioscience (Elite Edition). 2017;**9**:129-140

[104] Kim J et al. Silencing CCR2 in macrophages alleviates adipose tissue inflammation and the associated metabolic syndrome in dietary obese mice. Molecular Therapy-Nucleic Acids. 2016;**5**:e280

[105] Rodriguez-Prados JC et al. Substrate fate in activated macrophages: A comparison between innate, classic, and alternative activation. Journal of Immunology. 2010;**185**(1):605-614

[106] Freemerman AJ et al. Metabolic reprogramming of macrophages: Glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. The Journal of Biological Chemistry. 2014;**289**(11):7884-7896

[107] Johnson AR et al. Metabolic reprogramming through fatty acid transport protein 1 (FATP1) regulates macrophage inflammatory potential and adipose inflammation. Molecular Metabolism. 2016;**5**(7):506-526

[108] Xu J et al. NOTCH reprograms mitochondrial metabolism for proinflammatory macrophage activation. The Journal of Clinical Investigation. 2015;**125**(4):1579-1590

[109] Haschemi A et al. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. Cell Metabolism. 2012;**15**(6):813-826

[110] Byles V et al. The TSC-mTOR pathway regulates macrophage polarization. Nature Communications. 2013;**4**:2834

[111] Van den Bossche J et al. Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. Cell Reports. 2016;**17**(3):684-696

[112] Ip WKE et al. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science. 2017;**356**(6337):513-519

Chapter 3

# Wnt Signaling Regulates Macrophage Mediated Immune Response to Pathogens

*Suborno Jati and Malini Sen*

## Abstract

Infection with pathogenic microbes is a global threat. Macrophages play a fundamental role in promoting host resistance to deadly infections from pathogenic microbes by virtue of a well-orchestrated immune defense system. Phagocytosis and obliteration of invading pathogens by macrophages are an innate immune function that not only sustains immune homeostasis but also bolsters adaptive immune response through antigen processing and presentation. Wnt signaling, where Wnt, a secreted glycoprotein which interacts with Frizzled and ROR cell surface receptors to initiate cellular interactions, could be vital for the immune response executed and propagated by macrophages in both innate and adaptive immune responses. The goal of this chapter is to describe how Wnt signaling influences phagocytosis, autophagy, and transcriptional activation to enable the macrophage to exercise its immune response program to resist infection.

**Keywords:** macrophage, Wnt, phagocytosis, actin cytoskeleton, transcription, immunity

## 1. Introduction

### 1.1 Macrophages: innate and adaptive immunity

Macrophages are present as crucial members of a multitude of specialized cells that fortify our immune system by fighting against infection caused by pathogens [1]. Macrophages differentiate from tissue-infiltrated circulating monocytes, which originate from bone marrow resident myeloid precursors [2, 3]. All tissue macrophages, however, do not originate from monocytes. Although some macrophage origins have been studied carefully, the detailed molecular mechanisms toward the differentiation of different macrophage types remain mostly uncharacterized [4–7]. Irrespective of their origin, most macrophages eliminate encountered pathogens through phagocytosis (element of innate immunity) and additionally present the foreign antigens derived from pathogens via major histocompatibility complex (MHC) molecules to lymphocytes leading to lymphocyte activation (element of adaptive immunity) [2, 8]. Cytoskeletal modulations and transcriptional activation programs intrinsically associated with macrophage-mediated immune functions (e.g. phagocytosis, autophagy/xenophagy) conform to the in-built maneuvering of macrophages as they confront with different kinds of pathogens. Several lines of

evidence substantiate that Wnt signaling is important for the transcriptional programs and cytoskeletal modulations inherent to macrophages during immune surveillance and response to different kinds of infection [9–13].

### 1.2 Wnt signaling

Wnt signaling is an integral theme of tissue/organ morphogenesis, repair, and maintenance. Thus, it is not surprising that this central premise of life is also an important component of macrophage function [9–16]. Wnts constitute a large family of secreted glycoprotein ligands, which bind to Frizzled and/or ROR cell surface receptors during various phases of tissue and organ development, morphogenesis, and homeostasis. Frizzleds are seven transmembrane-spanning receptors bearing homology to heterotrimeric G protein-coupled receptors, and RORs bear homology to tyrosine kinase receptors [17–20]. Based on the gene database, there are about 19 Wnt ligands and about 12 and 2 Frizzled and ROR receptors, respectively [21, 22]. Whether all these gene products are expressed and functional in our system in different cellular contexts is unclear at this stage. Although there is evidence of co-receptor function by the ROR subtype receptors during Wnt-Frizzled signaling [22, 23], the degrees of coordination between the Frizzled and ROR receptors under different physiological conditions are yet to be characterized at the molecular level. Given the considerable homology among the respective members of the Wnt and Frizzled families, any one Wnt ligand may interact with multiple Frizzled receptors. Thus, the outcome of Wnt-Frizzled signaling in a particular cell type under a certain condition could be dependent precisely on the existing profile of Wnt-Frizzled stoichiometry [20].

Wnt signaling is broadly classified into two types—canonical or β-catenin-dependant and noncanonical or β-catenin-independent (**Figure 1**). The transcriptional coactivator β-catenin promotes gene expression by LEF/TCF family transcription factors in response to canonical Wnt signaling, and transcriptional activators such as NFκB, NFAT, and AP1 are associated with noncanonical Wnt signaling. Even though the ligands Wnt3A and Wnt5A are mostly considered as representatives of the canonical and noncanonical modes of Wnt signaling, respectively [21, 24], the mode of signaling is in reality governed by the receptor(s) receiving the Wnt signal as mentioned above and the associated adaptor molecule(s) transmitting it. Thus, some level of crosstalk between the two modes of signaling would not be uncommon. Interestingly, the intracellular adaptor molecule Disheveled acts as a mediator of both β-catenin-dependant and β-catenin-independent Wnt signaling. Heterotrimeric G proteins, which have been reported to couple with Frizzled receptors, add to the complexity of Wnt signaling [18, 25]. Whether heterotrimeric G proteins cooperate with Disheveled during canonical and noncanonical Wnt signaling is not known clearly. Although there is some evidence of the involvement of lipid molecules such as cholesterol in switching Disheveled between the canonical and noncanonical modes of Wnt signaling [25], the molecular details of such presumed conformational switches remain largely undefined. The reason behind the preference of cell surface coactivator receptors such as lipoprotein receptor-like protein (LRP) 5/6 for the canonical mode of Wnt signaling as opposed to the noncanonical mode also remains unclear (**Figure 1**).

### 1.3 Wnt signaling in immune system

Given that host cytoskeletal rearrangements encompassing phagocytosis and autophagy/xenophagy and transcriptional regulation of immune defense genes

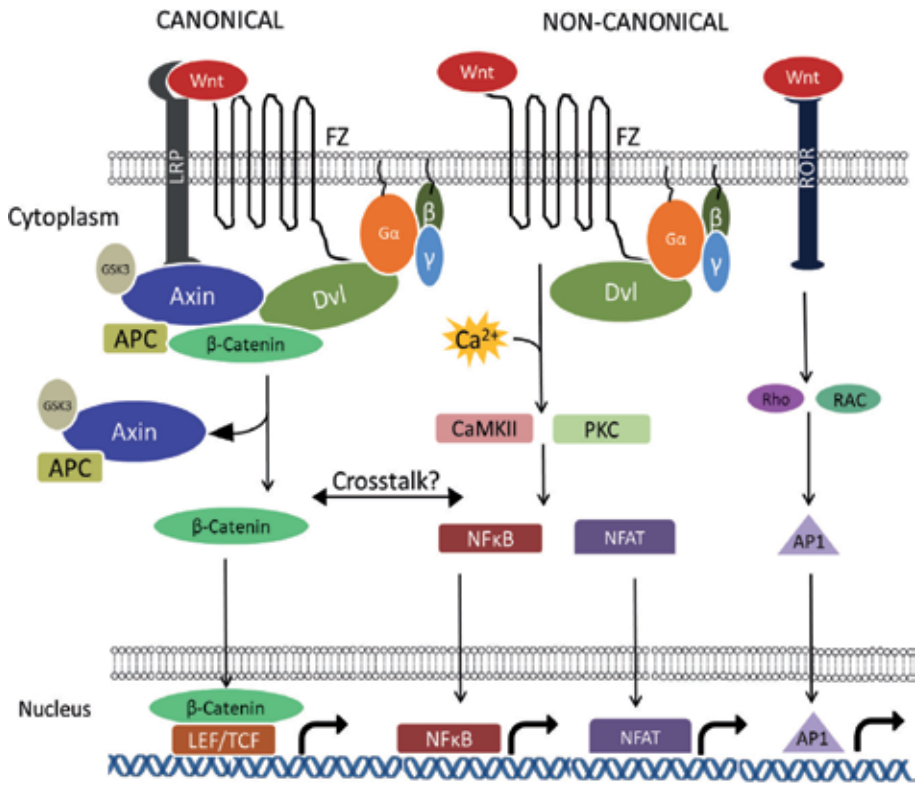


**Figure 1.**
*An overview of Wnt signaling cascade: in canonical mode of signaling, the association of Wnt-Fz and LRP activates a signaling cascade through Dvl and/or G-proteins that leads to inactivation of a GSK3 associated destruction complex which in the absence of Wnt would phosphorylate β-catenin for terminal destruction by proteasome. Via GSK3 inactivation, β-catenin gets stabilized and translocates to the nucleus where it acts as a co-activator of LEF/TCF (transcription factor). In the non-canonical mode of Wnt signaling (often β-catenin independent) the signaling cascade through Dvl and/or G-protiens leads to activation of Ca2+ mediated signaling where protein kinase C (PKC) and CaMKII gets activated and leads to translocation of NFκB, NFAT to the nucleus. Wnt also binds to ROR leading to activation of AP1. A crosstalk between the pathways is not uncommon.*

come into the direct line of control of pathogenic incursions and immune homeostasis [9–12, 26], Wnt signaling aptly associates with host-pathogen interactions of macrophages at the crossroads of innate and adaptive immunity. The attributes of Wnt signaling and the microbe world being diverse, their mutual interactions in the various host defense programs are expected to be manifold. Although Wnt3A and Wnt5A are often represented as the prototypes for the two different modes of Wnt signaling (canonical and noncanonical) in the regulation of immune response, several molecular details of the balancing act of the Wnts in relation to the interactions of macrophages with different microbes remain unclear.

The primary objective of this chapter is to briefly summarize the conceptual advancement in the context of Wnt signaling and immune defense by macrophages, focusing mainly on transcriptional activation and the actin cytoskeleton-associated phagocytosis and autophagy machineries. Our aim is to also address unanswered questions, which may prove instrumental in bridging existing gaps in our evaluation of the Wnts in the context of macrophage host defense programs.

## 2. Sustenance of immune defense by macrophages through a steady state of transcriptional activation by Wnt signaling

### 2.1 Significance of constitutive transcriptional activation in macrophages by Wnt signaling

Macrophages have long been acknowledged for executing immune defense against microbial pathogens through diverse means of signaling that include several transcription factors including NFκB, AP1, and NFAT [27–30]. The ability of macrophages to recognize and engulf pathogens, deliberate NADPH oxidase activity, and process antigens for presentation to MHC molecules and T cell activation place macrophages quite aptly at the crossroad of innate and adaptive immune defense programs [31–33]. Surely, macrophages have in-built mechanisms to execute innate immunity and translate it to adaptive immune response. However, not much is known about the molecular details of how macrophages are naturally geared to operate in such innate and adaptive modes of immune defense. We recently demonstrated that NF-κB (p65) [34], a transcription factor functioning at the core of our immune system, remains activated at a basal level in macrophages through a steady state of Wnt5A signaling. Administration of inhibitor of Wnt production2 (IWP2) to macrophages in culture or depletion of Wnt5A or Frizzled5 (putative Wnt5A receptor) gene expression in macrophages by silencing gene transcription through small interfering RNA blocks constitutive p65 activation and the steady-state immune activity of macrophages [10]. Sustained presence of the Wnt5A-p65 axis can potentially bridge innate and adaptive immune responses through regulation of the expression of immune response genes, such as CD14, interferons (IFN)s, and MHC, and elaboration of immune signaling networks that involve major immune response molecules such as the Toll-like receptors (TLR) and nucleotide-binding oligomerization domain-containing proteins (NOD) during challenge by pathogens [13, 35, 36]. The interrelation of this basal level Wnt5A-p65 signaling with other major transcription factors and coactivators of Wnt signaling that mediate immune response by macrophages remains to be deciphered at the molecular level.

### 2.2 NF-κB transcription factors

NF-κB transcription factors comprise a family of five members: p52, p50, p65 (RelA), c-Rel, and RelB, which regulate gene transcription as combinatorial dimers [34, 37, 38]. These dimers remain or become activated through different modes depending on the physiological context of cell signaling. In the classical mode of activation, the homo and heterodimers are translocated to the nucleus for gene expression after being released from the IκB-bound states in the cytoplasm in response to different stimuli that lead to proteasome-assisted IκB degradation through activation of the IκB kinase IKK2/β [34]. The p65 homo and heterodimers while being responsible for inflammatory gene expression are also significantly involved in the sustenance of innate immune response gene expression in a context-dependent manner [10]. Some of the NF-κB (p65) responsive immune response genes include CD14, MHC, and IFNs. A schematic of NF-κB activation is shown in **Figure 2**.

### 2.3 Wnt5A signaling-mediated activation of transcription

As mentioned earlier in this chapter, Wnt5A is one of several members of the large family of Wnt glycoprotein ligands. Frizzled-5, Frizzled-4, and ROR1 are putative receptors for Wnt5A. It is to be noted that although modified versions of selective Wnt-Frizzled complex structures have been solved [39], none of the ligand-receptor

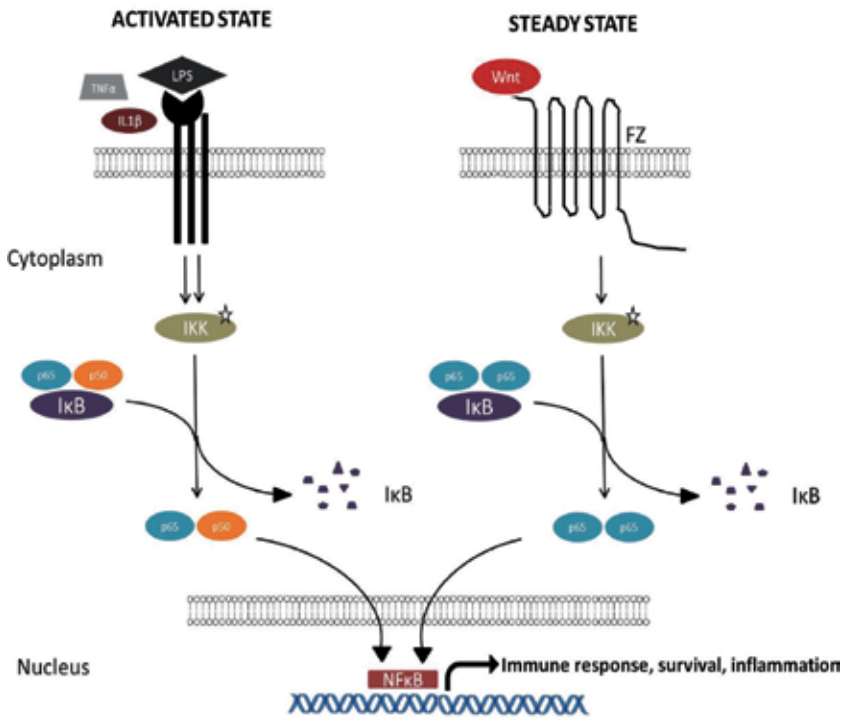


**Figure 2.**
*An overview of NFKB activation pathway in the macrophage: During steady state a basal level of stimulus by Wnt signaling keeps IKK enough activated to result in inactivation of IκB and translocation of a certain pool of NFκB transcription factor (p65 homodimer) to the nucleus. A minimum pool of transcription factors contributes to survival and vigilance for immune response. In the activated state, during inflammation and chronic infection, stimuli (TNFα, LPS, IL1β) lead to an increase in NFκB combinatorial dimers in the nucleus.*

complexes have been truly biochemically characterized in their physiological contexts. In the noncanonical mode of Wnt signaling of which Wnt5A is a representative, Wnt5A-Frizzled-ROR or Wnt5A-Frizzled-initiated signaling alters the activity of Rho/Rac family GTPases through differential activation of Disheveled [10, 40]. Within the Frizzled family of cell surface receptors, Frizzled2, Frizzled5, and Frizzled4 are some of the putative receptors for Wnt5A [17, 41, 42]. It is not known if Disheveled activation by Wnt5A signaling acts in concert with or is regulated by heterotrimeric G proteins, given that Frizzled receptors are homologous to heterotrimeric G protein-coupled receptors. The involvement of β-catenin by Wnt5A signaling is governed by the availability of receptors and cytoplasmic signaling intermediates [20, 43]. The subsequent activation of transcription factors such as AP1, NFAT, and NF-κB through complex signaling networks and crosstalk, either dependent or independent of nuclear translocation of β-catenin (explained in **Figures 1** and **2**), could lead to elaboration of context-dependent immune responses (**Figure 3**).

The basal Wnt5A-Frizzled5 signaling-dependent NF-κB (p65) activity in macrophages that we observed is at least partly accountable for the steady-state expression of CD14/IFNβ, the promoter sequence of which at the genome level contains p65 binding elements [10, 13] (**Figure 3**). The constitutive p65 activity in the nucleus also contributes to sustaining Wnt5A expression [10]. Accordingly, the self-sustaining Wnt5A-p65 axis responsive CD14 and IFNβ expression helps to initiate and coordinate several aspects of macrophage function including interaction of pathogen recognition with TLR signaling, thus enabling adaptation to protective immune responses to bacteria, bacterial LPS (lipopolysaccharide), and virus as explained in **Figure 3**. The Wnt5A-NF-κB (p65) responsive gene expression declines upon

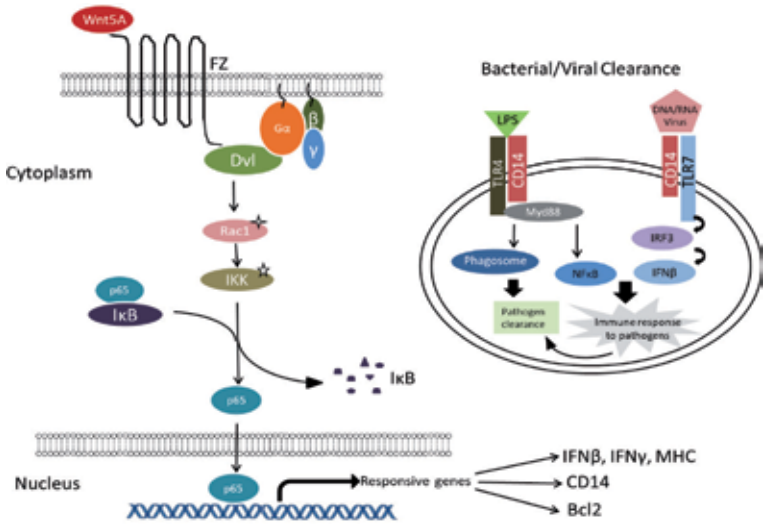


**Figure 3.**
*A schematic of Wnt5A-p65 axis: Wnt5A binds with it's putative receptor Frizzled5 (FZ) and transmits signal through intermediates like Disheveled (Dvl), trimeric G-proteins (Gα, β, γ) activating Rac1. Activated Rac1 helps in translocation of NFκB from cytosol to nucleus via activation of IKK and proteasomal degradation of IKK-phosphorylated IκB. The translocated p65 in the nucleus helps to maintain expression of proteins such as CD14, IFNγ, IFNb, MHC, needed for pathogen detection and clearance, and Bcl2, needed for cell survival. Amplification of signals by CD14-assisted molecules such as TLRs facilitate pathogen recognition and clearance.*

exposing macrophages to an IKK2-specific inhibitor [10]. Wnt5A signaling is also responsible for a basal level of secretion of IFN-γ, another important regulator of innate immune signaling in macrophages. The steady-state Wnt5A signaling and NF-κB activity also promote macrophage survival through the expression of NF-κB-responsive survival genes such as Bcl2 [10]. These data are consistent with the dearth of survival of NF-κB-deficient mice due to different kinds of infection and apoptotic cell death [44]. The Wnt5A-Frizzled5 signaling-assisted constitutive p65 activity is dependent on Rac1 activation, which lies upstream of IKK2 activity [10]. The detailed mechanism of how the Rac1 GTPase activates IKK in a Wnt5A signaling-dependent mode is yet to be explored. It also remains to be tested how Wnt5A-responsive innate immune functions in macrophages relating to pathogen recognition and activation of several intracellular signaling pathways translate to adaptive immune responses encompassing antigen processing/presentation and lymphocyte activation.

### 2.4 Signaling and transcriptional activation by other Wnts

In light of the fact that Wnts comprise a large family of glycoprotein ligands sharing considerable amino acid sequence homology and bind to cell surface receptors that are equally homologous [21], the schemes of regulation and sustenance of immune responses in macrophages by Wnt signaling are likely to be manifold. Several reports have outlined the importance of canonical Wnt signaling and β-catenin in the development, sustenance, and elaboration of memory and effector T cells that comprise a crucially important component of immunity to infectious pathogens [45]. The role of the TCF family of transcription factors in this respect has generated considerable interest in our understanding of the importance of Wnt signaling in immune homeostasis. However, the precise role of canonical Wnt signaling by β-catenin and TCF transcription factors in macrophages in the generation and sustenance of T cell-mediated immunity remains unclear.

## 3. Role of Wnt signaling in macrophage phagocytosis: involvement of the actin cytoskeleton

### 3.1 Significance of phagocytosis

Phagocytosis of pathogens is one of the most important features of the host-pathogen communications and interactions mediated by macrophages. This element of host defense by macrophages not only operates toward host protection at the onset of infection but also makes room for the initiation and amplification of intracellular signals that can potentially mature to the generation of antigen-specific T cell responses and creation of immunological memory (explained in **Figure 4**).

As described earlier in this chapter, Wnt5A signaling aids in maintaining a steady-state expression of CD14 and IFNβ, two of the many molecules involved in innate immune defense. Although it is not exactly clear how CD14 and IFNβ fit into the program of phagocytosis in exact molecular terms, it is documented that while CD14 is instrumental in the recognition of structural motifs like lipopolysaccharide (LPS) intrinsic to certain pathogens, both CD14 and IFNβ facilitate pathogen clearance through the initiation and propagation of macrophage TLR signaling during phagocytosis and activation of immune responses [10, 13] (**Figure 3**). Following pathogen engulfment and phagosome formation during phagocytosis, macrophages rely mostly on endosomal and lysosomal proteases and NADPH oxidase-generated reactive oxygen species for both pathogen clearance as well as processing and presentation of antigenic peptides to MHC molecules for presentation to T lymphocytes [31, 46] and translation to memory.

### 3.2 Need for Wnt5A signaling-assisted actin rearrangement/assembly

At the core of all phagocytosis-related processes lies the involvement of the actin cytoskeleton through its influence on protein sorting/trafficking and intracellular organelle fusions that are crucial for the activation of phagosomal enzymes such as

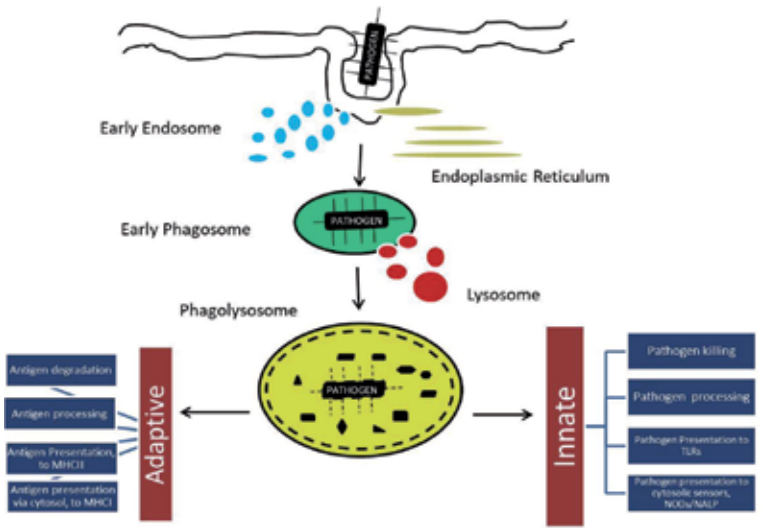


**Figure 4.**
*A schematic of maturation of pathogen containing vesicle and its outcome: After phagocytosis of pathogen there is fusion of early endosome and endoplasmic reticulum (ER) with the phagosome which helps in the maturation of the phagosome and fusion with lysosome. This is important for both innate and adaptive immunity.*

NADPH oxidase and phagosome maturation [31, 47]. Several cytoskeletal GTPases such as Rac1 and Disheveled, lipid rafts, and actin-nucleating proteins such as Arp2/3 and formins partake of the cytoskeletal actin modulations that accompany macrophage phagocytosis and phagosome maturation [47–50]. There is evidence that Wnt5A signaling is important for such rearrangements of the actin cytoskeleton. Accordingly, Wnt5A signaling facilitates Rac1- Disheveled-lipid raft-dependent phagocytosis of bacteria and other foreign matter through modulations of the actin cytoskeleton [9]. Blockers of any of the cytoskeletal actin-associated signaling intermediates—Rac1, Disheveled, or lipid raft and cytochalasin-D, an inhibitor of actin assembly—are antagonistic to the effect of Wnt5A signaling on phagocytosis [9]. The influence of Wnt5A signaling on phagocytic uptake is usually dependent on the microbe under consideration, because while most bacterial species tested undergo facilitated phagocytic uptake by Wnt5A signaling in macrophages, phagocytic uptake of *Leishmania donovani* remains unaffected by it [11]. Perhaps Wnt5A-facilitated internalization encompasses distinct membranous domains depending on the availability of cognate receptors, which are not equally compatible with all microbes. That Wnt5A signaling also facilitates phagosome-lysosome fusion during phagosome maturation which is evident from the augmented appearance of lysosomal markers such as cathepsins in Wnt5A-induced phagosomes of bacteria-infected macrophages [12]. Wnt5A-facilitated alteration in cytoskeletal actin assembly that correlates with phagosome-lysosome fusion is concomitant with the killing of several microbes including bacterial pathogens (*Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, etc.) and even *Leishmania donovani*, although it gets internalized independent of Wnt5A signaling [11, 12]. The mechanism of microbial killing is discussed at greater length in the following section of this chapter. Microbial killing is furthermore facilitated by Wnt5A-responsive NADPH oxidase activity, which is associated with cytoskeletal actin-dependent assembly of NADPH oxidase subunits [11]. Interestingly, nonpathogenic laboratory strains of bacteria that are engulfed by macrophages in increased numbers by Wnt5A signaling are not necessarily killed by it like the pathogenic bacterial strains [9, 12]. Such discrepancy in the fate of internalized microbes may be an outcome of notable differences in the interaction of different microbial components with Wnt5A-regulated cytoskeletal actin rearrangements. The interrelation between Wnt5A signaling and Ehrlichia infection is especially noteworthy in this context [51].

In light of the fact that the cytoskeletal actin-assisted phagosome is the originator and communicator of many signals generated by phagocytozed cargo-recognizing molecules such as TLR, NOD1, and NOD2 [35, 52, 53] (**Figure 4**), it is quite likely that the consequences of Wnt5A-assisted phagocytosis are numerous. Association of Wnt5A signaling with TLRs has already been reported [54]. Careful analysis of the consequences of such associations is important.

### 3.3 Role played by other Wnts and costimulatory molecules of Wnt signaling

Wnts other than Wnt5A are known to regulate macrophage phagocytosis as well. For example, the Drosophila Wnt has been reported to stimulate phagocytic uptake in the S2 cell, a macrophage-like line [55]. Moreover, Wnt1, Wnt7A, and Wnt3A have been reported as phagocytic modulators [56, 57]. The association or relation of these different modes of phagocytosis with Wnt5A signaling and cytoskeletal actin rearrangements is yet to be explored. At this point of our understanding of Wnt signaling with respect to phagocytosis, regulatory roles played by LRP5/6 and ROR, which act as co-receptors to Wnts [22, 58], remain unclear. It also remains to be seen if the influence of Wnt5A signaling on phagocytosis is in the canonical or noncanonical mode or is in fact an intermediary between the two depending on the context of infection, the available receptors, and coactivators.

## 4. Wnt signaling-induced actin-dependent autophagy-assisted xenophagy by macrophages and the potential link with antigen processing/presentation

### 4.1 Autophagy-assisted xenophagy

Several pathogenic microorganisms try to adapt to the intracellular milieu of macrophage creating a niche for their survival [59–61]. Nevertheless, as described earlier in this chapter, the host macrophage tries maneuvering elimination of infection by pathogens by several means. It has been reported that following phagocytosis of microbes by macrophages, the host autophagy machinery comes into play in the ultimate event of clearance of bacteria and other engulfed microbes (xenophagy) through coordinated alterations of the actin cytoskeleton. Autophagy involves the turnover and clearance of damaged organelles and proteins by the cell under both normal conditions as well as under stress in the maintenance of cellular homeostasis [62, 63]. During infection with pathogens, the autophagy program is often utilized for the incapacitation and eradication of engulfed pathogens [26, 64].

### 4.2 Role of Wnt signaling and cytoskeletal actin in autophagy-assisted xenophagy

Wnt signaling has been reported to play a significant role in the autophagy-assisted xenophagy of engulfed microbes by macrophages. Wnt5A signaling, for instance, has been documented to be an integral component of this theme in the killing of several bacterial pathogens through utilization of a Rac1-Disheveled-actin cytoskeleton circuit that involves interactions among several autophagy-associated proteins like microtubule-associated protein 1B-light chain 3B (LC3B), autophagy-related 5 (ATG5), ATG7, and Unc-51-like autophagy-activating kinase 1 (ULK1) [12]. The different nuances of Wnt5A signaling in connection with the actin cytoskeleton are depicted in **Figure 5**. Pathogen killing through autophagy machinery is blocked with the use of cytochalasin-D, an inhibitor of actin assembly as well as with inhibitors to Rac1 and Disheveled [12]. Although Wnt5A-assisted killing of *L. donovani* in macrophages has not been shown to directly involve autophagy, electron micrographs of *L. donovani* harboring parasitophorous vacuoles, which display distinct membranous aggregates, suggest that *L. donovani* containing parasitophorous vacuoles may be subjected to lysis by the host autophagy circuit activated by Wnt5A signaling [11]. The inactivation or lysis of microbe-carrying vacuoles, which happens in due course through fusion of autophagy-destined phagosome or autophagosome with the lysosome, may also be facilitated by Wnt5A signaling [12]. Although cholesterol and other lipids are known to partake of both Wnt5A signaling and actin dynamics [65, 66], at this stage much remains unknown about the specific roles of cholesterol and other lipids in the process of actin modulation during phagocytosis and autophagic clearance of bacteria and other microbes. It also remains to be seen if Wnt5A signaling during autophagy belongs strictly to the noncanonical mode or canonical mode based on the involvement of β-catenin.

### 4.3 Potential link with antigen presentation/adaptive immunity

In view of the fact that the autophagic or rather xenophagic removal of pathogens by macrophages involves reorganization and fusion of intracellular vesicles associated with at least partial lysis of pathogens, the processing and presentation of pathogen antigens to MHC molecules are a likely event during xenophagy in infected macrophages [67, 68]. Thus, autophagosome formation, autophagosome lysosome fusion, and T cell activation by the presentation of processed pathogenic

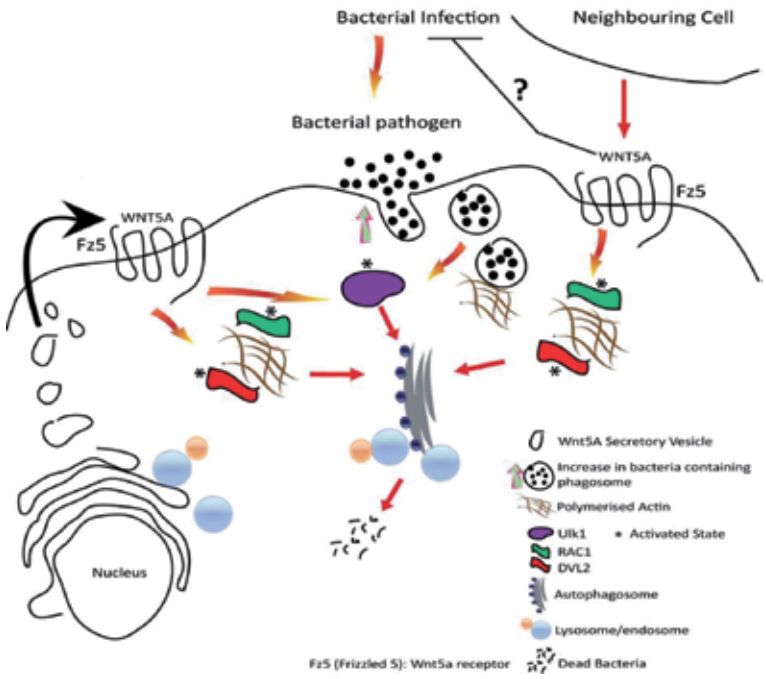


**Figure 5.**
*Schematic of Wnt5A signaling aided bacterial killing: Both autocrine and paracrine modes of Wnt5A signaling can lead to increase in phagocytosis of pathogenic bacteria. After phagocytosis, the Wnt5A mediated cytoskeletal modulation leads to fusion of early endosome and lysosome with the pathogen containing phagosome. Wnt5A signaling also activates Rac1 and Unc like kinase 1 (Ulk1) for initiation of autophagy. The subsequent steps of maturation lead to killing of pathogen in an autophagy dependent process (xenophagy).*

antigens may prevail as a continuum during immune defense depending on the nature and degree of the infection. Given the intrinsic association of Wnt signaling with cytoskeletal dynamics and autophagy [11, 12], it is quite likely that Wnt signaling will influence the antigen processing and presentation linked with autophagy in infected macrophages. Detailed investigation in this respect, although important, remains to be documented.

## 5. Concluding remarks

Given the important role played by Wnt ligands in the transmission of signals associated with cytoskeletal modulation and transcriptional regulation which are part and parcel of host-pathogen communications [27–29, 69], a combination of Wnt signal transduction cascades is expected to hold a fundamental standing in the immune defense program operated by macrophages in both innate and adaptive immunity. Phagocytosis, autophagy/xenophagy (intracellular microbial killing), and a steady-state expression of immune defense molecules through transcriptional regulation appear as some of the major players of the immune defense program operated by Wnt signaling.

In respect of transcriptional regulation of immune defense molecules by steady-state Wnt5A-signaling as described in this chapter [10], it is not understood exactly what dictates the nuclear translocation of p65 and not the other NFκB isoforms for specific modes of gene expression. Additionally, how this regulation fits in with the activity of other major transcription factors like NFAT and AP1 in the macrophage is also not clearly understood. Moreover, details of the context dependence of

Wnt5A signaling, wherein a certain level and mode of signal transmission will be beneficial for immune response, but excess will cause inflammation and disorder [70–72], remain largely unclear. Besides, a clear concept of how actin cytoskeleton-associated proteins such as Rac1 promote both NFκB activity as well as cytoskeletal rearrangements for phagocytosis and autophagy is yet to be achieved [10, 12]. Whether nuclear translocation of NFκB is a natural function of actin assembly or is executed by a separate pool of Rac1 associated cytoskeletal proteins is an important matter that deserves investigation.

With regard to phagocytosis and autophagy-assisted xenophagy, the molecular details of the actin rearrangements with actin binding proteins and the processing and presentation of antigens remain to be deciphered. This brings into question how different host-pathogen interactions within macrophages are guided by modulations of the actin cytoskeleton. Of special interest in this context is the interaction of the actin cytoskeleton with pathogenic mycobacteria, which thrive in self-generated niches within macrophages [60, 73]. The interrelation between different modes of Wnt signaling and mycobacterial infection, although much studied [74, 75], needs to be better understood with respect to actin dynamics. Now that Wnt5A signaling has been shown to play a major role in the regulation of actin cytoskeletal modulation and autophagy [11, 12, 76], future experiments addressing whether this can also facilitate the adaptive immune response through antigen processing and presentation may prove fruitful.

At this juncture of our understanding of Wnt signaling and immune response by macrophages, it is important to know how the different Wnt ligands operate in the regulation of immune response by the different types of macrophages that are distributed in different tissues under the varied conditions of intracellular milieu and infection. Macrophages (microglia) present in the brain and spinal cord maintain an active immune defense scheme against pathogens that affect the central nervous system. Alveolar and airway macrophages likewise protect the respiratory tract and lungs from the toxic effect of infectious agents. Peritoneal macrophages of the peritoneum and Kupffer cells of the liver also encounter and confront infectious agents for host protection. Quite naturally, the roles played by Wnt signaling in the combat mechanism of each macrophage type in its paradigm of immune defense is expected to be different at least to some extent on account of potential variations in cellular environmental cues and modes of host-pathogen interactions.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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## References

[1] Gordon S. The macrophage: Past, present and future. European Journal of Immunology. 2007;**37**(S1):S9-S17

[2] Geissmann F, Auffray C, Palframan R, Wirrig C, Ciocca A, Campisi L, et al. Blood monocytes: Distinct subsets, how they relate to dendritic cells, and their possible roles in the regulation of T-cell responses. Immunology and Cell Biology. 2008;**86**(5):398-408

[3] Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages and dendritic cells. Science. 2010;**327**(5966):656-661

[4] Anderson KL, Smith KA, Conners K, McKercher SR, Maki RA, Torbett BE. Myeloid development is selectively disrupted in PU. 1 null mice. Blood. 15 May 1998;**91**(10):3702-3710

[5] Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science. 2007;**317**(5838):666-670

[6] Davies LC, Taylor PR. Tissue-resident macrophages: Then and now. Immunology. 2015;**144**(4):541-548

[7] Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. Immunity. 2014;**41**(1):21-35

[8] Hume DA. Macrophages as APC and the dendritic cell myth. Journal of Immunology. 2008;**181**(9):5829-5835

[9] Maiti G, Naskar D, Sen M. The wingless homolog Wnt5a stimulates phagocytosis but not bacterial killing. Proceedings of the National Academy of Sciences. 2012;**109**(41):16600-16605

[10] Naskar D, Maiti G, Chakraborty A, Roy A, Chattopadhyay D, Sen M. Wnt5a-Rac1-NF-B Homeostatic circuitry sustains innate immune functions in macrophages. The Journal of Immunology. 2014;**192**(9):4386-4397

[11] Chakraborty A, Kurati SP, Mahata SK, Sundar S, Roy S, Sen M. Wnt5a signaling promotes host defense against *Leishmania donovani* infection. Journal of Immunology. 2017;**199**(3):992-1002

[12] Jati S, Kundu S, Chakraborty A, Mahata SK, Nizet V, Sen M. Wnt5A signaling promotes defense against bacterial pathogens by activating a host autophagy circuit. Frontiers in Immunology. 2018;**9**:679

[13] Guha I, Naskar D, Sen M. Macrophage as a mediator of immune response: Sustenance of immune homeostasis

[14] Clevers H. Wnt/β-catenin signaling in development and disease. Cell. 2006;**127**(3):469-480

[15] Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annual Review of Cell and Developmental Biology. 2004;**20**:781-810

[16] Staal FJT, Luis TC, Tiemessen MM. WNT signalling in the immune system: WNT is spreading its wings. Nature Reviews. Immunology. 2008;**8**(8):581-593

[17] Liu X, Liu T, Slusarski DC, Yang-Snyder J, Malbon CC, Moon RT, et al. Activation of a frizzled-2/beta-adrenergic receptor chimera promotes Wnt signaling and differentiation of mouse F9 teratocarcinoma cells via Galphao and Galphat. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**(25):14383-14388

[18] Schulte G, Bryja V. The frizzled family of unconventional G-protein-coupled receptors.

Trends in Pharmacological Sciences. 2007;**28**(10):518-525

[19] Wang H, Liu T, Malbon CC. Structure-function analysis of Frizzleds. Cellular Signalling. 2006;**18**(7):934-941

[20] Mikels AJ, Nusse R. Purified Wnt5a protein activates or inhibits β-catenin–TCF signaling depending on receptor context (Arias AM, editor). PLoS Biology. 2006;**4**(4):e115

[21] The Wnt Homepage [Internet]. Available from: https://web.stanford.edu/group/nusselab/cgi-bin/wnt/ [Accessed: 31 January 2019]

[22] Green J, Nusse R, van Amerongen R. The role of Ryk and Ror receptor tyrosine kinases in Wnt signal transduction. Cold Spring Harbor Perspectives in Biology. 1 Feb 2014;**6**(2):a009175

[23] Yu J, Chen L, Cui B, Widhopf GF, Shen Z, Wu R, et al. Wnt5a induces ROR1/ROR2 heterooligomerization to enhance leukemia chemotaxis and proliferation. Journal of Clinical Investigation. 2015;**126**(2):585-598

[24] Grumolato L, Liu G, Mong P, Mudbhary R, Biswas R, Arroyave R, et al. Canonical and noncanonical Wnts use a common mechanism to activate completely unrelated coreceptors. Genes & Development. 2010;**24**(22):2517-2530

[25] Aznar N, Ear J, Dunkel Y, Sun N, Satterfield K, He F, Kalogriopoulos NA, et al. Convergence of Wnt, growth factor, and heterotrimeric G protein signals on the guanine nucleotide exchange factor Daple. Science Signaling. 27 Feb 2018;**11**(519):eaao4220

[26] Bah A, Vergne I. Macrophage autophagy and bacterial infections. Frontiers in immunology. 6 Nov 2017;**8**:1483

[27] Fric J, Zelante T, Wong AYW, Mertes A, Yu H-B, Ricciardi-Castagnoli P. NFAT control of innate immunity. Blood. 2012;**120**(7):1380-1389

[28] Newton K, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harbor Perspectives in Biology. 1 Mar 2012;**4**(3):a006049

[29] Foletta VC, Segal DH, Cohen DR. Transcriptional regulation in the immune system: All roads lead to AP-1. Journal of Leukocyte Biology. 1998;**63**(2):139-152

[30] Zhong B, Tien P, Shu H-B. Innate immune responses: Crosstalk of signaling and regulation of gene transcription. Virology. 2006;**352**(1):14-21

[31] Rybicka JM, Balce DR, Khan MF, Krohn RM, Yates RM. NADPH oxidase activity controls phagosomal proteolysis in macrophages through modulation of the lumenal redox environment of phagosomes. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(23):10496-10501

[32] Underhill DM, Bassetti M, Rudensky A, Aderem A. Dynamic interactions of macrophages with T cells during antigen presentation. The Journal of Experimental Medicine. 1999;**190**(12):1909-1914

[33] Brode S, Macary PA. Cross-presentation: Dendritic cells and macrophages bite off more than they can chew! Immunology. 2004;**112**(3):345-351

[34] Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: Evolutionarily conserved mediators of immune responses. Annual Review of Immunology. 1998;**16**:225-260

[35] Zabucchi G, Trevisan E, Vita F, Soranzo MR, Borelli V. NOD1 and NOD2 interact with the phagosome cargo in mast cells: A detailed

morphological evidence. Inflammation. 2015;**38**(3):1113-1125

[36] Garcia-Rodriguez KM, Goenka A, Alonso-Rasgado MT, Hernández-Pando R, Bulfone-Paus S. The role of mast cells in tuberculosis: Orchestrating innate immune crosstalk?. Frontiers in Immunology. 17 Oct 2017;**8**:1290

[37] Sarnico I, Lanzillotta A, Benarese M, Alghisi M, Baiguera C, Battistin L, et al. NF-kappaB dimers in the regulation of neuronal survival. International Review of Neurobiology. 2009;**85**:351-362

[38] Sen M, Ghosh G. Transcriptional outcome of Wnt-frizzled signal transduction in inflammation: Evolving concepts. Journal of Immunology. 2008;**181**(7):4441-4445

[39] Janda CY, Waghray D, Levin AM, Thomas C, Garcia KC. Structural basis of Wnt recognition by frizzled. Science. 2012;**337**(6090):59-64

[40] Schlessinger K, Hall A, Tolwinski N. Wnt signaling pathways meet Rho GTPases. Genes and Development. 2009;**23**(3):265-277

[41] He X, Saint-Jeannet J-P, Wang Y, Nathans J, Dawid I, Varmus H. A member of the frizzled protein family mediating axis induction by Wnt-5A. Science. 1997;**275**(5306):1652-1654

[42] Sato A, Yamamoto H, Sakane H, Koyama H, Kikuchi A. Wnt5a regulates distinct signalling pathways by binding to Frizzled2. The EMBO Journal. 2010;**29**(1):41-54

[43] Bryja V, Schulte G, Rawal N, Grahn A, Arenas E. Wnt-5a induces dishevelled phosphorylation and dopaminergic differentiation via a CK1-dependent mechanism. Journal of Cell Science. 2007;**120**(4):586-595

[44] Sha WC, Liou HC, Tuomanen EI, Baltimore D. Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. Cell. 1995;**80**(2):321-330

[45] Staal FJT, Arens R. Wnt Signaling as master regulator of T-lymphocyte responses: Implications for transplant therapy. Transplantation. 2016;**100**(12):2584-2592

[46] Vyas JM, Van der Veen AG, Ploegh HL. The known unknowns of antigen processing and presentation. Nature Reviews. Immunology. 2008;**8**(8):607-618

[47] Blocker A, Severin FF, Burkhardt JK, Bingham JB, Yu H, Olivo J-C, et al. Molecular requirements for bi-directional movement of phagosomes along microtubules. The Journal of Cell Biology. 1997;**137**(1):113-129

[48] Rotty JD, Brighton HE, Craig SL, Asokan SB, Cheng N, Ting JP, et al. Arp2/3 complex is required for macrophage integrin functions but is dispensable for FcR phagocytosis and in vivo motility. Developmental Cell. 2017;**42**(5):498-513 e6

[49] Clarke M, Engel U. Mechanically induced actin-mediated rocketing of phagosomes. Molecular Biology of the Cell. 2006;**17**:10

[50] Nagao G, Ishii K, Hirota K, Makino K, Terada H. Role of lipid rafts in innate immunity and phagocytosis of polystyrene latex microspheres. Colloids and Surfaces. B, Biointerfaces. 2011;**84**(2):317-324

[51] Luo T, Dunphy PS, Lina TT, McBride JW. *Ehrlichia chaffeensis* exploits canonical and noncanonical host Wnt signaling pathways to stimulate phagocytosis and promote intracellular survival. Infection and Immunity. 2016;**84**(3):686-700

[52] Kong L, Ge B-X. MyD88-independent activation of a novel actin-Cdc42/Rac pathway is required for toll-like receptor-stimulated phagocytosis. Cell Research. 2008;**18**(7):745

[53] Blander JM, Medzhitov R. Regulation of phagosome maturation by signals from toll-like receptors. Science. 2004;**304**(5673):1014-1018

[54] Trinath J, Holla S, Mahadik K, Prakhar P, Singh V, Balaji KN. The WNT signaling pathway contributes to dectin-1-dependent inhibition of toll-like receptor-induced inflammatory signature. Molecular and Cellular Biology. 2014;**34**(23):4301-4314

[55] Zhu F, Zhang X. The Wnt signaling pathway is involved in the regulation of phagocytosis of virus in Drosophila. Scientific Reports. 25 Jun 2013;**3**:2069

[56] Wallace J, Lutgen V, Avasarala S, St Croix B, Winn RA, Al-Harthi L. Wnt7a induces a unique phenotype of monocyte-derived macrophages with lower phagocytic capacity and differential expression of pro- and anti-inflammatory cytokines. Immunology. 2018;**153**(2):203-213

[57] Chen K, Fu Q, Li D, Wu Y, Sun S, Zhang X. Wnt3a suppresses *Pseudomonas aeruginosa*-induced inflammation and promotes bacterial killing in macrophages. Molecular Medicine Reports. 2016;**13**(3):2439-2446

[58] Goel S, Chin EN, Fakhraldeen SA, Berry SM, Beebe DJ, Alexander CM. Both LRP5 and LRP6 receptors are required to respond to physiological Wnt ligands in mammary epithelial cells and fibroblasts. The Journal of Biological Chemistry. 2012;**287**(20):16454-16466

[59] Moradin N, Descoteaux A. Leishmania promastigotes: Building a safe niche within macrophages. Frontiers in Cellular and Infection Microbiology. 19 Sep 2012;**2**:121

[60] Stutz MD, Pellegrini M. *Mycobacterium tuberculosis*: Preparing and maintaining the replicative niche. Trends in Microbiology. 2018;**26**(10):813-814

[61] Ribet D, Cossart P. How bacterial pathogens colonize their hosts and invade deeper tissues. Microbes and Infection. 2015;**17**(3):173-183

[62] Monastyrska I, Klionsky DJ. Autophagy in organelle homeostasis: Peroxisome turnover. Molecular Aspects of Medicine. 2006;**27**(5-6):483-494

[63] Farré JC, Krick R, Subramani S, Thumm M. Turnover of organelles by autophagy in yeast. Current Opinion in Cell Biology. 2009;**21**(4):522-530

[64] Chargui A, El May MV. Autophagy mediates neutrophil responses to bacterial infection. APMIS. 2014;**122**(11):1047-1058

[65] Iliev AI, Djannatian JR, Nau R, Mitchell TJ, Wouters FS. Cholesterol-dependent actin remodeling via RhoA and Rac1 activation by the *Streptococcus pneumoniae* toxin pneumolysin. Proceedings of the National Academy of Sciences. 2007;**104**(8):2897-2902

[66] Woods A, James CG, Wang G, Dupuis H, Beier F. Control of chondrocyte gene expression by actin dynamics: A novel role of cholesterol/Ror-α signalling in endochondral bone growth. Journal of Cellular and Molecular Medicine. 2009;**13**(9b):3497-3516

[67] Crotzer VL, Blum JS. Autophagy and its role in MHC-mediated antigen presentation. The Journal of Immunology. 2009;**182**(6):3335-3341

[68] English L, Chemali M, Duron J, Rondeau C, Laplante A, Gingras D, et al.

Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. Nature Immunology. May 2009;**10**(5):480

[69] Mostowy S, Shenoy AR. The cytoskeleton in cell-autonomous immunity: Structural determinants of host defence. Nature Reviews. Immunology. 2015;**15**(9):559-573

[70] Sen M, Chamorro M, Reifert J, Corr M, Carson DA. Blockade of Wnt-5A/frizzled 5 signaling inhibits rheumatoid synoviocyte activation. Arthritis and Rheumatism. 2001;**44**(4):772-781

[71] Sen M, Lauterbach K, El-Gabalawy H, Firestein GS, Corr M, Carson DA. Expression and function of wingless and frizzled homologs in rheumatoid arthritis. Proceedings of the National Academy of Sciences. 2000;**97**(6):2791-2796

[72] Sen M. Wnt signalling in rheumatoid arthritis. Rheumatology (Oxford, England). 2005;**44**(6):708-713

[73] Nguyen L, Pieters J. The Trojan horse: Survival tactics of pathogenic mycobacteria in macrophages. Trends in Cell Biology. 2005;**15**(5):269-276

[74] Brandenburg J, Reiling N. The Wnt blows: On the functional role of Wnt signaling in Mycobacterium tuberculosis infection and beyond. Frontiers in Immunology. 26 Dec 2016;**7**:635

[75] Villaseñor T, Madrid-Paulino E, Maldonado-Bravo R, Urbán-Aragón A, Pérez-Martínez L, Pedraza-Alva G. Activation of the Wnt pathway by Mycobacterium tuberculosis: a Wnt–Wnt situation. Frontiers in Immunology. 1 Feb 2017;**8**:50

[76] Witze ES, Litman ES, Argast GM, Moon RT, Ahn NG. Wnt5a control of cell polarity and directional movement by polarized redistribution of adhesion receptors. Science. 2008;**320**(5874):365-369

Chapter 4

# Macrophages in the Pathogenesis of Leprosy

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## Abstract

Leprosy is a chronic infectious disease caused by the intracellular pathogen *Mycobacterium leprae*. The disease may present different clinical forms depending on the immunological status of the host. *M. leprae* may infect macrophages and Schwann cells, and recent studies have demonstrated that macrophages are fundamental cells for determining the outcome of the disease. Skin lesions from patients with the paucibacillary form of the disease present a predominance of macrophages with a pro-inflammatory phenotype (M1), whereas skin lesions of multibacillary patients present a predominance of anti-inflammatory macrophages (M2). More recently, it was shown that autophagy is responsible for the control of bacillary load in paucibacillary macrophages and that the blockade of autophagy is involved in the onset of acute inflammatory reactional episodes in multibacillary cells. So, strategies that aim to induce autophagy in infected macrophages are promising not only to improve the efficacy of multidrug therapy (MDT) but also to avoid the occurrence of reactional episodes that are responsible for the disabilities observed in leprosy patients.

**Keywords:** macrophages, leprosy, innate immunity, scavenger receptors, autophagy

## 1. Introduction

Macrophages are highly plastic and heterogeneous in several aspects, presenting a spectrum of distinct phenotypes according to the microenvironment [1–3]. During mycobacterial infection, its membrane components have the ability to induce polarization and interaction with this type of cell [4]. The cell wall of *M. leprae* consists of lipids and contains large amounts of phthiocerol dimycocerosate and phenolic glycolipid-1 (PGL-1) [5, 6]. PGL-1 has been identified as an important antigen and virulence factor, which has also been shown to be a promising diagnostic molecule by inducing the production of IgM class antibodies [7, 8]. Interestingly, the presence of lipids and sugars in the cell wall also induces an increase in phagocytosis [9], both by macrophages and by other cell types. Besides that, the presence of *M. leprae*-PGL-1 interacting with resident macrophages is able to lead to the

production of nitric oxide, thus causing peripheral nerve damage characteristic of patients with leprosy [10]. Other studies have shown the ability of *M. leprae* to induce the production of oxidative mediators and their products, peroxynitrite and nitrotyrosine [11–14].

Studies have demonstrated the ability of *M. leprae* to interact with a range of scavenger receptors of macrophages culminating in a tolerogenic response profile. The scavenger receptors are membrane receptors whose main function is the removal of molecules and cellular debris from the body, binding through a variety of polyanions, leading to phagocytosis of the target, being found in several cell types such as macrophages [15]. The ability of *M. leprae* to interact with the CD163 receptor, a scavenger receptor, which, during this interaction, can act as a co-receptor for *M. leprae* entry in macrophages, has been described [16]. It is known that activation of this receptor is related to the activation of the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2), leading to the synthesis and increase of the activity of the enzyme heme oxygenase-1 (HO-1), which, through anti-inflammatory and antioxidant pathways, releases interleukin (IL)-10 and generates carbon monoxide, contributing to the polarization of these cells [17–19]. Bonilla and colleagues [20] demonstrated that autophagy, a mechanism of metabolic control, regulates the expression of scavenger receptors macrophage receptor with collagenous structure (MARCO) and scavenger receptor type A (SRA-I) that increase phagocytosis and NRF2 activity during *Bacillus Calmette-Guérin* (BCG) or *M. tuberculosis* (H37Rv) infection.

*M. leprae* is able to induce macrophage SRA-I and CD36 expression [6] that contributes to the uptake of lipids, culminating in an increase in the uptake and accumulation of oxidized lipids within the macrophages, leading to a foamy cell phenotype, associated with an inhibition of the pro-inflammatory response with downregulation of major histocompatibility complex (MHC) II and toll-like receptor (TLR) 2 [21, 22]. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN or CD209) is another scavenger receptor present in macrophages that interacts with *M. leprae*, and it is involved in the phagocytosis of the bacilli [23, 24]. Other receptors have been described with great importance in the initial interaction and polarization of the response of macrophages to bacteria. It has recently been observed that *M. leprae* is able to activate innate receptors such as TLR4 [25], through PGL-1 that induces the irregular production of interferon (IFN)-β, chemokine (C-X-C motif) ligand (CXCL)-10/interferon gamma-induced protein 10 (IP-10), and inducible nitric oxide synthase (iNOS), thus decreasing the production and activation via tumor necrosis factor (TNF) [26].

The persistence of *M. leprae* infection depends on the type of the host immune response. Macrophages are crucial modulators of innate and adaptive immune responses are the main cell types directly infected by the bacillus, and can lead to different immune responses. The initial interaction of the macrophage with *M. leprae* is essential for the polarization of the response toward a susceptible phenotype, favoring the survival of the bacilli. In this way, studies that elucidate this contact may favor the protective response against infection, thus contributing to strategies of control of the disease.

## 2. Macrophage polarization and *M. leprae* infection

Macrophages are specialized cell types present in most mammalian tissue. Recently, many studies have been highlighting the "general" and "tissue-specific" functions of macrophages, including their roles in systemic metabolism, fibrosis, development, cancer, and tissue homeostasis [27]. However, these cells are best

known for their role in the innate immunity, which was first addressed by Ilya Metchnikoff in 1884 in his work describing the "phagocytes" [28]. Several subsets of macrophages were described in different pathological conditions and tissues of humans and mice based on their phenotype and biological functions [1, 29–31]. Despite their high plasticity, macrophages are classically described in two main functionally distinct phenotypes—classically activated or inflammatory macrophages (M1) and alternatively activated or healing macrophages (M2)—reflecting the T helper type (Th) 1 and Th2 response profiles [2, 3, 30].

In summary, M1 macrophages are induced by lipopolysaccharide and IFN-γ in a pro-inflammatory environment promoting a microbicidal and inflammatory phenotype, while polarization to M2 macrophages, induced in response to IL-4 (M2a), immune complexes (M2b) or IL-13 and IL-10 (M2c), is rather anti-inflammatory and associated with healing and tumor progression. In addition, granulocyte and macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) induce the differentiation of macrophages into, respectively, M1 and M2 phenotypes [2, 3, 32, 33]. Previously, it was demonstrated that macrophages differentiated with GM-CSF or M-CSF were able to phagocytose *M. leprae* [34]. Despite this, only GM-CSF-differentiated M1 cells were able to stimulate T cells to produce IFN-γ, after treatment of the macrophages with IFN-γ and CD40 ligand; furthermore, this treatment induced expression of major membrane protein (MMP)-II on the macrophage cell surface, suggesting its ability to process the phagocytosed bacteria [34]. In addition, *M. leprae* was able to induce IL-10 production in M-CSF-differentiated M2 cells, but not in GM-CSF-differentiated M1 macrophages.

In 2016, the protein jagged 1 (JAG1) was identified as a potential regulator of macrophage polarization in leprosy [35]. While unstimulated endothelial cells lead to M2 macrophage polarization, in the presence of IFN-γ, endothelial cells induce the differentiation to M1 macrophages. JAG1 is preferentially expressed in the vascular endothelium in skin lesions of paucibacillary tuberculoid patients, stimulating the differentiation of M1 antimicrobial macrophages by the IFN-γ-JAG1 axis [35].

Due to increased systemic pro-inflammatory mediators, a higher frequency of apoptosis was described in paucibacillary tuberculoid patients [36]. Curiously, the phagocytosis of apoptotic cells in the presence of *M. leprae* induces a shift from M1 to M2 phenotype in GM-CSF-differentiated macrophages with increased expression of scavenger receptors as SRA-I, production of IL-10 and transforming growth factor beta (TGF-β) anti-inflammatory cytokines, and decreased levels of pro-inflammatory IL-15 and IL-6 by a mechanism mediated by arginase [37] (**Figure 1**). Based on those results, it was suggested that in paucibacillary tuberculoid skin lesions, the phagocytosis of apoptotic cells would induce an M2 phenotype in some macrophages, explaining the persistence of the disease besides the ability to mount an effective cellular immune response to *M. leprae* infection [37].

Analysis of paucibacillary tuberculoid and reversal reaction (an acute inflammatory clinical condition associated with increased levels of IFN-γ in leprosy patients) patients' skin lesions demonstrated that macrophage subtypes with microbicidal and homeostatic functions are spatially distributed in tuberculoid granulomas according to the specific microenvironments [38]. The center of the tuberculoid granulomas appears to be populated by pro-inflammatory $CD68^+$ $CD163^-$ M1 macrophages, responsible for containing the infection, while the periphery is composed of anti-inflammatory $CD68^+$ $CD163^+$ M2 macrophages, tasked with limiting tissue damage caused by the M1 macrophage antimicrobial activity [39]. Accordingly, Montoya and colleagues [22] proposed two different macrophage functional programs for the polar clinical forms of leprosy. They suggested that in tuberculoid paucibacillary patients, IL-15 induces the vitamin D-mediated antimicrobial program in the macrophages, resulting in killing of the

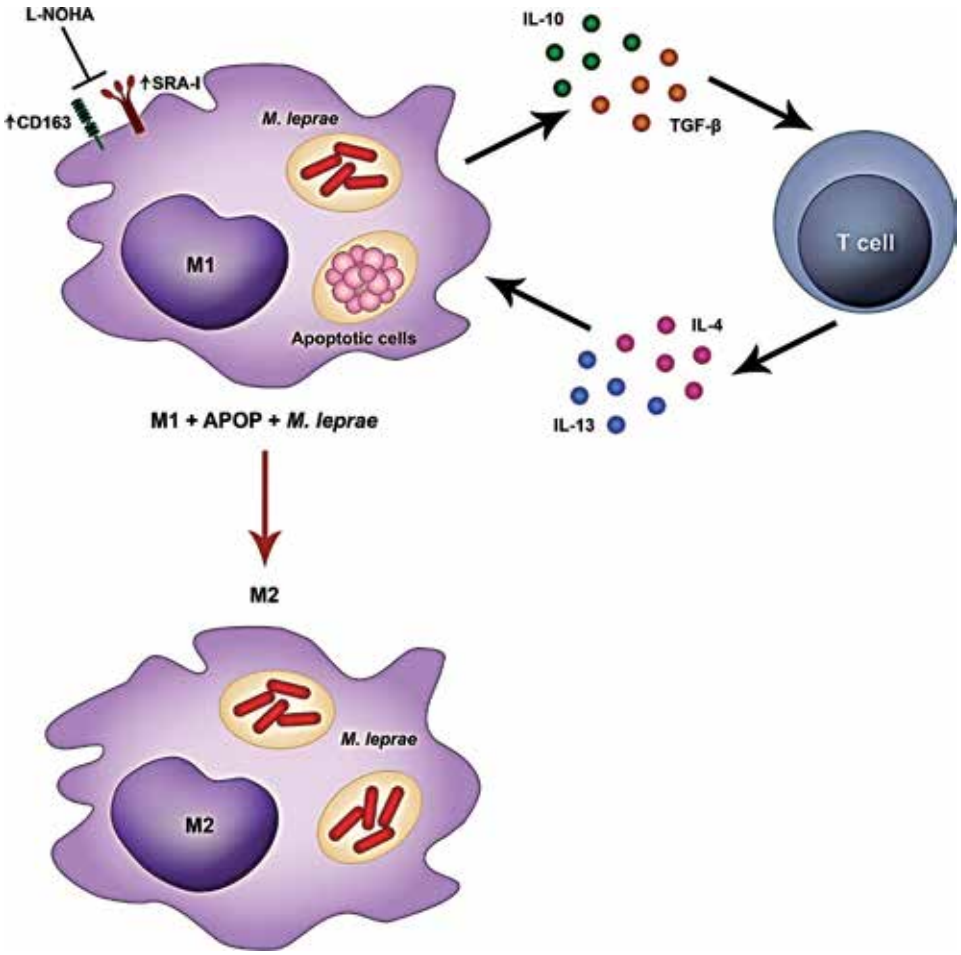


**Figure 1.**
*Macrophage plasticity in tuberculoid skin cells. M1 phenotype prevails in tuberculoid cells. The pro-inflammatory cytokines present in the tissue may contribute to increased host cell apoptosis, and the removal of the apoptotic cells may contribute to changes in macrophage plasticity. M1 macrophages that uptake* M. leprae *and apoptotic cells have an increase in the percentage of M2 markers CD163 and SRA-I that is dependent on arginase production, since the arginase inhibitor N-hydroxy-nor-L-arginine (nor-NOHA) blocks these phenotype changes. In addition, the stimuli with* M. leprae *and apoptotic cells induce an increase in the production of IL-10 and TGF-β that contributes for inducing the secretion of IL-4 and IL-13 by Th2 cells that sustain some M2 cells in tuberculoid lesions.*

mycobacteria, while in multibacillary lepromatous patients, the higher levels of IL-10 would induce the phagocytic pathway by increasing the expression of CD209 and scavenger receptors as CD163 in the macrophage cell surface, resulting in phagocytosis of *M. leprae* and oxidized low-density lipoproteins (LDL) favoring the formation of foam cells and persistence of the infection [22]. In addition, antimicrobial M1 macrophages differentiated with IL-15 could be repolarized into the phagocytic M2 phenotype after treatment with IL-10, while phagocytic IL-10-differentiated M2 macrophages could only be repolarized into the M1 phenotype after co-stimulation with TLR2/1 ligand and IFN-γ or TLR2/1 ligand and anti-IL-10-neutralizing antibodies, but not IL-15 or IFN-γ alone, suggesting that production IL-10 by M2 macrophages might create a barrier for M1 reprogramming [39].

*M. leprae* infection of IL-10-differentiated M2 cells results in induction of type I IFN and suppression of the vitamin D directed pathway, suggesting that *M. leprae* evades the intrinsic capacity of human cells to activate the vitamin

D-mediated antimicrobial pathway via the induction of type I IFN [40]. Although previous studies have demonstrated the activation of antimicrobial pathways in IL-15-differentiated macrophages, there is no study demonstrating how vitamin D status modulates IL-15-differentiated macrophage phenotype and function. More recently, it was demonstrated that the presence of vitamin D during macrophage differentiation bestows the capacity of human macrophages to mount an antimicrobial response against *M. leprae* [41]. However, more studies are needed to evaluate if the plasma levels of vitamin D could be a predictor of the outcome of the disease.

Several studies demonstrated the predominance of M2 markers like CD68, CD209, CD163, SRA-I, HO-1, arginase-1, IL-10, IL-13, TGF-β, and basic fibroblast growth factor in multibacillary lepromatous patients' skin lesion macrophages [16, 22, 37, 42–44]. In the same way, CD163, the hemoglobin (Hb) scavenger receptor, might contribute to the polarization of multibacillary lepromatous macrophages to an anti-inflammatory profile by increasing the expression of indoleamine 2,3-dioxygenase (IDO) and IL-10, in addition to increasing the internalization of *M. leprae* and iron, contributing to the mycobacterial persistence [16, 45]. The increase in the internalization of Hb-haptoglobin (Hp) complex by CD163 contributes to the activation of the enzyme HO-1 via IL-10 [46]. de Mattos Barbosa and colleagues [42] proposed that *M. leprae*-infected skin macrophages would increase the acquisition of iron both by transferrin and heme-bound, via transferrin receptor 1 and CD163, activating the enzyme HO-1 that catalyzes heme into carbon monoxide, biliverdin, and free iron, increasing the intracellular iron pool and the iron storage in the protein ferritin (Ft), due to a reduction in expression of the sole iron exporter, ferroportin 1 (Fpn-1) [42] **(Figure 2)**. Iron retention via Ft and reduced secretion of iron by Fpn-1 are classical traits of microbicidal inflammatory M1 macrophages, while tissue repair-associated M2 are characterized by enhanced HO-1-mediated heme catalysis and increased iron exportation via Fpn-1 [46]. Even though there is a prevalence of M1 or M2 markers in the polar clinical forms of leprosy, skin lesion macrophages present themselves in a spectrum of heterogeneous phenotypes sharing characteristics of both subtypes, and more than one specific population can be present at the same time [38, 42, 47].

A different subset of macrophages, known as M4, was described in skin lesions from lepromatous patients. M4 macrophages in lepromatous skin lesions were described as CD68-positive cells that express myeloid-related protein 8 (MRP8) and matrix metalloproteinase (MMP)-7 [48]. This particular subset of macrophages is differentiated with the platelet chemokine CXCL4 and is mostly related to the formation of foamy cells present on atherosclerotic lesions due to increased expression of LDL receptors. Macrophages differentiated with this chemokine present a functionally distinct phenotype characterized by increased expression of CD206, CD68, IL-6, TNF, MRP8, MMP7, and MMP12, suppressed phagocytic capacity, and the complete lack of CD163 accompanied by the inability to induce HO-1 in response to Hb-Hp complexes, which is irreversible even after removal of CXCL4 and stimulation with M-CSF or IL-10 [32, 48, 49]. Expression of IL-6 and TNF, cytokines associated with the promotion of microbicidal M1 macrophages responses, was increased on skin lesions of paucibacillary tuberculoid patients [48]. Additionally, in vitro exposure to *M. leprae* or PGL-1 impairs the capacity of healthy donor's monocytes to differentiate to M1 macrophages, reducing the cell surface expression of M1 markers and the production of M1-associated chemokines and cytokines [50]. It was hypothesized that previous contact with *M. leprae* might limit the functional capacity of monocytes, reducing the ability to mount an effective immune response in a secondary contact [50]. Together, these data support the idea that an anti-inflammatory regenerative environment restrictive of microbicidal response is promoted in lepromatous patient's skin lesions, leading to

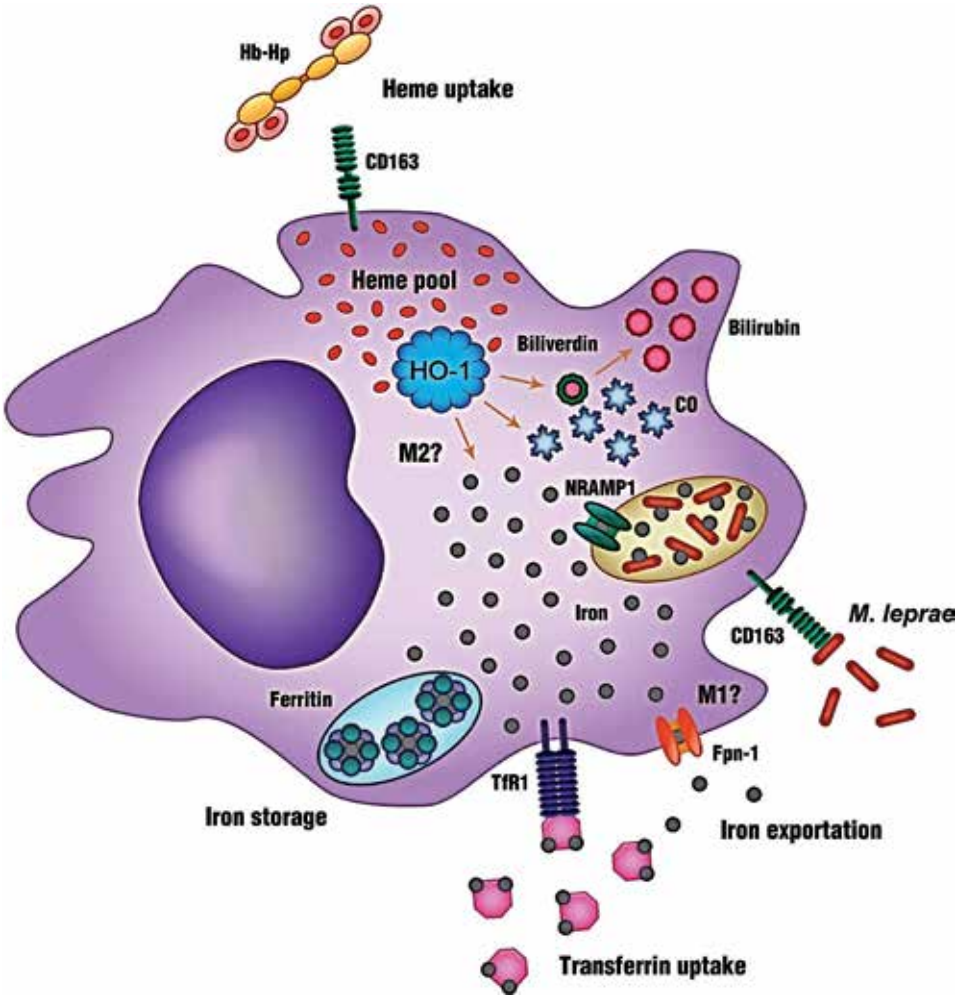


**Figure 2.**
*Lepromatous leprosy macrophage iron metabolism. Skin lesion macrophages of lepromatous patients present high expression of M2 markers as CD163, a scavenger receptor that recognizes hemoglobin-haptoglobin (Hb-Hp) complex and was previously implied in* M. leprae *internalization. The heme molecules are degraded by heme oxygenase-1 (HO-1) that catalyzes heme in free iron, carbon monoxide (CO), and biliverdin that is converted to bilirubin by the enzyme biliverdin reductase; these are classically upregulated in M2 macrophages. Transferrin receptor 1 (TfR1) is also increased in lepromatous macrophages. This receptor recognizes iron-bound transferrin, which is endocytosed, and the iron is later liberated to the cytoplasm. Lepromatous macrophages also present a lower expression of ferroportin 1, the iron cellular exporter, characteristic from M1 macrophages, contributing to increasing the cellular iron pool. The free iron present in the cytoplasm is quickly stored in the form of ferritin but can also be available for* M. leprae *use and increased growth in the phagosomes, as observed in this clinical form. Natural resistance-associated macrophage protein 1 (NRAMP1) is also increased in lepromatous macrophages, but its role in* M. leprae*-infected cells is still to be determined.*

differentiation of a heterogeneous subset of highly phagocytic iron and lipid-loaded foamy macrophages that create an ideal environment for survival and vast propagation of the *M. leprae* infection and consequently the increase in the number of skin lesion in this pole of the disease [16, 22, 37, 42–44, 48].

## 3. The role of macrophages in the immune response to *M. leprae*

One of the most crucial steps in a human innate immune response is how the host cells recognize a microbial pathogen. The TLR family has a vital role in the

mycobacterial recognition and subsequently induction of antimicrobial defenses and adaptive immune response [51]. Recognition of *M. leprae* pathogen-associated molecular patterns (PAMPs) occurs through the TLR2/1 heterodimer to the tri-acylated lipopeptides, leading to the differentiation of monocytes into macrophages and dendritic cells and triggering the production of TNF as part of an acute inflammatory response [52]. The tissue expression of TLRs correlated with the immunological spectrum of the disease, once both TLR1 and TLR2 were prominently observed in the self-limited tuberculoid lesions when compared to the disseminated lepromatous lesions [53]. Another pattern recognition receptor (PRR) involved in *M. leprae* detection is nucleotide-binding oligomerization domain-containing 2 (NOD2). Human NOD2 receptor recognizes structurally unique muramyl dipeptides from *M. leprae*, triggering an IL-32-mediated innate immune response that induces the differentiation of monocytes into dendritic cells [54, 55]. Interestingly, activation of monocytes via NOD2 agonist was more efficient in the induction of dendritic cell differentiation than TLR2/1 ligand treatment [54].

The activation of PRR can induce the antimicrobial autophagy pathway, a biological process regulated by multiple specialized proteins known as autophagy-related proteins (ATG), and can be started in response to various cellular stresses and signals such as nutrient withdrawal, growth factor deprivation, and cytokine stimulation and also by pathogen infection [47]. In addition to the role of autophagy in the elimination of potentially toxic protein aggregates and in the prevention of neurodegeneration [56], autophagy plays a key role in the host's response to mycobacterial infection, because it is able to reverse the blockade of phagosome maturation, inhibiting the intracellular survival of the pathogen [57]. It has been shown that autophagy is an important innate mechanism associated with leprosy immunopathogenesis [58]. Recently, it was demonstrated that autophagy enhances the ability of *M. leprae*-infected Langerhans cells to present antigens to CD1a T cells [59].

As mentioned earlier, the paucibacillary tuberculoid skin macrophages activate the vitamin D pathway and produce antimicrobial peptides that could be involved in autophagy induction. In addition, Silva and colleagues [58] demonstrated that autophagy is differentially regulated between leprosy polar forms. In paucibacillary tuberculoid skin lesion macrophages, IFN-γ/beclin 1-induced autophagy contributes for *M. leprae* control, whereas in lepromatous macrophages B cell lymphoma 2 (BCL2)-mediated blockade of beclin 1 autophagic pathway promotes mycobacterial persistence [58]. Indeed, the *M. leprae* can take advantage of host antiviral protein 2′-5′-oligoadenylate synthetase like (OASL) to inhibit autophagy and promote its own survival through a stimulator of interferon genes (STING)-mediated type I IFN response [60]. Furthermore, the autophagy levels were restored in lepromatous patients undergoing reversal reaction episodes [57]. More recently, de Mattos Barbosa et al. [61] elegantly demonstrated a role for autophagy in the development of reversal reaction. This study showed a downregulation of autophagy associated with inflammasome activation in skin lesion macrophages of multibacillary leprosy patients who developed reversal reaction episodes in the future. Thus, the autophagic pathway is a key factor in multibacillary leprosy patients to avoid exacerbated inflammasome activation and the onset of reversal reaction. A newly published study showed that Th17-derived cytokine IL-26 has a direct bind capacity and antimicrobial effect against mycobacteria in cell-free cultures [62]. In *M. leprae*-infected macrophages, IL-26 treatment was associated with autophagy induction via STING (probably due to its ability to bind DNA) as well targeting of mycobacteria to lysosomal compartments [62]. Curiously, it has been shown in *M. tuberculosis*-infected macrophages that the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS), an upstream receptor to STING, controls both pro-mycobacterial type I IFN production and the activation of antimycobacterial selective autophagy

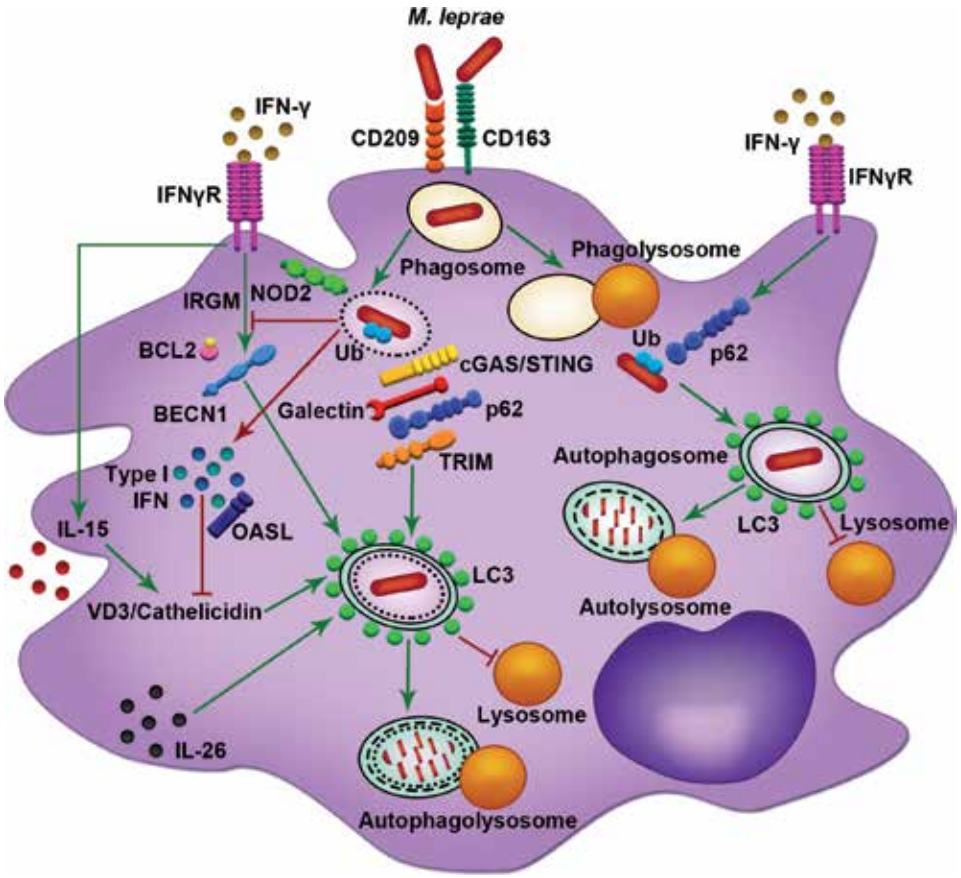


**Figure 3.**
*Targeting* M. leprae *to autophagy (xenophagy) in macrophages of leprosy patients. (Left) After phagocytosis,* M. leprae *causes phagosome maturation arrest and phagosomal permeabilization through the bacterial ESX-1 secretion system, which allows the detection of extracellular mycobacterial DNA by the cGAS/STING-sensing cytosolic surveillance pathway that licenses the ubiquitination of mycobacteria and recognition by the ubiquitin-binding autophagy adaptors p62 and NBR1 (and probably NDP52 and OPTN), which finally interact with LC3, allowing the mycobacterial phagosome to become sequestered within an autophagosome. The autophagosome-sequestered phagosome maturates in a degradative autophagolysosome (which also contains antimicrobial peptides) by fusing with a lysosome and leads to the pathogen destruction and antigen presentation. Other molecules such as TBK1, IRF3, DRAM1, UBQLN1, PARKIN, and SMURF1 might be also involved in this process. Microbial invasion can be also detected and targeted to autophagy pathway by galectins that act as a receptor for vacuole-damaging pathogens or by TRIM-mediated precision autophagy, which can directly recognize the bacterial target without required intermediary autophagic tags such as ubiquitin and galectins. TRIMs and galectins also cooperate during selective autophagy. TRIM proteins use galectins and ubiquitins to detect and tag damaged mycobacteria-containing phagosomes and promote the assembly of autophagic machinery via MTOR inhibition and AMPK activation. IFN-γ-mediated autophagy requires IRGM, which interacts with ULK1 and BECN1 and dissociates BCL2 from BECN1-PIK3C3 complex, thus governing the assembly of autophagy initiation complexes that will further promote the incorporation of mycobacterial phagosomes into autophagosomes. Autophagy initiation step is amplified by the detection of* M. leprae*-derived MDP by NOD2, which enhances NOD2-IRGM interaction and induces IRGM ubiquitination. IRGM can also activate BECN1 via AMPK induction. IFN-γ can also induce autophagy through the IL-15/VD3/cathelicidin pathway. IL-26 is reported to activate autophagy via STING.* M. leprae *can dampen autophagy initiation by increasing the BCL2 levels and its interaction with BECN1 or by induction of type I IFN signaling pathway (which includes OASL) that inhibits the VD3-dependent autophagy.* M. leprae *can also hamper the autophagy maturation step by an unknown mechanism, which might involve the BECN1-BCL2 association. Autophagy activating pathways are prominently observed in tuberculoid macrophages, whereas autophagy inhibition processes are predominantly found in lepromatous macrophages. (Right) Another possible pathway is that right after phagocytosis,* M. leprae *is incorporated into phagolysosomes but avoids lysosomal degradation via translocation from the phagolysosomes to the cytosol by using the ESX-1 secretion system. The* M. leprae *cytosolic entry is followed by ubiquitin-mediated autophagy recognition and degradation into mature autolysosomes. Green arrows indicate steps activating autophagy. Red arrows and inhibition bars represent steps inhibiting autophagy.*

pathway, which can be uncoupled from intracellular immune responses mediated by NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome activation [63–65].

Galectins are a family of β-galactoside-binding cytosolic lectins that monitors endosomal and lysosomal integrity. These danger receptors can detect bacterial invasion by detecting unusual exposure of glycans to the cytosol and activate antibacterial autophagy [66–68]. Immunohistochemistry analysis of leprosy lesions revealed a higher expression of galectin-3 protein on lepromatous macrophages than tuberculoid cells. The increased galectin-3 expression in lepromatous cells was associated with the reduction of dendritic cell differentiation and T-cell antigen presentation [69]. Interestingly, galectin-3 was associated with both bacterial control and survival, as well as autophagy activation and inhibition [66, 68], whereas galectin-8 was related to antibacterial autophagy activation [67, 68]. The underlying cellular mechanisms of target *M. leprae* as an autophagic cargo destination in human macrophages are still not fully understood; some of them displayed in **Figure 3** are insights from *M. tuberculosis* infection model.

Although the innate activation of macrophages orchestrates antimicrobial responses that contribute to host defense against intracellular pathogens such as *M. leprae*, those responses have been also implicated in the initiation of nerve damage in leprosy. The axonal damage is not directly mediated by *M. leprae* itself, but by *M. leprae*-specific PGL-1 induction of nitric oxide synthase in infected macrophages, which leads to axon damage by injuring their mitochondria and inducing demyelination [10]. Taken together, these findings illustrate the plasticity of human macrophages and how they deploy different strategies to fight against mycobacterial infections. Most of the time, these approaches begin with microbe sensing and culminate in the targeting of the pathogen for destruction in the autolysosomal pathway (**Figure 3**), the tuberculoid leprosy macrophages being more efficient than the lepromatous ones in these processes. Hence macrophages are essential components of mammalian tissues in which they perform a variety of biologic functions; understanding their difference is an essential step toward the development of innate immune countermeasures.

## 4. Macrophage autophagy as a target for the control of the disease

Leprosy remains a major global problem. Early detection of cases and immediate treatment with multidrug therapy (MDT) remain the main intervention strategies [70]. Despite the effectiveness of MDT in controlling the polar forms of the disease, limitations in terms of persistent activity in paucibacillary patients, in combination with the persistence of live and/or dead bacilli in multibacillary patients, have been observed, which has repercussions on the frequency of relapses and reactional episodes after treatment [71, 72]. Recent studies have demonstrated that autophagy is an important molecular mechanism for controlling the viability of mycobacteria in the host cell and of the bacillary load in patients with leprosy [58–60]. Autophagy can be induced by oxidative stress or by an infectious agent and is closely associated with the immune response and host defense [73, 74]. In addition to its homeostatic role, the autophagic degradation pathway is involved in several human diseases, including metabolic disorders, neurodegenerative diseases, cancer, and infectious diseases. Given these observations, pharmacological approaches to regulate positively or negatively this pathway are receiving considerable attention. For example, positive regulation of autophagy may be of therapeutic benefit in certain neurodegenerative diseases (e.g., Huntington's disease), while inhibition of autophagy is

being investigated as a strategy for treatment of some cancers [75, 76]. The molecular regulators interconnecting autophagy and apoptosis, including BCL2, BCL2-associated X protein (BAX), and beclin 1, have been suggested to act as switching points that are critical for the outcome of tumor cells, and lysosomes have been reported to initiate the cell death pathway in autophagic cells [77, 78]. Regarding leprosy, it was observed that in skin cells of patients with the lepromatous form of the disease there is a blockade of the autophagic flux that can be attributed to the increased expression of the antiapoptotic protein BCL2, which inhibits autophagy mediated by beclin 1 [58]. Blockade of the autophagic machinery in lepromatous cells may contribute to the persistence of mycobacteria in host cells. Genetic studies on leprosy have shown that several polymorphisms in genes associated with the control of autophagic pathways such as IFN, immunity-related GTPase family M protein (IRGM), NOD, and TLR play a prominent role in susceptibility to the disease, thus demonstrating the importance of understanding, inducing, and controlling this biological process in leprosy [79–85].

When the initial studies aiming at induction of autophagy were conducted, the only known drug capable of inducing autophagy chronically was rapamycin. However, the adverse effects of rapamycin (which were not associated with the induction of autophagy) made this drug unattractive to use. Several drugs and nutritional supplements can induce autophagy, such as verapamil, statins, metformin, resveratrol, vitamin D, and omega 3 [86]. Although it is not known whether these agents exert their beneficial clinical effects through the induction of autophagy or other pathways, there is a considerable overlap between diseases occurring in an environment of poor autophagy and diseases that respond to drugs that may induce autophagy. With regard to infectious diseases, there are limited data on the usefulness of autophagy-inducing pharmaceutical agents as potential therapeutics against human pathogens. Drug screening studies that aim to identify molecules with pro-autophagic effects have been performed, and promising results demonstrated a pro-autophagic effect of drugs capable of inhibiting the growth of *M. tuberculosis* in human macrophages in vitro [87–89]. In addition, the antibiotics isoniazid and pyrazinamide, two first-line cocktail drugs used to treat tuberculosis, exert their antimycobacterial activity through autophagy [90]. The treatment with statins, drugs that inhibit cholesterol synthesis, reduces the bacillary load of *M. tuberculosis* in human macrophages and mice by increasing autophagy and phagosome maturation [91]. Furthermore, statins also have an antimicrobial effect against *M. leprae* and potentiate the antimycobacterial effect of rifampicin, a first-line cocktail drug used in leprosy treatment [92]. Vitamin D3, which activates autophagy, has been successfully used in the treatment of patients with tuberculosis and could be one of the components of an ideal treatment for leprosy and other chronic infectious diseases in which the cellular immune response is deregulated [93–96].

Activation of autophagy by verapamil has been demonstrated by several groups. Initial studies evaluating the effect of verapamil and its analogs on macrophages infected with *M. tuberculosis* showed an association between the induction of autophagy and inhibition of intracellular replication of mycobacteria, and one of the structural analogs had an additive effect on the inhibitory antimicrobial activity of isoniazid and rifampicin [97, 98]. Metformin is an antidiabetic of the biguanide class. Mechanisms of autophagy induction by metformin are known, but no relationship with infectious processes caused by mycobacteria has been described so far. Similarly, resveratrol has also been studied for its autophagy-inducing role, and no studies in the literature have been found correlating with the mycobactericidal role.

Together, these data show the importance of autophagy in the pathogenesis of leprosy, contributing to a better understanding of the mechanisms of mycobacterial control associated with the lepromatous and tuberculoid leprosy poles, which

may lead to the establishment of new targets and therapeutic strategies to control leprosy. Moreover, the identification of autophagy as an important factor during the establishment of resistant and susceptible forms of the disease opens the door for the development of new therapeutic strategies of disease control through the modulation of autophagy.

## 5. Conclusion

Considering the aspects observed during the course of this chapter, macrophages have a crucial role in inducing the immune response to *M. leprae*, and their uptake capacity, phagocytosis, and microbicidal activity may depend on the microenvironment. Macrophages, after the interacting with either the bacilli or its wall components, are able to induce oxidative stress [10–14] and to induce various receptors as scavenger receptors [6, 16, 23, 24, 34, 42–44] and PRR [53–55, 69], leading to the polarization of their response. In an anti-inflammatory profile (M2), this cell induces increased uptake of lipids [21, 22] and Hb-haptoglobin [16, 42], which aid the growth of *M. leprae* by the activation of the enzymes IDO [42, 45], HO-1 [42] and arginase [37]. On the other hand, in a pro-inflammatory and microbicidal profile (M1), the macrophage produces TNF [26, 48], IL-6 [37, 48, 49], and IL-15 [22, 37, 39] besides being able to stimulate T cells to produce IFN-γ [34]. In addition, these M1 macrophages induce autophagy [57, 58], an important process of homeostatic regulation recently described with the immunological role [56], which acts on infection control. Several drugs have been described as autophagy inducers and have been studied as treatment for neurodegenerative diseases [76] and to control of *M. tuberculosis* infection [89]. Autophagy-inducing drugs are promising targets as adjuvants to MDT.

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## Conflict of interest

None.

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## References

[1] Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili S-A, Mardani F, et al. Macrophage plasticity, polarization, and function in health and disease. Journal of Cellular Physiology. 2018;**233**:6425-6440

[2] Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. The Journal of Pathology. 2013;**229**:176-185

[3] Sica A, Mantovani A. Macrophage plasticity and polarization: In vivo veritas. The Journal of Clinical Investigation. 2012;**122**:787-795

[4] Agdestein A, Jones A, Flatberg A, Johansen TB, Heffernan IA, Djønne B, et al. Intracellular growth of *Mycobacterium avium* subspecies and global transcriptional responses in human macrophages after infection. BMC Genomics. 2014;**15**(1)

[5] Brennan P, Nikaido H. The envelope of mycobacteria. Annual Review of Biochemistry. 1995;**64**:29-63

[6] Kaur G, Kaur J. Multifaceted role of lipids in *Mycobacterium leprae*. Future Microbiology. 2017;**12**(4):315-335

[7] Hunter SW, Brennan PJ. A novel phenolic glycolipid from *Mycobacterium leprae* possibly involved in immunogenicity and pathogenicity. Journal of Bacteriology. 1981;**147**:728-735

[8] Lastória JC, Abreu MAMM. Leprosy: Review of the epidemiological, clinical, and etiopathogenic aspects—Part 1. Anais Brasileiros de Dermatologia. 2014;**89**:205-218

[9] Saito H, Tomioka H, Watanabe T, Sato K. Mechanisms of phagocytosis of *Mycobacterium leprae* and other mycobacteria by human oligodendroglial cells. Infection and Immunity. 1986;**51**:163-167

[10] Madigan CA, Cambier CJ, Kelly-Scumpia KM, Scumpia PO, Cheng TY, Zailaa J, et al. A macrophage response to *Mycobacterium leprae* phenolic glycolipid initiates nerve damage in leprosy. Cell. 2017;**170**:973-985

[11] Adams LB, Job C, Krahenbuhl JL. Role of inducible nitric oxide synthase in resistance to *Mycobacterium leprae* in mice. Infection and Immunity. 2000;**68**:5462-5465

[12] Holzer TJ, Nelson KE, Schauf V, Crispen RG, Andersen BR. *Mycobacterium leprae* fails to stimulate phagocytic cell superoxide anion generation. Infection and Immunity. 1986;**51**:514-520

[13] Schalcher TR, Vieira JLF, Salgado CG, Borges R, Monteiro MC. Short communication antioxidant factors, nitric oxide levels, and cellular damage in leprosy patients. Revista da Sociedade Brasileira de Medicina Tropical. 2013;**466**:45-49

[14] Schön T, Hernandez-Pando RH, Negesse Y, Leekassa R, Sundqvist T, Britton S. Expression of inducible nitric oxide synthase and nitrotyrosine in borderline leprosy lesions. The British Journal of Dermatology. 2001;**145**:809-815

[15] Penberthy KK, Ravichandran KS. Apoptotic cell recognition receptors and scavenger receptors. Immunological Reviews. 2016;**269**(1):44-59

[16] Moura DF, De Mattos KA, Nery AC, Pinheiro RO, Sarno EN. CD163 favors *Mycobacterium leprae* survival and persistence by promoting anti-inflammatory pathways

in lepromatous macrophages. European Journal of Immunology. 2012;**42**:2925-2936

[17] Philippidis P, Mason JC, Evans BJ, Nadra I, Taylor KM, Haskard DO, et al. Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: Antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary by pass surgery. Circulation Research. 2004;**94**:119-126

[18] Sierra-Filardi E, Vega MA, Sánchez-Mateos P, Corbí AL, Puig-Kröger A. Heme oxygen-ase-1 expression in M-CSF-polarized M2 macrophages contributes to LPS-induced IL-10 release. Immunobiology. 2010;**215**:788-795

[19] Yang H, Wang H, Levine YA, Gunasekaran MK, Wang Y, Addorisio M, et al. Identification of CD163 as an antiinflammatory receptor for HMGB1-haptoglobin complexes. JCI Insight. 2016;**1**(7)

[20] Bonilla DL, Bhattacharya A, Sha Y, Xu Y, Xiang Q, Kan A, et al. Autophagy regulates phagocytosis by modulating the expression of scavenger receptors. Immunity. 2013;**39**:537-547

[21] Cruz D, Watson AD, Miller CS, Montoya D, Ochoa M, Sieling PA, et al. Host-derived oxidized phospholipids and HDL regulate innate immunity in human leprosy. The Journal of Clinical Investigation. 2008;**118**

[22] Montoya D, Cruz D, Teles RMB, Lee DJ, Ochoa MT, Krutzik SR, et al. Divergence of macrophage phagocytic and antimicrobial programs in leprosy. Cell Host & Microbe. 2009;**6**:343-353

[23] Barreiro LB, Quach H, Krahenbuhl J, Khaliq S, Mohyuddin A, Mehdi SQ, et al. DC-SIGN interacts with *Mycobacterium leprae* but sequence variation in this lectin is not associated with leprosy in the Pakistani population. Human Immunology. 2006;**67**:102-107

[24] Soilleux EJ, Sarno EN, Hernandez MO, Moseley E, Horsley J, Lopes UG, et al. DC-SIGN association with the Th2 environment of lepromatous lesions: Cause or effect? The Journal of Pathology. 2006;**209**:182-189

[25] Polycarpou A, Holland MJ, Karageorgiou I, Eddaoudi A, Walker SL, Willcocks S, et al. *Mycobacterium leprae* activates toll-like receptor-4 signaling and expression on macrophages depending on previous Bacillus Calmette-Guerin vaccination. Frontiers in Cellular and Infection Microbiology. 2016;**6**(72):1-12

[26] Oldernburg R, Mayau V, Prandi J, Arbus A, Astarie-Dequeker C, Guilhot C, et al. Mycobacterial phenolic glycolipids selectively disable TriF-dependent Tlr4 signaling in macrophages. Frontiers in Immunology. 2018;**9**:1-12

[27] Okabe Y, Medzhitov R. Tissue biology perspective on macrophages. Nature Immunology. 2016;**17**:9-17

[28] Taube AI. Metchnikoff and the phagocytosis theory. Nature Reviews. Molecular Cell Biology. 2003;**4**:897-901

[29] Parisi L, Gini E, Baci D, Tremolati M, Fanuli M, Bassani B, et al. Macrophage polarization in chronic inflammatory diseases: Killers or builders? Journal of Immunology Research. 2018;**2018**:8917804

[30] Funes SC, Rios M, Escobar-Vera J, Kalergis AM. Implications of macrophage polarization in autoimmunity. Immunology. 2018;**154**:186-195

[31] Murray PJ. Macrophage polarization. Annual Review of Physiology. 2017;**79**:541-566

[32] Gleissner CA. Macrophage phenotype modulation by CXCL4 in atherosclerosis. Frontiers in Physiology. 2012;**3**

[33] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends in Immunology. 2004;**2512**:677-686

[34] Makino M, Maeda Y, Fukutomi Y, Mukai T. Contribution of GM-CSF on the enhancement of the T cell-stimulating activity of macrophages. Microbes and Infection. 2007;**9**:70-77

[35] Kibbie J, Teles RMB, Wang Z, Hong P, Montoya D, Krutzik S, et al. Jagged1 instructs macrophage differentiation in leprosy. PLoS Pathogens. 2016;**128**:e1005808

[36] Walsh DS, Lane JE, Abalos RM, Myint KSA. TUNEL and limited immunophenotypic analyses of apoptosis in paucibacillary and multibacillary leprosy lesions. FEMS Immunology and Medical Microbiology. 2004;**413**:265-269

[37] de Oliveira FT, Andrade PR, de Mattos Barbosa MG, Pinto TGT, Ferreira PF, Ferreira H, et al. Effect of apoptotic cell recognition on macrophage polarization and mycobacterial persistence. Infection and Immunity. 2014;**82**:3968-3978

[38] Fachin LRV, Soares CT, Belone AFF, Trombone APF, Rosa PS, Guidella CC, et al. Immunohistochemical assessment of cell populations in leprosy-spectrum lesions and reactional forms. Histology and Histopathology. 2017;**32**:385-396

[39] Montoya D, Mehta M, Ferguson BG, Teles RMB, Krutzik SR, Cruz D, et al. Plasticity of antimicrobial and phagocytic programs in human macrophages. Immunology. 2019;**156**:164-173

[40] Zavala K, Gottlieb CA, Teles RM, Adams JS, Hewison M, Modlin RL, et al. Intrinsic activation of the vitamin D antimicrobial pathway by *M. leprae* infection is inhibited by type I IFN. PLoS Neglected Tropical Diseases. 2018;**12**:e0006815

[41] Kim EW, Teles RMB, Haile S, Liu PT, Modlin RL. Vitamin D status contributes to the antimicrobial activity of macrophages against *Mycobacterium leprae*. PLoS Neglected Tropical Diseases. 2018;**12**:e0006608

[42] de Mattos Barbosa MG, da Silva Prata RB, Andrade PR, Ferreira H, de Andrade Silva BJ, da Paixão de Oliveira JA, et al. Indoleamine 2,3-dioxygenase and iron are required for *Mycobacterium leprae* survival. Microbes and Infection. 2017;**19**:505-514

[43] de Sousa JR, de Sousa RPM, Aarão TLS, Dias LB, Carneiro FRO, Fuzii HT, et al. In situ expression of M2 macrophage subpopulation in leprosy skin lesions. Acta Tropica. 2016;**157**:108-114

[44] Mattos KA, Oliveira VCG, Berrêdo-Pinho M, Amaral JJ, Antunes LCM, Melo RCN, et al. *Mycobacterium leprae* intracellular survival relies on cholesterol accumulation in infected macrophages: A potential target for new drugs for leprosy treatment. Cellular Microbiology. 2014;**16**:797-815

[45] de Souza SJ, Lara FA, Amadeu TP, de Oliveira FT, da Costa Nery JA, Sampaio EP, et al. The role of indoleamine 2,3-dioxygenase in lepromatous leprosy immunosuppression. Clinical and Experimental Immunology. 2011;**165**:251-263

[46] Recalcati S, Locati M, Gammella E, Invernizzi P, Cairo G. Iron levels in polarized macrophages: Regulation of immunity and autoimmunity. Autoimmunity Reviews. 2012;**11**:883-889

[47] Pinheiro RO, Schmitz V, Silva BJA, Dias AA, De Souza BJ, De Mattos Barbosa MG, et al. Innate immune responses in leprosy. Frontiers in Immunology. 2018;**9**. DOI: 10.3389/fimmu.2018.00518

[48] de Sousa JR, Lucena Neto FD, Sotto MN, Quaresma JAS. Immunohistochemical characterization of the M4 macrophage population in leprosy skin lesions. BMC Infectious Diseases. 2018;**18**:576

[49] Chinetti-Gbaguidi G, Colin S, Staels B. Macrophage subsets in atherosclerosis. Nature Reviews. Cardiology. 2015;**12**:10-17

[50] Fallows D, Peixoto B, Kaplan G, Manca C. *Mycobacterium leprae* alters classical activation of human monocytes in vitro. Journal of Inflammation. 2016;**13**:8

[51] Medzhitov R. Toll-like receptors and innate immunity. Nature Reviews. Immunology. 2001;**1**(2):135-145

[52] Krutzik SR, Tan B, Li H, Ochoa MT, Liu PT, Sharfstein SE, et al. TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells. Nature Medicine. 2005;**11**:653-660

[53] Krutzik SR, Ochoa MT, Sieling PA, Uematsu S, Ng YW, Legaspi A, et al. Activation and regulation of toll-like receptors 2 and 1 in human leprosy. Nature Medicine. 2003;**9**:525-532

[54] Schenk M, Krutzik SR, Sieling PA, Lee DJ, Teles RMB, Ochoa MT, et al. NOD2 triggers an interleukin-32-dependent human dendritic cell program in leprosy. Nature Medicine. 2012;**18**:555-563

[55] Schenk M, Mahapatra S, Le P, Kim HJ, Choi AW, Brennan PJ, et al. Human NOD2 recognizes structurally unique muramyl dipeptides from *Mycobacterium leprae*. Infection and Immunity. 2016;**84**:2429-2438

[56] Nixon RA. Autophagy in neurodegenerative disease: Friend, foe or turncoat? Trends in Neurosciences. 2006;**29**:528-535

[57] Deretic V, Singh S, Master S, Harris J, Roberts E, Kyei G, et al. Mycobacterium tuberculosis inhibition of phagolysosome biogenesis and autophagy as a host defence mechanism. Cellular Microbiology. 2006;**8**:719-727

[58] Silva BJA, Barbosa MGM, Andrade PR, Ferreira H, Nery JAC, Côrte-Real S, et al. Autophagy is an innate mechanism associated with leprosy polarization. PLoS Pathogen. 2017;**13**:e1006103

[59] Dang AT, Teles RMB, Liu PT, Choi A, Legaspi A, Sarno E, et al. Autophagy links antimicrobial activity with antigen presentation in Langerhans cells. JCI Insight. 2019;**4**:e126955

[60] de Toledo-Pinto TG, Ferreira ABR, Ribeiro-Alves M, Rodrigues LS, Batista-Silva LR, Silva BJA, et al. STING-dependent 2′-5′-oligoadenylate synthetase-like production is required for intracellular mycobacterium leprae survival. The Journal of Infectious Diseases. 2016;**214**:311-320

[61] de Mattos Barbosa MG, de Andrade Silva BJ, Assis TQ, da Silva Prata RB, Ferreira H, Andrade PR, et al. Autophagy impairment is associated with increased inflammasome activation and reversal reaction development in multibacillary leprosy. Frontiers in Immunology. 2018;**9**:1223

[62] Collins AC, Cai H, Li T, Franco LH, Li X-D, Nair VR, et al. Cyclic GMP-AMP synthase is an innate immune DNA

sensor for mycobacterium tuberculosis. Cell Host & Microbe. 2015;**17**:820-828

[63] Dang AT, Teles RM, Weiss DI, Parvatiyar K, Sarno EN, Ochoa MT, et al. IL-26 contributes to host defense against intracellular bacteria. The Journal of Clinical Investigation. 2019;**130**:1926-1939

[64] Wassermann R, Gulen MF, Sala C, Perin SG, Lou Y, Rybniker J, et al. Mycobacterium tuberculosis differentially activates cGAS- and inflammasome-dependent intracellular immune responses through ESX-1. Cell Host & Microbe. 2015;**17**:799-810

[65] Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, Olivas J, et al. The cytosolic sensor cGAS detects Mycobacterium tuberculosis DNA to induce type I interferons and activate autophagy. Cell Host & Microbe. 2015;**17**:811-819

[66] Chauhan S, Kumar S, Jain A, Ponpuak M, Mudd MH, Kimura T, et al. TRIMs and galectins globally cooperate and TRIM16 and galectin-3 co-direct autophagy in endomembrane damage homeostasis. Developmental Cell. 2016;**39**:13-27

[67] Thurston TLM, Wandel MP, von Muhlinen N, Foeglein A, Randow F. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature. 2012;**482**:414-418

[68] Weng I-C, Chen H-L, Lo T-H, Lin W-H, Chen H-Y, Hsu DK, et al. Cytosolic galectin-3 and -8 regulate antibacterial autophagy through differential recognition of host glycans on damaged phagosomes. Glycobiology. 2018;**28**:392-405

[69] Chung AW, Sieling PA, Schenk M, Teles RMB, Krutzik SR, Hsu DK, et al. Galectin-3 regulates the innate immune response of human monocytes. The Journal of Infectious Diseases. 2013;**207**:947-956

[70] Prasad PVS, Kaviarasan PK. Leprosy therapy, past and present: Can we hope to eliminate it? Indian Journal of Dermatology. 2010;**55**:316-324

[71] Lockwood DN. Steroids in leprosy type 1 (reversal) reactions: Mechanisms of action and effectiveness. Leprosy Review. 2000;**71**:111-114

[72] Sales AM et al. A comparative study between 12 and 24-dose therapeutic regimens for multibacillary leprosy patients. International Journal of Leprosy and Other Mycobacterial Diseases. 2004;**72**(3):320-323

[73] Espert L et al. Autophagy in Mycobacterium tuberculosis and HIV infections. Frontiers in Cellular and Infection Microbiology. 2015;**2**:49

[74] Netea-Maier RT et al. Modulation of inflammation by autophagy: Consequences for human disease. Autophagy. 2016;**12**:245-260

[75] Fleming A, Noda T, Yoshimori T, Rubinsztein DC. Chemical modulators of autophagy as biological probes and potential therapeutics. Nature Chemical Biology. 2011;**7**:9-17

[76] Rubinsztein DC, Codogno P, Levine B. Autophagy modulation as a potential therapeutic target for diverse diseases. Nature Reviews. Drug Discovery. 2012;**II**:709-730

[77] Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl 2 antiapoptotic proteins inhibits Beclin1 dependent autophagy. Cell. 2005;**122**:927-939

[78] Yang J, Yao S. JNK Bcl xL Bax/ Bak pathway mediates the crosstalk

between matrine induced autophagy and apoptosis via interplay with Beclin1. International Journal of Molecular Sciences. 2015;**16**:25744-25758

[79] Kang TJ, Chae GT. Detection of toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. FEMS Immunology and Medical Microbiology. 2001;**31**:53-58

[80] Johnson CM, Lyle EA, Omueti KO, Stepensky VA, Yegin O, Alpsoy E, et al. Cutting edge: A common polymorphism impairs cell surface trafficking and functional responses of TLR1 but protects against leprosy. Journal of Immunology. 2007;**178**:7520-7524

[81] Bochud P-Y, Sinsimer D, Aderem A, Siddiqui MR, Saunderson P, Britton S, et al. Polymorphisms in toll-like receptor 4 (TLR4) are associated with protection against leprosy. European Journal of Clinical Microbiology & Infectious Diseases. 2009;**28**:1055-1065

[82] Zhang F-R, Huang W, Chen S-M, Sun L-D, Liu H, Li Y, et al. Genomewide association study of leprosy. The New England Journal of Medicine. 2009;**361**:2609-2618

[83] Berrington WR, Macdonald M, Khadge S, Sapkota BR, Janer M, Hagge DA, et al. Common polymorphisms in the NOD2 gene region are associated with leprosy and its reactive states. The Journal of Infectious Diseases. 2010;**201**:1422-1435

[84] Cardoso CC, Pereira AC, Brito-de-Souza VN, Dias-Baptista IM, Maniero VC, Venturini J, et al. IFNG +874 T>a single nucleotide polymorphism is associated with leprosy among Brazilians. Human Genetics. 2010;**128**:481-490

[85] Yang D, Chen J, Shi C, Jing Z, Song N. Autophagy gene polymorphism is associated with susceptibility to leprosy by affecting inflammatory cytokines. Inflammation. 2014;**37**:593-598

[86] Levine B et al. Development of autophagy inducers in clinical medicine. The Journal of Clinical Investigation. 2015;**125**(1):14-24

[87] Floto RA, Sarkar S, Perlstein EO, Kampmann B, Schreiber SL, Rubinsztein DC. Small molecule enhancers of rapamycin-induced TOR inhibition promote autophagy, reduce toxicity in Huntington's disease models and enhance killing of mycobacteria by macrophages. Autophagy. 2007;**3**:620-622

[88] Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, et al. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. Nature Chemical Biology. 2007;**3**:331-338

[89] Fabri M, Realegeno SE, Jo EK, Modlin RL. Role of autophagy in the host response to microbial infection and potential for therapy. Current Opinion in Immunology. 2011;**23**:65-70

[90] Kim J-J, Lee H-M, Shin D-M, Kim W, Yuk J-M, Jin HS, et al. Host cell autophagy activated by antibiotics is required for their effective antimycobacterial drug action. Cell Host & Microbe. 2012;**11**:457-468

[91] Parihar SP, Guler R, Khutlang R, Lang DM, Hurdayal R, Mhlanga MM, et al. Statin therapy reduces the mycobacterium tuberculosis burden in human macrophages and in mice by enhancing autophagy and phagosome maturation. The Journal of Infectious Diseases. 2014;**209**:754-763

[92] Lobato LS, Rosa PS, Ferreira JS, Neumann AS, Da Silva MG, Do Nascimento DC, et al. Statins

increase rifampin mycobactericidal effect. Antimicrobial Agents and Chemotherapy. 2014;**10**:5766-5774

[93] Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. Vitamin D in the treatment of pulmonary tuberculosis. The Journal of Steroid Biochemistry and Molecular Biology. 2007;**103**:793-798

[94] Fabri M, Stenger S, Shin D-M, Yuk J-M, Liu PT, Realegeno S, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Science Translational Medicine. 2011;**3**:104ra102

[95] Selvaraj P. Vitamin D, vitamin D receptor, and cathelicidin in the treatment of tuberculosis. Vitamins and Hormones. 2011;**86**:307-325

[96] Liu PT, Wheelwright M, Teles R, Komisopoulou E, Edfeldt K, Ferguson B, et al. MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy. Nature Medicine. 2012;**18**:267-273

[97] Abate G, Ruminiski PG, Kumar M, Singh K, Hamzabegovic F, Hoft DF, et al. New verapamil analogs inhibit intracellular mycobacteria without affecting the functions of mycobacterium-specific T cells. Antimicrobial Agents and Chemotherapy. 2015;**60**:1216-1225

[98] Juárez E et al. Loperamide restricts intracellular growth of mycobacterium tuberculosis in lung macrophages. American Journal of Respiratory Cell and Molecular Biology. 2016;**55**:837-847

Chapter 5

# Macrophage Polarization Is Decisive for Chronic Bacterial Infection-Induced Carcinogenesis

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## Abstract

Macrophages are the special cells of the immune system and play both immunological and physiological role. One of the peculiar characteristics of macrophages is that they are double-edged and highly plastic component of immune system. Due to this characteristic, they are responsible for both progressions as well control of a variety of inflammatory, infectious and metabolic diseases and cancer. These are found in the body in three major phenotypes, which are known as M0 (also known as naïve); M1 (classically activated macrophages); and/or M2 (alternatively activated macrophages) at normal physiological conditions. We have been exploring macrophages in context of bacterial infection and previously demonstrated that M2 polarization of M1 effector alveolar macrophages during chronic/persistent *Chlamydia pneumonia*, *Mycobacterium tuberculosis* and *Helicobacter pylori* pathogens are decisive for the infection induced cancer development in host. Since chronic infection with these pathogens has been associated with adenocarcinoma, therefore, we feel that disruption of macrophage plasticity plays crucial role in the host for the development of cancer. On the basis of this, we propose that in such pathological conditions, management of M1/M2 imbalance is paramount for minimizing the risk of developing cancer by chronic and persistent infection.

**Keywords:** macrophages, immuno-epigenetics, metabolic programming, sterile inflammation, cancer

## 1. Introduction

Recent studies have demonstrated that macrophages display high grade of phenotypic plasticity due to which they can both enhance and inhibit immune response. This phenotypical plasticity of macrophages enables them to contribute to pathogenesis of large variety of diseases as well as homeostasis mechanisms. Due to this characteristic, these cells are now known as double-edge component of immunity as well. Many studies have demonstrated that these cells can enhance the progression as well as control many infectious and tumor [1] diseases. Both peripheral and tissue macrophages together constitute the reticuloendothelium system which plays a major role in both sensing microbial antigens and their subsequent eradication [2]. Macrophages are recruited to the inflamed/infected

tissues, react to a variety of stimuli, and acquire either classical phenotype also known as M1 or alternative phenotype also known as (AAM, M2). Classically activated macrophages are immunostimulatory in nature and have Th1-orienting capacity while M2 are immunoregulatory in nature and have Th2 programming capacity [3]. The latter ones are anticipated to support the survival of various intracellular pathogens during persistency and believed to promote neoplastic transformation of infected tissue micromilieu (**Figure 1**). AAM accumulation in majority of adenocarcinoma (around 10% cases) confers poor prognosis during microbial persistency. Therefore in such abnormal pathological conditions, selective elimination of macrophages by ablating colony-stimulating factor 1(CSF-1) in LySMcre and op/op mouse model [4] or by the use of pharmacological drugs such as clodronate liposomes [5], which are among few possible modalities for mitigating macrophage-associated neoplasia. Within the frame of the above mentioned, this chapter will discuss various strategies to repolarize tumor-associated macrophages (TAM) during cancer development and uncover how selective activation of M1 macrophages could control infection-induced cancer but also existing anti-tumor immune therapies in both mouse and human model of tumors with special emphasis on gastric and lung tumors and inflammatory diseases like inflammatory bowel disease (IBD), which are responsible for global mortality. This may be achieved by targeting the major intracellular signaling component such as sphingolipids and Th2/Th17 responses, which promote M2 phenotype during persistent infection and potentially involve in the development of cancer.

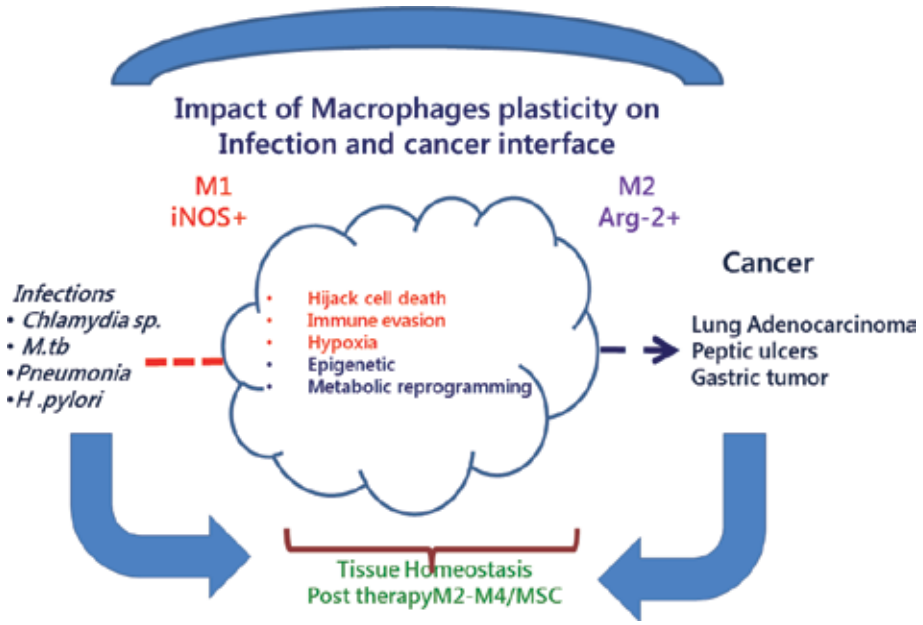


**Figure 1.**
*Schematic representation of various approaches by which persistent infection with human pathogens disrupts functional plasticity of effector macrophages and promote cancer progression. The figure depicts how certain pathogens exploit various cellular and genetic mechanisms and promote M2 polarization of iNOS+ effector macrophages which are the special and double-edge component of the immune system. Phenotypic and functional polarization of effector macrophage is decisive event and anticipated to escort pathogens for neoplastic transformation of infected tissues during latent infections.*

## 2. Pathogens disrupt macrophage plasticity and effector response during persistency

Recent study has demonstrated that a bacterial product known as trabectedin is toxic to macrophages. This product inhibits NF-Y and KLF-2/4 which is important for the differentiation of macrophages in tumor micromilieu [6]. Similarly mitigating NF-κB, STAT3 and HIF-1 are involved in the activation of naïve macrophage to M1 effector phenotype and hold tremendous therapeutic option for modulating macrophages activation. Histopathological analysis of persistently infected lungs reveals the infiltration by specialized macrophage known as foamy macrophages. These are lipid-loaded macrophages and quite refractory in their nature. These macrophages behave more like AAM and are actively involved in the clearance of cellular debris and dead bacteria containing neutrophils and DC [7]. In some cases of coronary atheroma patients, these macrophages acquire phenotype similar to TAM (tumor-associated macrophages) and harbor dead bacteria in their endosome [8]. The presence of these macrophages thus promotes non-immunogenic inflammation which is similar to cancer-associated inflammation and supports opportunistic survival of deadly pathogens. Both phenotypic and functional polarizations of M1/M2 effector phenotype of macrophages are believed to be one of the prognostic factors contributing to the development of tumor during persistent/latent infections (**Figure 1**) in host. Once infiltrated in the infected lungs, these AAM/foamy macrophages potentially modify effector T cells and predispose them also as refractory which are otherwise proficient in the killing of infected cells. These macrophages secrete a plethora of cytokines/growth factors like VEGF-β, TGF-β, hypoxia-inducible factor, and sphingolipids which altogether contribute to neoplastic transformation of infected tissue. High gradient of VEGF and TGF-β promotes the differentiation of regulatory T cells [9] and inhibits the effector response of CD8+ T cells [10]. On the other hand, sphingolipids particularly S-1P/ceramide (either host or pathogen-derived) are known to promote mitophagy [11], M2 polarization of infiltrating M1, or naïve monocyte/macrophage populations [12]. In view of this, and to restore Th1 effector immune response during latent infection, reactivation of M1 effector phenotype of macrophage thus represents the most suitable therapeutic interventions. Apart from this, modulating the cytokine network also seems to be the most effective strategy for boosting immunity for the management of latent/persistent infections.

## 3. Bacterial persistency hijacks programmed cell death and autophagy and promotes immune metabolic reprogramming

Pathogenic bacteria have evolved several ways to survive efficiently in the phagocytes during their dissemination across the lymphatic system. Various pathogens adapt various strategies to this purpose which range from conferring resistance to the apoptosis [13], immune evasion [14], and metabolic programming of myeloid cells [15] as shown in **Figure 2**. Of these, conferring resistance and insensitivity for cell death in the infected cell seems to be one of the most fundamental processes. A range of bacterial pathogens like *Chlamydia trachomatis* (*C. tr*), *Chlamydia pneumonia* (*C. pn*), and *Helicobacter pylori* (*H. pylori*) which are associated with the pathogenesis of lung [16] and stomach cancer [17] respectively, exploit death and immune signaling for surviving in the hostile environment of antigen presenting (**Figure 2**) and effector cells. We [18, 19] and others [20] have demonstrated that *C. pn* and *C. tr* increase the stability of various endogenous regulators of apoptosis

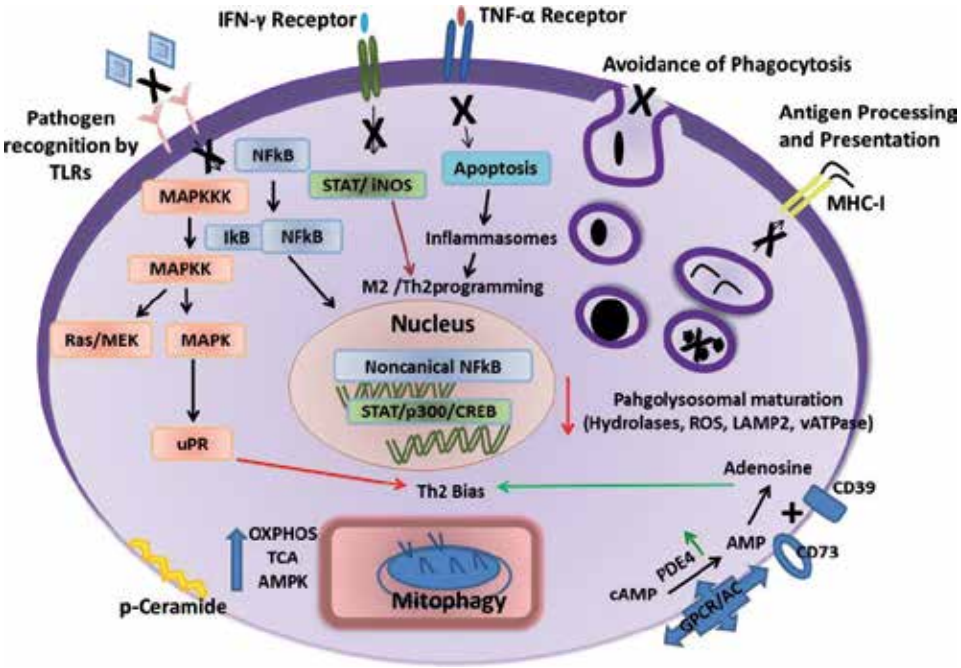


**Figure 2.**
*Bacterial pathogens potentially exploit and interfere in various pathways in committed macrophage for subverting effector mechanisms during latency. Pathogenic bacteria interfere with various key signaling pathways which are important for the effector responses, e.g., recognition by receptors, uptake, and phagocytosis, lysosomal degradation, and alter signaling pathways and secretion of Th1 cytokines for establishing Th2 bias.*

proteins called IAP and inhibit the activity of both apoptotic and inflammatory Caspases 3, 8, and 11 during latent infection. Upregulation of CIAP2 and XIAP proteins [19] during acute and persistent/latent infection has shown the increase in noncanonical signaling of NF-κB which is a master transcription factor involved in both cell death inhibition and inflammatory programming and autophagy for Th1 effector response during infection. Our recent study has shown that *C. pneumonia* potentially interfere with M1 programming of infected macrophages [21] when stimulated with their cognate innate and inflammatory stimuli which is due to increased expression of HIF-1 and p38MAPK proteins [22] which are known to promote unfolded proteins response (UPR) in the infected macrophages [23] which in turn predispose macrophages refractory to immune stimulation. Within macrophages, mitochondria is potentially involved in the innate immune response of macrophages against a variety of successful intracellular pathogens mainly by flushing catatonic peptides like LL37, CAP 12, and CAP 18 to cytoplasmic compartment for efficient capture and killing of pathogen in mature phagolysosomes [24]. Recent studies have amply demonstrated that most of the opportunistic pathogens interfere in the mitochondrial physiology by promoting mitophagy which jeopardizes innate immune defense of macrophages against pathogens. In such cases, tweaking mitochondria by using Smac mimetic-based interventions holds promises in the management of persistent infection. Although we have recently demonstrated that Smac mimicry [21] is capable of mounting an efficient immune response against mild pathogens, it fails to do so against pathogenic microbes like *Leishmania donovani*. Since pathogenic microbes enhance the expression of p38MAPK/HIF-1 pathways [25] for sabotaging macrophages functions, therefore we feel that at the moment targeting p38MAPK/HIF-1 in conjunction to Smac mimetics would be paramount for controlling pathogenic microbes. This is a quite intriguing aspect of

the field and needs further in-depth investigation. In the same line, many pathogens are known to interfere with autophagy process which is yet another potential mechanism which influences antigen presentation by APC to T cells for the clearance of pathogens. Many pathogens attack mTORc1 complex and disrupt the autophagosome apparatus [26, 27] and inhibit their presentation by APC to T cells for immune surveillance during latent phase. Therefore pharmacological tweaking of autophagy may offer one potential strategy for clearing dead pathogens effectively from the lung tissue. Irrespective of acute or persistent phase, all pathogens utilize host metabolism for their survival inside the host. During persistency, many pathogens consume carbohydrate and protein reservoir of infected macrophages and alter their metabolic rates. L-Tryptophan [28] and glucose are critical for macrophages, and their fluctuation largely dictates the effector response of infected macrophages as well as the fate of pathogen in APC/phagocytes. During persistent infection, *Chlamydia* sp. utilize cellular depot of L-tryptophan amino acid by activating IDO gene and metabolize it to L-kynurenine which impedes glycolysis [29] in the macrophages. M1 effector macrophages rely on cellular depot of glucose for both activation and differentiation into effector phenotype; therefore, during latent infection, pathogens disrupt in the glucose metabolism and promote hypoglycemia rendering them refractory for optimum defense against pathogens.

## 4. Human pathogens promote epigenetic changes in macrophages during persistency

At genome level, *C. tr* infection causes global hypoacetylation and hypermethylation of lysine residues on core histones which alter histone post-translational modifications which differ between acute and persistent infections. Upregulation of pH2AX (Ser139) and H3K9me3 which are hallmarks of DNA double-strand breaks (DSBs) and senescence-associated heterochromatin foci (SAHF), respectively, during *Chlamydophila trachomatis* [30] infection suggested teratogenic manifestation of *Chlamydia* persistency. This is largely due to increasing levels of reactive oxygen species (ROS) which is produced during latent infection. ROS contribute to DNA double-stranded breaks leading to persistent DNA damage, which in turn triggers SAHF formation in an ERK-dependent manner [30]. CPAF and CADD proteins from *Chlamydophila* pathogens are known to perturb host cell cycle machinery and inhibit recruitment of the DNA damage response proteins pATM and 53BP1 to damaged sites interfering with DNA repair mechanisms [30]. Despite impaired DNA repair, infected cells continue to proliferate which are in turn supported by enhanced oncogenic signals such as ERK, CyclinE, and SAHF. These changes altogether lead to the malignant transformation of infected tissue. Similarly, other pathogens like *Campylobacter rectus* [31], which is associated with oral cancer, downregulate Igf2 gene and enhance DNA methylation at its promoter which can be attributed to bacteria-mediated epigenetic modifications to the host genome. Other pathogens like *Salmonella enterica* serovar Typhi, which is one of the prognostic factors for the susceptibility for gallbladder carcinoma, exploit MAPK and AKT pathways [32] which initiate and sustain neoplastic transformation of infected host. Macrophages sense and trigger immune response against pathogens via TLR-linked signaling cascade. Under normal circumstance, almost all pathogens trigger TLR signaling pathways for activating macrophages; however, only few obligate intracellular pathogens, in hitherto, interfere with TLR signaling directly or indirectly and limit defense mechanisms [33] of effector macrophage like pattern of cytokines secretions, their uptake, and phagocytosis by macrophages. Although there are multiple ways how a pathogen can interfere with TLR signaling, so far TLR2/4 triggered

hypoxia and associated sterile inflammatory response, and/or TLR masking/shedding mechanisms have been identified and proposed [34, 35]. *Yersinia enterocolitica* and *Candida albicans* are known to induce immunosuppression through TLR2-mediated IL-10 release and differentiation of T-helper cells to CD4+CD25+ regulatory T cells [36]. *Yersinia* species secrete a virulence (V) antigen, LcrV, which binds to CD14 and TLR2, trigger IL-10 secretion, and mediate immunosuppression. It has recently been shown that a particular residue in the N-terminal region of LcrV targets TLR2 and is required for altering IL-10 induction via TLR2 [37]. Likewise, *H. pylori* escapes from recognition by the TLRs due to the removal of phosphate groups from the 1′ to 4′ positions of lipid A in LPS, which confers low negative charge to this molecule and increases the chance of escaping TLR recognition. The recognition of non-LPS ligands by TLR2 leads to anti-inflammatory responses that are associated with IL-10 production [38]. Flagellin of *H. pylori* is one of the PAMP which potentially modifies the N-terminal recognition domain of TLR5 and helps in escaping the innate immune responses. Manipulation of amino acid 89–96 of the recognition domain of TLR5 results in low affinity to flagellin binding [39]. Under recurrent/latent infection state, TLR2-mediated signaling, hitherto, inhibits IFN-γ response and hijacks Th1 programming of macrophages. A pathogen like *M. avium* inhibits IFN-γ signaling in TLR2-dependent manner where it enhances the expression of dominant-negative STAT1b. Similarly, 19KD protein of *Mycobacterium tuberculosis* inhibits IFN-γ-induced expression of HLA-DR and FcγR1 expression on human macrophages [40]. In addition to the induction of anti-inflammatory signals by TLRs, certain pathogenic microbes have developed strategies to either block or avoid their recognition by TLRs and subsequent activation of the innate defense. According to one recent study, phospholipids and Ypk protein of *Treponema pallidum* interfere in TLRs (TLR3, TLR4, and TLR9) signaling [41] by blocking the function of LPS-binding protein and CD14. Several bacterial pathogens have altered specific PAMP structures to circumvent recognition by TLR4 or TLR5; pathogens, such as *Porphyromonas gingivalis* or *Leptospira*, which have specialized LPS structures that only interact with TLR2; likewise, in *Helicobacter pylori*, the flagellin [39, 42] is not appropriately recognized by TLR5, approving the survival of the bacteria without loss of virulence. Virulent strains of *Salmonella typhi* escape from their recognition by host PRR by various mechanisms, which predominately include modifying their lipid A by various mechanisms including deacylation, palmitoylation, and the addition of aminoarabinose [43]. Pathogens have evolved in several ways of avoiding NO-mediated killing that plays a central role in effector response in phagocytes. *Salmonella typhi* reside in a specialized membrane compartment called the *Salmonella*-containing vacuole (SCV), which is similar to inclusion in the case of *Chlamydia* sp. in macrophages, and use a T3SS called *Salmonella* pathogenicity island 2 (Spi2), which protects them from reactive nitrogen intermediates. Spi2-deficient strains of *S. typhi* get colonized in iNOS+ compartment efficiently [44] with the intracellular organisms in the SCV. Intracellular organisms have also developed mechanisms to detoxify NO-mediated effects. These include the ability to repair damage caused by reactive nitrogen intermediates and to detoxify these molecules. Pathogens have evolved the strategies of inhibiting iNOS activity which is the characteristic feature of M1 effector macrophages. Mucosal pathogen *Citrobacterro dentium* causes a marked reduction in the level of iNOS activity in macrophages [45]. There are many reported examples of bacterial pathogens altering inflammatory cytokines related to signaling. *Staphylococcus aureus* proteins A and M bind directly to the TNF-α receptor 1, on respiratory epithelium, which then potentiates a chemokine and cytokine cascade and subsequent disease [46]. Similarly, *Shigella flexneri*, through exploring type III effector, OspG, which is a protein kinase, activates ubiquitin-conjugated enzymes, thereby affecting

phospho-ikBα degradation and subsequent NF-κB activation. Both *Chlamydia pneumonia* and *Chlamydia trachomatis* promote shedding of TNF receptor 1 by activating TACE activity and shunt TNF signaling though TNFR2 [47] and resist antibacterial and inflammatory action of TNF which is major component of effector macrophages.

## 5. Potential interventions for reactivating refractory macrophages for therapy outcome

While the application of antibiotics is sufficient to control acute infection however during persistent infection, the outcome of treatment mostly remains refractory. This is due to increased density of refractory macrophages in various affected tissues which resists many therapies as seen in many similar diseases like cancer and metabolic disease which is mediated with tissue accumulation of type 2 or tumor-associated macrophages. It is now well accepted by medical community that increased densities of these macrophages are associated with poor prognosis in many infectious, tumor, and metabolic disease. In such conditions antibiotics and/or chemotherapy would require an additional regimen for effective treatment. During past decades, the growing evidence suggested that TAMs clearly play an important role in tumor progression, metastasis, and resistance to available chemotherapies by modulating the microenvironment inside the tumor mass as well as in the stoma. Therefore, it is important to reeducate or target the TAMs (M2-like) to antitumor M1-like macrophage phenotype for successful treatment of several human malignancies. In the remaining sections of this chapter, we have discussed various macrophage-specific and nonspecific interventions for reactivating refractory population of macrophages for improving existing therapies.

### 5.1 Neoadjuvant for retuning refractory macrophages

Many interventions have been made to reactivate or retune the TAM, but most of them could not influence the disease outcome profoundly. In this context our recent studies have shown neoadjuvant impact of low-dose radiation for retuning TAM, T cell-aided therapy [48], and subsequent normalization of vasculature in solid tumor-bearing animals. Since infection induced adenocarcinoma is manifested with high grade infiltration of foamy macrophages, which are like M2 TAM, therefore, on the basis of our tumor studies, we propose low dose gamma irradiation as one of the non-specific therapeutic interventions for the management of persistent infection-induced tumor development.

### 5.2 Nanomedicine as immune adjuvant for refractory macrophages

Nanomedicine has emerged as one of the new modalities for reprogramming of both naïve as well as refractory macrophages toward their effector phenotype and thus represents one potential intervention for the management of latent infectious disease. We and others have recently demonstrated that due to their size and unspecific adjuvant properties, nanocarriers/nanocapsules can penetrate inflamed tissue microenvironment effectively and deliver drug in controlled and sustained rate for exerting adjuvant actions on macrophages in the inflamed and fibrotic lesions of infected tissue. Nanomedicine-based approaches may impact refractory macrophages at various levels, namely, (i) enhanced infiltration of fresh monocyte/macrophages, (ii) direct killing, and (iii) in situ polarization of AAM/foamy-like macrophages during chronic infection to assist clearing of infection. One of the interesting

mechanisms by which nanoparticle may improve the therapy outcome is to control the differentiation of naive monocytes toward iNOS+ M1 effector macrophages and replace CD11b+/iNOS-/Arginase-1+ AAM during chronic infections. In this context, our recent work has shown that a certain biodegradable amino acid-based pNAPA nanocapsule can potentially stimulate naïve macrophage to the M1 effector phenotype. On the basis of these merits, the nanocapsules may be used as adjuvant for activating innate immune system for the management of infectious diseases and cancer. Another potential application of nanoparticles is to deliver drugs or biopharmaceuticals for preventing differentiation of effector phenotype of macrophage to refectory. In this context one study has shown that delivery of CCR2 and CCR5 siRNA-loaded nanoparticles was able to reduce the recruitment of monocytes to inflamed tissue [49]. Nanocarrier-based approaches can be used for the direct killing of the refractory macrophages as well. For instance, liposomal formulations have been developed for the delivery of bisphosphonates such as zoledronates and clodronates. Subcutaneous/orthotropic injections of these nanocarriers result in the depletion of AAM accompanied with impaired angiogenesis and reduction in metastasis. Nonspecific targeting is the major issue with nanocarriers which can be addressed by tagging these nanocapsules with specific ligands such as LyP1 and mannose receptors (e.g., CD206) which are highly expressed by TAM/AAM [50] for effective targeting of macrophages. PLA-PEG nanoparticles, cyclodextrin nanoparticles, and liposomal formulations have been developed for loading drug cargoes such as sunitinib, IL-12 plasmids, TGF-β inhibitors, and VEGF siRNA for reprogramming of refractory macrophages for skewing in situ Th1 effector immune response against latent infections [51–54].

### 5.3 Immunotherapeutics are the next-generation treatment modalities

One of the key characteristics of both AAM and TAM is to restrict Th1 immune response/T-cell programming by virtue of their releasing of Th2 cytokines and growth factors, which stimulate the neoplastic differentiation of inflamed fibroblast in tissue [55]. One of the major mechanisms by which these cells limit effector T-cell response is to engage programmed cell death ligands 1 and 2 (PD-L1, PD-L2) [56] which are expressed by the AAM/TAMs. Pulmonary infiltration of lipid rich foamy macrophages is a typical evidence of persistent infection-induced non-immunogenic/sterile inflammatory immune response during persistent/latent *C. pn* and *M. tb* infections. Foamy macrophages are special kinds of AAMs which have poor antibacterial defense mechanisms and serve as carriers of many pathogens during dissemination. These macrophages inhibit Th1 programming of CD4/8+ T cells and promote Th2 bias by secreting IL-4 and IL-13 in infected tissue micromilieu and help these bacteria in immune escape. These macrophages are known to express PD-1L which after binding to PD-1 T cells drives anergy in T cells. Binding of PD-1L to PD-1 receptor triggers functional anergy in cytotoxic T lymphocytes (CTL) which are otherwise effective in eradicating persistently infected dead cells. Many pathogens exploit these pathways as a major immune evasion mechanism for securing their opportunistic survival. For example, *Chlamydia* sp., *H. pylori*, and *Leishmania donovani* pathogens are known to modulate the expression of PD-L1 on macrophages [57–59] for dumping adaptive immune responses. In such conditions, blocking PD-L1 pathway by monoclonal antibody against PD-1/PD-L1 has been found to be effective in restoring phagocytic potential of macrophages for dead cell clearance and subsequent control of tumor in mice models. For this study, it is anticipated that co-administration of antibody against PD-1/checkpoint inhibitors (CTLA4) along with antibiotics would be beneficial for the management of latent infectious diseases. In the same line, vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β), fibroblast growth factor (FGF), and platelet-derived growth factor

(PDGF), which are potentially secreted by AAM and promote sterile inflammation, also represent potential pharmacological targets for enhancing immunogenic death of cells during latent phase. Colony-stimulating factor 1 receptor (CSF1R) represents yet another promising target for therapeutic interventions because CSF1R signaling is crucial for differentiation, recruitment, and survival of TAMs [60].

### 5.4 Antibody/small molecule inhibitor targeting polarization of refractory macrophages

Intracellular pathogens, during both acute and latent infection, fiddle with various signaling pathways which range from receptor-associated cell death and innate immune signaling, antigen presentation, vesicular transport, and phagocytosis pathways. Although we have disused these in earlier section, here we will discuss the pharmacological and clinical significance of various approaches which may be decisive for mitigating cellular perturbations in the host for restoring immune defenses of macrophage during persistency. In this context, our recent study [21] has proposed that Smac mimetic (IAP-specific inhibitors)-based strategy has potential for enhancing immunogenic cell death of infected cells and reactivating refractory macrophages for improved clearance. Due to these virtues, several Smac mimetics have entered in the second-phase clinical trial against cancer, and we anticipate that the same is expected to help immune system for the management of persistent bacterial infection as well. Other than this, many pathogens exploit MAPK pathways [61] for their benefits and induce production of IL-10 cytokines in the macrophages which further inhibits T-cell programming mainly by promoting T-cell exhaustion [62]. Other than this, elevated levels of p38MAPK promote sterile/anti-inflammatory response, which supports opportunistic survival of pathogen inside macrophages. Likewise, many pathogens exploit cAMP/PKA pathways and acquire Th2 bias during their persistency [63] for securing their survival; TNF-α is a major and key cytokine responsible for receptor-mediated killing of infected cells. We (unpublished data) and others have shown that many intracellular pathogens, during persistency, potentially target this cytokine and inactivate cell death pathways in TACE- or ADAM-dependent manner. Pathogens like *Chlamydia* sp. secrete CPAF, CADD, and hsp60 exerting TACE activity and cause shedding of TNFR1 [21], which actually induces cell death. Interestingly these proteins which are secreted by chlamydial pathogens require MAPK activation for efficient shedding of TNFR1 [64]. Therefore on the basis of the above observations, designing a suitable MAPK/phosphodiesterase 4 (PDE4) inhibitors [65] thus represents a compelling approach for controlling bacterial persistency and associated immune evade mechanism. Sphingolipids are yet another dual-specific cellular targets [66] of many pathogens for deviating Th1 effector immune response [67]. We have recently demonstrated that S1P/ceramide rheostat is an important parameter which can largely dictate whether pathogen would undergo persistency or not [66]. In this direction, we have recently demonstrated that the gain of S-1P during acute *M. tb* infection affords protective immunity in host for controlling pathogen burden; however, the same is anticipated to promote mycobacterial persistency and thus in such conditions, employment of sphingolipid-based inhibitors, in hitherto, would favor host for breaking persistency and induction of protective immune responses.

### 5.5 Future perspectives: macrophage-based palliative strategies for tissue homeostasis post-antibiotic purging

Successful therapy post-antibiotic treatment infection should normalize the tissue microenvironment and restore homeostasis. This could be achieved by chelating

oxidative stress and remnants of inflammatory response for the replenishment of tissue mass, which normally gets lost during various therapeutic procedures. Management of M1/M2 imbalance is believed to be the key for minimizing the risk of having cancer by chronic and persistent infection with intracellular pathogens. In the clinics, this can be achieved by exchanging refractory populations of macrophage with effector ones which can control the sterile reactions and tumorigenesis. However, due to the pro-inflammatory nature of iNOS+ effector macrophages, this may elicit another sequence of destruction, which alone may not be beneficial. Therefore in such delicate conditions, co-administration of M1 macrophage with mesenchymal stem cell regenerative approach seems to be optimum for reconstituting the affected tissues and organs. The potential inclusion of macrophage-mesenchymal cell-based therapeutic intervention could be categorized under prospective palliative therapies for restoration of physiological function post-treatment.

## 6. Conclusion

Since chronic infection with bacterial pathogens has been associated with adenocarcinoma, therefore, we believe that the management of M1/M2 imbalance is paramount for minimizing the risk of developing cancer by chronic and persistent infection of the lung, stomach, and cervix. This may be achieved by targeting major signaling pathways such as sphingolipids and Th2/Th17 responses which drive M2 phenotype and which are potentially involved in the development of cancer. In the light of the above, we propose that selective activation of M1 macrophages could improve existing antitumor immune therapies in both mouse and human models of tumors with special emphasis on gastric and lung tumors and inflammatory diseases like inflammatory bowel disease (IBD) which are responsible for global mortality.

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## Conflict of interest

The authors have no competitive/financial interest.

## Acronyms and abbreviations

| | |
|---|---|
| MAPK | mitogen-activated protein kinase |
| MAPKK | MAPK kinase |
| MAPKKK | MAPKK kinase |
| NF-κB | nuclear factor κB |
| STAT | signal transducer and activator of transcription |
| TLR | toll-like receptor |
| TNF-α | tumor necrosis factor-α |

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## References

[1] Nadella V, Singh S, Prakash H. Macrophages directed approaches are paramount for effective cancer immunotherapies. Integrative Cancer Science and Therapeutics. 2016;**3**:465-472

[2] Gordon S, Plüddemann A. Tissue macrophages: Heterogeneity and functions. BMC Biology. 29 Jun 2017;**15**(1):1-18

[3] Atri C. Role of human macrophage polarization in inflammation during infectious diseases. International Journal of Molecular Sciences. 2018;**19**:1801

[4] Hua L, Shi J, Shultz LD, Ren G, Harbor B. Genetic models of macrophage depletion. Methods in Molecular Biology. 2019;**1784**:1-15

[5] Weisser SB, Van Rooijen N, Sly LM. Depletion and reconstitution of macrophages in mice. 2. Derivation of polarized macrophages from bone marrow aspirates. Journal of Visualized Experiments. 2012;**1**(66):5-11

[6] Sessa C, De Braud F, Perotti A, Bauer J, Curigliano G. Trabectedin for women with ovarian carcinoma after treatment with platinum and taxanes fails. Journal of Clinical Oncology. 2019;**23**:1867-1874

[7] Teng O, Ke C, Ang E, Guan XL. Macrophage-bacteria interactions—A lipid-centric relationship. Frontiers in Immunology. 2017;**8**:1836

[8] Kozarov E. Bacterial invasion of vascular cell types. Future Cardiology. 2012;**8**:123-138

[9] Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, Colussi O, et al. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Research: 2013;**73**:539-549

[10] Gavalas NG, Tsiatas M, Tsitsilonis O, Politi E, Ioannou K, Ziogas AC, et al. VEGF directly suppresses activation of T cells from ascites secondary to ovarian cancer via VEGF receptor type 2. British Journal of Cancer. 2012;**107**:1869-1875

[11] Dany M, Ogretmen B. Ceramide induced mitophagy and tumor suppression. Biochimica et Biophysica Acta. 2016;**1853**:2834-2845

[12] Rodriguez YI, Campos LE, Castro MG, Aladhami A, Alvarez SE. Sphingosine-1 phosphate: A new modulator of immune plasticity in the tumor microenvironment. Frontiers in Oncology. 2016;**6**:1-16

[13] Kobayashi SD, Braughton KR, Whitney AR, Voyich JM, Schwan TG, Musser JM, et al. Bacterial pathogens modulate an apoptosis differentiation program in human neutrophils. PNAS. 2003;**100**:10948-10953

[14] Finlay BB, Mcfadden G. Review anti-immunology: Evasion of the host immune system by bacterial and viral pathogens. Cell. 2006;**124**:767-782

[15] Kelly B, Neill LAJO. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Research. 2015;**25**:771-784

[16] Chanudet E, Adam P, Nicholson AG, Wotherspoon AC, Ranaldi R, Goteri G, et al. *Chlamydiae* and mycoplasma infections in pulmonary MALT lymphoma. British Journal of Cancer. 2007;**97**:949-951

[17] Applications S. The infection connection. *Helicobacter pylori* is more than just the cause of gastric ulcers. Science & Society. 2006;**7**:5-8

[18] Prakash H, Becker D, Böhme L, Albert L, Witzenrath M, Rosseau S, et al. cIAP-1 controls innate immunity

to *C. pneumoniae* pulmonary infection. PLoS One. 2009;**4**:e6519

[19] Prakash H, Albrecht M, Becker D, Kuhlmann T, Rudel T. Deficiency of XIAP leads to sensitization for *Chlamydophila pneumoniae* pulmonary infection and dysregulation of innate immune response in mice. The Journal of Biological Chemistry. 2010;**285**:20291-20302

[20] Rajalingam K, Sharma M, Paland N, Hurwitz R, Thieck O, Oswald M. IAP-IAP complexes required for apoptosis resistance of *C. trachomatis*-infected cells. PLoS Pathogens. 2006;**2**:e114

[21] Nadella V, Mohanty A, Lalita Sharma SY, Mollenkopf H-J, Mazumdar VB, Palaparthi R, et al. Inhibitors of apoptosis protein antagonists (Smac mimetic compounds) control polarization of macrophages during microbial challenge and sterile inflammatory responses. 2018;**8**:1-21

[22] Lin N, Simon MC, Lin N, Simon MC. Hypoxia-inducible factors: Key regulators of myeloid cells during inflammation find the latest version: Hypoxia-inducible factors: Key regulators of myeloid cells during inflammation. The Journal of Clinical Investigation. 2016;**126**:3661-3671

[23] Grootjans J, Kaser A, Kaufman RJ, Blumberg RS, Program D, Jolla L. The unfolded protein response in immunity and inflammation Joep. Nature Reviews. Immunology. 2017;**16**:469-484

[24] Van Harten RM, Van Woudenbergh E, Van Dijk A, Haagsman HP. Cathelicidins: Immunomodulatory antimicrobials. Vaccine. 2018;**14**:63

[25] Yamala AK, Nadella V, Mastai Y, Prakash H, Paik P. Poly N-acryloyl-(L-phenylalanine methyl ester) hollow core nanocapsules facilitate sustained delivery of immunomodulatory drugs and exhibit adjuvant properties. Nanoscale. 2017;**28**:14006-14014

[26] Steele S, Brunton J, Kawula T. The role of autophagy in intracellular pathogen nutrient acquisition. Frontiers in Cellular and Infection Microbiology. 2015;**5**:1-11

[27] Marcelo C, Milton F, Aguilera O. Autophagy response: Manipulating the mTOR-controlled machinery by amino acids and pathogens. Amino Acids. 2014;**47**:2101-2112

[28] Diskin C, Pålsson-mcdermott EM. Metabolic modulation in macrophage effector function. Frontiers in Immunology. 2018;**9**:1-17

[29] Badawy AA. Kynurenine pathway of tryptophan metabolism: Regulatory and functional aspects. International Journal of Tryptophan Research. 2017;**10**:1178646917691938

[30] Chumduri C, Gurumurthy RK, Zadora PK, Mi Y, Meyer TF. Chlamydia infection promotes host DNA damage and proliferation but impairs the DNA damage response. Cell Host & Microbe. 2013;**13**:746-758

[31] Bobetsis YA, Barros S. Bacterial infection promotes DNA hypermethylation. Journal of Dental Research. 2007;**86**:169-174

[32] Scanu T, Spaapen RM, Bakker JM, Holden DW, Nath G, Neefjes J. *Salmonella* manipulation of host signaling pathways provokes cellular transformation associated with article *Salmonella* manipulation of host signaling pathways provokes cellular transformation. Cell Host & Microbe. 2015;**17**:763-774

[33] Van Avondt K, Van Sorge NM, Meyaard L. Bacterial immune evasion through manipulation of host inhibitory immune signaling. PLoS Pathogens. 2015;**2**:1-8

[34] Tan Y, Zanoni I, Cullen TW, Goodman AL, Jonathan C. Mechanisms

of toll-like receptor 4 endocytosis reveal a common immune-evasion strategy used by pathogenic and commensal bacteria. Immunity. 2016;**43**:909-922

[35] Gopalakrishnan A, Salgame P. Toll-like receptor 2 in host defense against *Mycobacterium tuberculosis*: To be or not to be-that is the question. Current Opinion in Immunology. 2017;**42**:76-82

[36] Netea MG, Sutmuller R, Hermann C, Van Der Graaf CAA, Van Der Meer JWM, Van Krieken JH, et al. Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. Journal of Immunology. 2019;**2004**:3712-3718

[37] Sing A, Rost D, Tvardovskaia N, Roggenkamp A, Wiedemann A, Kirschning CJ, et al. Yersinia V—Antigen exploits toll-like receptor 2 and CD14 for interleukin 10—Mediated immunosuppression. The Journal of Experimental Medicine. 2002;**196**:1017-1024

[38] Jr RMP, Fiske C, Wilson KT. Role of innate immunity in *Helicobacter pylori*-induced gastric malignancy. Physiological Reviews. 2010;**90**:831-858

[39] Gewirtz AT, Yu Y, Krishna US, Israel DA, Lyons SL, Peek RM. *Helicobacter pylori* flagellin evades toll-like receptor 5—Mediated innate immunity. The Journal of Infectious Diseases. 2004;**2279**:1914-1920

[40] Gehring AJ, Rojas RE, Canaday DH, Lakey DL, Harding CV, Boom WH, et al. The *Mycobacterium tuberculosis* 19-kilodalton lipoprotein inhibits gamma interferon-regulated HLA-DR and Fc gamma R1 on human macrophages through toll-like receptor 2. Infection and Immunity. 2003;**71**:4487-4497

[41] Hashimoto M, Asai Y, Ogawa T. Treponemal phospholipids inhibit innate immune responses induced by pathogen-associated molecular patterns. Journal of Biological Chemistry. 2003;**278**:44205-44213

[42] Nigou J, Zelle-rieser C, Gilleron M, Thurnher M, Puzo G. Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: Evidence for a negative signal delivered through the mannose receptor. Journal of Immunology. 2001;**166**:7477-7485

[43] Ernst RK, Guina T, Miller SI. *Salmonella typhimurium* outer membrane remodeling: Role in resistance to host innate immunity. Microbes and Infection. 2001;**3**:1327-1334

[44] Vazquez-Torres A, Xu Y, Jones-Carson J, Holden DW, Lucia SM, Dinaer MC, et al. *Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. Science. 2000;**287**:7-10

[45] Vallance BA, Deng W, De Grado M, Chan C, Jacobson K, Finlay BB. Modulation of inducible nitric oxide synthase expression by the attaching and effacing bacterial pathogen *Citrobacter rodentium* in infected mice. Infection and Immunity. 2002;**70**:6424-6435

[46] Gómez MI, Lee A, Reddy B, Muir A, Soong G, Pitt A, et al. *Staphylococcus aureus* protein a induces airway epithelial inflammatory responses by activating TNFR1. Nature Medicine. 2004;**10**:842-848

[47] Kim DW, Lenzen G, Page A, Legrain P, Sansonetti PJ, Parsot C. The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. PNAS. 2005;**102**:14046-14051

[48] Nadella V, Singh S, Jain Aklank, Jain M, Vasquez KM, Sharma A, et al. Low dose radiation primed

iNOS + M1macrophages modulate angiogenic programming of tumor derived endothelium. 2018;**57**:1-8

[49] Leuschner F, Dutta P, Gorbatov R, Novobrantseva TI, Donahoe JS, Courties G, et al. Articles therapeutic siRNA silencing in inflammatory monocytes in mice. Nature Biotechnology. 2011;**29**:1005-1010

[50] Yan Z, Wang F, Wen Z, Zhan C, Feng L, Liu Y, et al. LyP-1-conjugated PEGylated liposomes: A carrier system for targeted therapy of lymphatic metastatic tumor. Journal of Controlled Release. 2012;**157**:118-125

[51] Liao D, Liu Z, Wrasidlo WJ, Luo Y, Nguyen G, Chen T. Targeted therapeutic remodeling of the tumor microenvironment improves an HER-2 DNA vaccine and prevents recurrence in a murine breast cancer model. Cancer Research. 2011;**71**:5688-5697

[52] Liu X, Gao X, Zheng S. Modified nanoparticle mediated IL-12 immunogene therapy for colon cancer. Nanomedicine Nanotechnology, Biology, and Medicine. 2017;**13**:1993-2004

[53] Park J, Wrzesinski SH, Stern E, Look M, Criscione J, Ragheb R, et al. Combination delivery of TGF-β inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy. Nature Materials. 2013;**11**:895-905

[54] Xu Z, Wang Y, Zhang L, Huang L. Nanoparticle-delivered transforming growth factor-β siRNA enhances vaccination against advanced melanoma by modifying tumor. ACS Nano. 2014;**8**:3636-3645

[55] Burkholder B, Huang R, Burgess R, Luo S, Sloane V, Zhang W, et al. Tumor-induced perturbations of cytokines and immune cell networks. Biochimica et Biophysica Acta. 2014;**1845**:182-201

[56] Mino-kenudson M. Programmed cell death ligand-1 (PD-L1) expression by immunohistochemistry: Could it be predictive and/or prognostic in non-small cell lung cancer? Mechanisms of PD-L1 expression. Cancer Biology & Medicine. 2016;**1**:157-170

[57] Starkey MR, Nguyen DH, Brown AC, Essil A, Kim RY, Yagita H, et al. Programmed death ligand 1 promotes early-life chlamydia respiratory infection—Induced severe allergic airway disease. American Journal of Respiratory Cell and Molecular Biology. 2016;**54**:493-503

[58] Roy S, Gupta P, Palit S, Basu M, Ukil A, Das PK. The role of PD-1 in regulation of macrophage apoptosis and its subversion by *Leishmania donovani*. Clinical & Translational Immunology. 2017;**6**:e137-e110

[59] Silva R, Gullo I, Carneiro F. The PD-1:PD-L1 immune inhibitory checkpoint in *Helicobacter pylori* infection and gastric cancer: A comprehensive review and future perspectives. Porto Biomedical Journal. 2016;**1**:4-11

[60] Cannarile MA, Weisser M, Jacob W, Jegg A, Ries CH, Rüttinger D. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. Journal for Immunotherapy of Cancer. 2017;**5**:1-13

[61] Krachler AM, Woolery AR, Orth K. Manipulation of kinase signaling by bacterial pathogens. The Journal of Cell Biology. 2011;**195**:1083-1092

[62] Garra AO, Murphy KM. From IL-10 to IL-12: How pathogens and their products stimulate APCs to induce T(H)1 development. Nature Immunology. 2009;**10**:929-932

[63] Rodriguez KAM. The myriad roles of cyclic AMP in microbial pathogens, from signal to sword. Nature Reviews. Microbiology. 2013;**10**:27-38

[64] Paland N, Böhme L, Gurumurthy RK, Maurer A, Szczepek AJ, Rudel T. Reduced display of tumor necrosis factor receptor I at the host cell surface supports infection with *Chlamydia trachomatis*. The Journal of Biological Chemistry. 2008;**283**:6438-6448

[65] Li H, Zuo J, Tang W. Phosphodiesterase-4 inhibitors for the treatment of inflammatory diseases. Frontiers in Pharmacology. 2018;**9**:1-21

[66] Sharma L, Prakash H. Sphingolipids are dual specific drug targets for the management of pulmonary infections: Perspective. Frontiers in Immunology. 2017;**8**:1-6

[67] Baumruker T, Prieschl EE. Sphingolipids and the regulation of the immune response. Seminars in Immunology. 2002;**14**:57-63

Chapter 6

# Polarization of Tumor-Associated Macrophages by Chinese Medicine Intervention: Mechanisms and Applications

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## Abstract

Macrophage polarization is a spectrum of phenotypes and generally can be classified into two states: (1) classically activated or M1 macrophages, which can be driven by lipopolysaccharide (LPS) alone or in association with Th1 cytokines and produce pro-inflammatory cytokines such as TNF-α, IL-6 and, IL-12, and (2) alternatively activated M2 macrophages, which can be promoted by Th2 mediators IL-4 and IL-13 and produce anti-inflammatory cytokines such as TGF-β and IL-10. Current studies have found that the phenotypic switch between M1 and M2 macrophages governs the fate of an organ in inflammation or injury. The imbalance of M1/M2 polarization is closely involved in various pathological processes and is becoming a potential target for therapeutic strategies. Traditional Chinese medicine is an integrated healthcare system composed of many practices and is characterized by multi-target, multi-level, and coordinated intervention effects. Chinese medicines nowadays are applied to regulate phenotype polarization of macrophages to improve the microenvironment, thus ameliorating or even eliminating the symptoms. In this chapter, we will discuss the molecular mechanisms of macrophage polarization, their roles in health and disease, and the intervention with Chinese medicines to modulate the polarization of macrophages in tumor microenvironment (TME) for therapeutic purpose.

**Keywords:** tumor microenvironment, tumor-associated macrophage, polarization, Chinese medicine

## 1. Introduction

Primary and metastatic tumors are generally known as a complex ecosystem containing tumor cells and the surrounding environment, called tumor microenvironment (TME). Apart from autonomous changes by genetic alteration of tumor cells, the dynamic changes of TME progress the tumor progression [1]. TME is a multifaceted pool that consists of various cell types including neoplastic cells, stromal cells, and immune cells that interact with one another via numerous secreted cytokines, growth factors, and chemokines. Tumor-associated macrophages (TAMs) take up a large portion of recruited immune cells and constitute up

to 50% of the tumor mass. It was reported that the high level of TAMs is associated with poor prognosis and decreasing overall survival in many cancers, such as liver, breast, gastric, and thyroid cancers, suggesting that TAMs certainly play essential roles during tumor development [2–6].

TAM recruitment and accumulation are regulated by various cytokines and chemokines, such as CCL2, CCL5, CCL7, CXCL12, etc., and growth factors including VEGF, PDGF, and CSF1, as well as other factors such as fibronectin and fibrinogen [7–10]. CSF1 is the major regulator for monocyte proliferation and differentiation. CCL2 is a dominant attractant in many tumors. Since monocytes highly expressed the receptor of CCL2 (CCR2), most of tumors produced a high level of CCL2 that can intensely attract monocytes migrating toward CCL2-CCR2 axis [11–17]. However, CCL2 inhibition studies show that it could not completely suppress TAM accumulation, indicating that other factors affect this process [7, 17–21]. The CCL12-CXCR4 axis is reported to promote TAM regional accumulation under therapeutic treatments. In mice model, breast cancer highly expressed CCL20 and CCL5; Either inhibited CCL20 expression or treated with CCR5 antagonist, the number of TAMs was significantly reduced within tumors. These studies have shown that in breast cancer, CCL20-CCR6 and CCL5-CCR5 axes contribute to TAM accumulation. Another chemokine CCL11 can be induced under hypoxia condition and subsequently recruit TAMs to the hypoxic region.

In turn, TAMs can produce different molecules to remodel TME and influence fundamental aspects of tumor pathology. For instance, TAMs secrete endothelial growth factor (EGF) to increase neoplastic proliferation directly [22]; TAMs release vascular endothelial growth factors (VEGF) [23], angiogenic factor thymidine phosphorylase, and other chemokines including CCL2 and CXCL8 to enhance angiogenesis; TAMs produce metalloproteases (MMPs) to change TME matrix architecture for tumor metastasis [24]; and TAMs express immune regulatory molecules such as arginase-1 (ARG1), IL-10, and IL12 to modulate immune response [2]. The role of TAMs is accomplished by their phenotypic plasticity, either pro-inflammatory or anti-inflammatory phenotype, in response to the complex stimuli in TME. The double-edged sword feature of TAM polarization makes them as a novel and potent target for cancer prevention and treatments.

Traditional Chinese medicine is an integrated healthcare system composed of many practices that were rooted in China for over 5000 years. Due to its multi-target, multi-level, and coordinated intervention effects, Chinese medicine is widely used for therapeutic strategies. Recent studies reported that some of the Chinese herbal medicines have beneficial effects on cancer therapy via modulating TAM polarization, indicating a new mechanism for Chinese medicine treatment. In this chapter, we will explore the molecular mechanisms of TAM polarization and their roles in health and disease, and we will review the intervention by some of the Chinese herbal medicines on TAM polarization.

## 2. TAM polarization and molecular mechanisms

### 2.1 TAM polarization

It is widely accepted that the majority of TAMs are derived from circulating monocytes via cytokine recruitment and then differentiate to macrophages. And those at the metastatic sites are called metastatic-associated macrophages (MAMs) according to their location [25]. While recent studies have shown that the tissue-resident macrophages also contribute to TAM population [26, 27], these

progenitors, also called embryonic macrophages, are derived from the yolk sac or fetal liver-derived progenitors, and they can maintain themselves by local proliferation in a hematopoietic system-independent way [28]. The selective depletion studies found that only the tissue-resident macrophages support the established tumor growth. Therefore, TAMs are heterogeneous cell populations from both tissue-resident macrophages and monocyte-derived macrophages and assist TME remodeling.

Besides their heterogeneity, TAMs are also characterized by high plasticity. In the general regard, macrophages can be overgeneralized to two extreme subsets based on the stimuli, surface markers, and secreted molecules, as well as functional properties: the classically activated M1 and alternatively activated M2 macrophages. The M1 phenotype is induced by the Th1 cytokine interferon-γ (IFN-γ), bacterial moieties such as lipopolysaccharide (LPS), and Toll-like receptor (TLR) agonists. The M1 macrophages are characterized by their capacity to produce inflammatory cytokines (e.g., IL-6, IL-1, IL-12, IL-23, and TNF-α) and stimulate immune response, express reactive oxygen species (ROS) and inducible nitric oxide synthase (iNOS), and have a cytotoxic effect toward neoplastic cells and phagocytic microorganisms [29–34]. Generally, the M1-like macrophages act as sentries and display tumoricidal function, antimicrobial activity, and tissue destruction effect [33, 35].

In contrast, the M2 phenotype is promoted by Th2 mediators and produces immunosuppressive factors (e.g., IL-10, TGF-β) and growth factors (e.g., VEGF) and exerts anti-inflammatory and pro-tumorigenic activities [34, 36, 37]. Moreover, the M2-like macrophages can be further subdivided into three categories, M2a, M2b, and M2c, based on the type of stimuli. The M2a macrophages are driven by type II cytokines including IL4 and IL13 and expressed a high level of arginase-1; M2b macrophages are activated by immune complexes/TLR, while M2c macrophages by anti-inflammatory cytokines (e.g., IL-10) and glucocorticoids [38]. The M2-like macrophages promote angiogenesis, wound repair, and tumor growth, as well as resistance to parasitic infection. Many studies reported that TAMs mostly represent M2-like macrophages and play pro-tumoral roles.

## 2.2 Molecular mechanisms in regulating TAM polarization

### *2.2.1 The JNK signaling pathway*

The c-Jun N-terminal kinase (JNK) proteins are a group of stress-activated serine threonine protein kinases of the MAPK and can be activated by various external stimuli including inflammatory cytokines, environmental stresses, growth factors, and GPCR agonists. The outside signals can be transduced by small GTPase to MAP3Ks and further activate MKK4/7. The MAP3Ks play key roles in the JNK pathway and affect tremendous downstream transcription factors including AP-1, Smad3, and STAT3, thus controlling many biological processes [39]. The studies on adipose tissue macrophages (ATMs) have demonstrated that the JNK pathway is indispensable in regulating M1/M2 phenotype formation. In HFD-/NAFLD-induced inflammation and obesity, the activated JNK pathway can promote the expression of the M1-associated genes via CCR2 and NF-κB signaling. The M1-like ATMs are related to the resistance to insulin [40, 41]. Recent studies found that normal adipocytes produce Th2 cytokines, such as IL-13 and IL-4 which can enhance M2-like macrophage polarization via activating STAT6 and PPAR$\delta/\beta$, as well as ACE to block the JNK pathway-induced M1-like phenotype [42]. Studies also found that vigorous exercise can promote M2 state through decrease phosphor-JNK [43].

### 2.2.2 The PI3K/Akt signaling pathway

Among different pathways, the PI3K/Akt pathway is playing a central role in regulating polarized phenotype alteration. It can be activated by many stimuli such as TLR4, PRRs, FcRs, and cytokines and modify downstream cytokine production [44–47]. In turn, the PI3K/Akt pathway can affect the expression of stimuli and form a feedback loop. For example, the activated PI3K/Akt pathway can inhibit the transcription factors of TLR4 including TRAF6 and FOXO1 either directly or indirectly to suppress TLR4 stimulation. The PI3K has two transducers PIP2 and PIP3 which exert opposite functions during stimulation. It has been reported that PIP2 can enlarge LPS-induced M1-like macrophage polarization, while PIP3 can target mTORC2 via Akt recruitment and promote M2-like macrophage polarization. Other studies found that PTEN and SHIP play an inhibitory effect on PI3K/Akt transduction by transforming PIP3 to PIP2. The downstream signals mTORC1 and mTORC2 also participate in regulating M1/M2 alteration. Deletion of TSC1 can promote LPS-induced M1 polarization and inhibit IL-4-triggered M2 polarization via inhibiting mTORC1-induced Akt signaling, while the deletion of TSC2 gives an opposite response. Furthermore, the isoforms of Akt also contribute to influence the M1-/M2-polarized phenotype transformation in the opposite way. In knocked out Akt1, the expressions of iNOS and IL-12 were enhanced which is a hallmark of M1-like macrophages, and the transcription factor C/EBPβ of M2-related genes was decreased. The deletion of Akt2 led to C/EBPβ and M2 markers enhanced, including Arg1, Fizz1, and Ym1 [48].

### 2.2.3 The JAK/STAT signaling pathway

The JAK/STAT pathway is one of the principle regulators for transducing different signals and affects various gene expressions. The JAK family consists of JAK1–3 and TYK2 and can be recruited and bind to the intracellular domains of activated receptors. JAKs will subsequently become dimers after autophosphorylation and then phosphorylate their downstream STAT family which has seven members including STAT1–4, STAT5A/B, and STAT6. The activated STAT family will translocate to the nucleus and modulate the expression of their target genes [49]. Increasing evidence found that the JAK/STAT pathway is closely related to M1/M2 phenotypic polarization. Among different stimuli of JAK/STAT signaling pathway, the IFN-γ has been known as a strong inducer of M1 phenotype through STAT1 activation [50]. It is controlled by IRF5 and IRF4 which exert promotive and inhibitory effects, respectively [51]. The IL-4 and IL-10 can activate STAT3 and STAT6 to program the M2-like phenotype and also have cross talk with JNK pathway as mentioned in the JNK signaling pathway. The IL-13 can activate both M1- and M2-associated genes through STAT1, STAT3, and STAT6 activation [52]. There are two regulators of JAK/STAT pathway that affect M1/M2 reprogramming, SOCS1 and SOCS3. The SOCS1 exhibits a suppressive function on STAT1, thus leading to the M1-like phenotype inhibition, while activating STAT6 to induce M2 polarization. The SOCS3 can activate STAT1 activity to contribute M1 polarization [53, 54].

### 2.2.4 The Notch signaling pathway

The Notch pathway is generally known to play a fundamental role in regulating development and assist to govern the fate in response to different stimuli. There are four members of transmembrane receptors including Notch1–4. When the Notch receptors bind to their ligand family, such as Delta-like proteins (DLLs) and Jagged

proteins, the Notch intracellular domain (NICD) receptors will be released into the cell nucleus and binds to RBP-J to form a transcription complex, thus driving the target gene expression [55]. For example, LPS stimulation can upregulate DLL4 which is one of the DLLs in the TLR4/NF-κB-dependent way. The increased DLL4 can lead to activated Notch signaling and induce pro-inflammatory genes, such as IL-12 and iNOS [56]. Apart from the direct function of RBP-J, it can also positively regulate IRF8 activation to promote pro-inflammatory cytokine production. And this regulation is associated with PI3K/Akt and TLR4/NF-κB pathways [57].

*2.2.5 Other molecular mechanisms*

Apart from the signaling pathways mentioned above, there are many other pathways involving in M1/M2 reprogramming. For example, the TLR/NF-κB pathway is important in regulating the innate immune response. TLRs can sense the microbial components and transduce signals to affect NF-κB activity. When the NF-κB is formed as p50/p65, it promotes M1-associated gene expression, while p50/p50 form has beneficial effects on M2-associated gene expression [58, 59]. It is worth noting that the hypoxia-dependent pathway also participates in M1/M2 phenotypic switch. The HIF-1α is induced under hypoxia condition and serves as a transcription factor to regulate protein production. It has been reported that HIF-1α promotes M1-like polarization by enhancing iNOS production and HIF-2α promotes M2 phenotype via increasing Arg-1 expression [60].

## 3. Roles of TAM polarization and Chinese medicine intervention

The roles of TAMs under physiological and pathological conditions depend on their dichotomic polarization. Generally, when infection of tissue or damage occurs, The first-responding TAMs show M1-like phenotype and secrete pro-inflammatory cytokines to defend against invading pathogens and eliminate necrotic cells. And at the latter stage, the M2-like macrophages have shown as a compensation mechanism to prohibit extensive inflammation and assist in wound healing. In cancers, the M1-like TAMs predominantly exert cytotoxicity effect on cancer cells, while the M2-like TAMs assist in modulating immunosuppressive and pro-tumoral TME for cancer progression. Nowadays, TAMs are becoming promising targets for therapeutic strategies [61, 62]. Many Chinese herbal medicines have been identified to have anti-microbial, anti-inflammatory, immune regulatory, and antitumor effects. It would be interesting to review the intervention of Chinese medicines on TAM polarization in different cancers and diseases. Here, we select some of the Chinese medicines to describe as examples.

### 3.1 Baicalein

Baicalein (5,6,7-trihydroxyflavone) is isolated from the Chinese herb *Scutellaria baicalensis* root and has many beneficial effects on antitumor, anti-inflammation, anti-fibrosis, and antimicrobial [63, 64]. The treatment of baicalein in breast cancer is the first to explain its effect on TAM regulation. In breast cancer, TAMs showed M2-like phenotype that produced TGF-β1 and enhanced tumor growth and EMT process via PI3K/Akt signaling pathway. In turn, the tumor cells secreted TGF-β1 to maintain TAMs in M2-like phenotype. The positive feedback loop between tumor cells and M2-like macrophages was formed and further contributed to tumor metastasis in the lung. Baicalein administration could block TGF-β1 via inhibiting PI3K/Akt pathway. Besides, instead of altering the population of TAMs, baicalein could

drive M2-like macrophages to M1-like macrophage differentiation, with M1 markers increased. Therefore, the application of baicalein in regulating TAM polarization in breast cancer may provide a new understanding of other cancer treatments [65].

### 3.2 *Panax notoginseng*

The root of *Panax notoginseng* (PN) (Burk.) F.H. Chen is one of the popular Chinese herbs also known as sanqi, tianqi, or sanchi in Asia [66]. It has been widely used in many disorders for over 400 years due to its anticancer, anti-inflammatory, antiatherosclerotic, and hemostatic properties [67, 68]. Recent studies have shown that PN not only has cytotoxicity on cancer cells but also can redirect TAM polarization. It is commonly known that M2-like macrophages exert pro-tumorigenic effects on cancer, and to redirect M2 phenotype to antitumor M1 phenotype would be one of the promising strategies in cancer treatment. In many lung cancer studies, it has been reported that high doses of PN administration have direct cytotoxic effects on cancer cells, while the lower dose of PN still have inhibitory effects on tumor growth, suggesting there are other regulatory mechanisms. The in vitro study found that a lower dose of PN did not affect cancer cells, but it could reeducate M2-like macrophages toward M1 phenotypic differentiation [69]. It would help to better explain the pharmacological mechanism of PN.

### 3.3 Osthole

Osthole [7-methoxy-8-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one] is isolated from *Cnidium monnieri* (*Fructus Cnidii*) and belongs to coumarin family, which is a benzopyrone and used as tumor-target drug carrier [70]. Osthole not only has cytotoxicity to cancer cells, such as breast cancer, lung cancer, HCC, and nasopharyngeal cancer (NPC) [71–73], but also has immunomodulatory effects on different tumors. In pancreatic tumors, osthole decreased M2-like macrophage population both in tumor site and spleen. But it did not affect M1-like macrophages. An in vitro study found that osthole could significantly inhibit STAT6 pathway and p-ERK1/2-C/EBP β signal, thus further inhibiting the M2-like macrophage polarization [74].

### 3.4 Emodin

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a natural anthraquinone derivative from many Chinese herbs, and it has multiple pharmacological effects [75]. One study focused on the effects of emodin on macrophage polarization has shown that it could bidirectionally regulate both M1 and M2 phenotype programs via different signaling pathways, as well as participated in the epigenetic modification. It seems like that emodin can restrain excessive M1- or M2-like macrophages and assist in maintaining homeostasis in different pathologies. For example, in breast cancer, emodin decreased TAM infiltration and inhibited M2-polarized phenotype by suppressing STAT6 and C/EBPβ signaling pathway. Moreover, it could increase H3K27m3 to downregulate M2-related genes.

### 3.5 Other Chinese medicine

Many other Chinese medicines have protective functions on different diseases through regulating M1/M2 phenotypic switch (as shown in **Table 1**). For example, curcumin can promote macrophages toward M2-like phenotype to ameliorate liver fibrosis, and it also assists wound healing [76]. Smiglaside A and Ginsenoside Rb3 have protective functions against acute lung injury via inducing M2-like

| Chinese medicine | M1/M2 phenotype switch | Disease | Reference |
|---|---|---|---|
| *Angelica sinensis* | M2 | Cardiac fibrosis | [79] |
| Baicalein | M1 | Breast cancer | [65] |
| Berberine | M2 | Colitis; insulin resistance | [80, 81] |
| Bergenin | M2 | Colitis | [82] |
| Celastrol | M2 | Diet-induced obesity; acute ischemic stroke | [83, 84] |
| Corilagin | M1 | Schistosome egg-induced hepatic fibrosis | [85] |
| Crocin | M2 | Atherosclerosis | [86] |
| Curcumin | M2 | Liver fibrosis; wound healing | [76, 87] |
| Dioscin | M1 | Melanoma | [88] |
| Diosgenin glucoside | M2 | Neuroinflammatory diseases | [89] |
| Emodin | M1 | Breast cancer | [90] |
| *Ganoderma lucidum Karst* | M1 | Inflammation | [91] |
| Gastrodin | M2 | Cerebral palsy | [92] |
| Ginkgolide B | M2 | Ischemic stroke | [93] |
| Ginsenoside Rb1 | M2 | Atherosclerosis | [94] |
| Ginsenoside Rb3 | M2 | Acute lung injury | [78] |
| Isoliquiritigenin | M2 | Acute kidney injury | [95] |
| Kumatakenin | M1 | Ovarian cancer | [96] |
| Magnesium lithospermate B | M2 | Neuronal injury | [97] |
| *Mylabris phalerata* | M1 | Lung carcinoma | [98] |
| Osthole | M1 | Pancreatic cancer | [74] |
| Paeoniflorin | M2 | Neuronal injury | [99] |
| *Panax notoginseng* | M1 | Lung carcinoma; influenza A virus infection | [69, 100] |
| Pentacyclic triterpene Lupeol | M2 | Inflammatory bowel disease | [101] |
| Pterostilbene | M1 | Lung cancer | [102] |
| Punicalagin | M2 | Inflammation | [103] |
| Saponin | M2 | Intestinal polyps | [104] |
| Smiglaside A | M2 | Acute lung injury | [77] |
| Tanshinone IIA | M2 | Acute kidney injury Inflammation | [105] |
| Timosaponin AIII | M2 | Colitis | [106] |
| *Trichosanthes Kirilowii* lectin | M2 | Streptozocin-induced kidney injury | [107] |

**Table 1.**
*The intervention of Chinese medicine on M1/M2 switch in different diseases.*

macrophage polarization [77, 78]. These findings may throw a new light for the regulatory mechanisms of Chinese medicines and promote their applications in health and diseases.

## 4. Conclusions

Current studies have described the heterogeneity and adaptive plasticity of TAMs in the intrinsic and dynamic TME. They are composed of both tissue-resident macrophages and monocyte-derived macrophages and interplay with TME. The latter one is attracted and recruited to the tumor site via various signals in TME, while TAMs can produce different molecules to remodel TME. In response to different stimuli, TAMs can differentiate into either classically activated/M1 macrophages or alternatively activated/M2 macrophage which involves multiple signaling pathways. The role of TAMs depends on their dichotomic polarization in health and disease. Therefore, they are becoming potential targets for many therapeutic strategies. Chinese medicine has been widely used in a long history of Asia and shows multiple effects on different diseases. Knowing the intervention of Chinese medicine on TAMs polarization may help to better understand the principle of Chinese medicine and contribute to the comprehensive applications in many diseases.

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## Conflict of interest

The authors have no conflict of interest.

## Abbreviations

| | |
|---|---|
| AP-1 | activator protein 1 |
| ARG1 | arginase-1 |
| ATM | adipose tissue macrophage |
| CCR | C-C motif chemokine receptors |
| CSF1 | colony stimulating factor |
| DLL | Delta-like protein |
| EGF | endothelial growth factor |
| FcR | Fc receptor |
| FOXO1 | Forkhead Box O1 |
| GPCR | G-protein-coupled receptors |
| HFD | high-fat diet |
| HIF | hypoxia inducible factor |
| IFN | interferon |
| IL | interleukin |
| iNOS | inducible nitric oxide synthase |
| IRF | interferon regulatory factor |
| JAK | Janus kinase |
| JNK | c-Jun N-terminal kinase |
| LPS | lipopolysaccharide |

| | |
|---|---|
| MAM | metastatic-associated macrophage |
| MAPK | mitogen-activated protein kinase |
| MMP | metalloprotease |
| mTOR | mammalian target of rapamycin |
| NAFLD | nonalcoholic fatty liver disease |
| NICD | intracellular domain of notch receptor |
| NPC | nasopharyngeal cancer |
| PDGF | platelet-derived growth factor |
| PI3K | phosphoinositide-3-kinase |
| PIP2 | phosphatidylinositol 4,5-bisphosphate |
| PIP3 | phosphatidylinositol 3,4,5-trisphosphate |
| PN | *Panax notoginseng* |
| PPAR | peroxisome proliferator-activated receptor |
| PRR | pattern recognition receptor |
| RBP-J | recombination signal binding protein for immunoglobulin Kappa J region |
| ROS | reactive oxygen species |
| Smad3 | SMAD family member 3 |
| SOCS | suppressor of cytokine signaling |
| STAT | signal transducer and activator of transcription |
| TAM | tumor-associated macrophages |
| TGF | transforming growth factor |
| Th1 | type 1 T helper |
| Th2 | type 2 T helper |
| TLR | Toll-like receptor |
| TME | tumor microenvironment |
| TRAF | TNF receptor-associated factor |
| VEGF | vascular endothelial growth factor |
| Ym1 | chitinase-like 3 |

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## References

[1] Alamoud KA, Kukuruzinska MA. Emerging insights into Wnt/β-catenin signalling in head and neck cancer. Journal of Dental Research. 2018;**97**(6):665-673

[2] Noy R, Pollard JWJI. Tumor-associated macrophages: From mechanisms to therapy. Immunity. 2014;**41**(1):49-61

[3] Zhang QW et al. Prognostic significance of tumor-associated macrophages in solid tumor: A meta-analysis of the literature. Plos One. 2012;7(12):e50946

[4] Guo B et al. Meta-analysis of the prognostic and clinical value of tumor-associated macrophages in adult classical Hodgkin lymphoma. BMC Medicine. 2016;**14**(1):159

[5] Mei J et al. Prognostic impact of tumor-associated macrophage infiltration in non-small cell lung cancer: A systemic review and meta-analysis. Oncotarget. 2016;7(23):34217

[6] Yin S et al. The prognostic and clinicopathological significance of tumor-associated macrophages in patients with gastric cancer: A meta-analysis. PloS One. 2017;**12**(1):e0170042

[7] Nakatsumi H, Matsumoto M, Nakayama KI. Noncanonical pathway for regulation of CCL2 expression by an mTORC1-FOXK1 axis promotes recruitment of tumor-associated macrophages. Cell Reports. 2017;**21**(9):2471-2486

[8] Qian BZ et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature. 2011;**475**(7355):222

[9] Halama N et al. Tumoral immune cell exploitation in colorectal cancer metastases can be targeted effectively by anti-CCR5 therapy in cancer patients. Cancer Cell. 2016;**29**(4):587-601

[10] Abraham D et al. Stromal cell-derived CSF-1 blockade prolongs xenograft survival of CSF-1-negative neuroblastoma. International Journal of Cancer. 2010;**126**(6):1339-1352

[11] Leung SY et al. Monocyte chemoattractant protein-1 expression and macrophage infiltration in gliomas. Acta Neuropathologica. 1997;**93**(5):518-527

[12] Negus R et al. Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of CC chemokines. The American Journal of Pathology. 1997;**150**(5):1723

[13] Arenberg DA et al. Macrophage infiltration in human non-small-cell lung cancer: The role of CC chemokines. Cancer Immunology, Immunotherapy. 2000;**49**(2):63-70

[14] Ueno T et al. Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. Clinical Cancer Research. 2000;**6**(8):3282-3289

[15] Ohta M et al. Monocyte chemoattractant protein-1 expression correlates with macrophage infiltration and tumor vascularity in human esophageal squamous cell carcinomas. International Journal of Cancer. 2002;**102**(3):220-224. DOI: 10.1002/ijc.10705

[16] Pena CG et al. LKB1 loss promotes endometrial cancer progression via CCL2-dependent macrophage recruitment. The Journal of Clinical Investigation. 2015;**125**(11):4063-4076. DOI: 10.1172/JCI82152

[17] Arakaki R et al. CCL2 as a potential therapeutic target for clear cell renal cell carcinoma. Cancer Medicine. 2016;**5**(10):2920-2933

[18] Peña CG et al. LKB1 loss promotes endometrial cancer progression via CCL2-dependent macrophage recruitment. The Journal of Clinical Investigation. 2015;**125**(11):4063-4076

[19] Liu B et al. ATF4 regulates CCL2 expression to promote endometrial cancer growth by controlling macrophage infiltration. Experimental Cell Research. 2017;**360**(2):105-112

[20] Fujimoto H et al. Stromal MCP-1 in mammary tumors induces tumor-associated macrophage infiltration and contributes to tumor progression. International Journal of Cancer. 2009;**125**(6):1276-1284

[21] Yoshimura T et al. Monocyte chemoattractant protein-1/CCL2 produced by stromal cells promotes lung metastasis of 4T1 murine breast cancer cells. PloS One. 2013;**8**(3):e58791

[22] O'sullivan C, Lewis CE, Harris ALJTL. Secretion of epidermal growth factor by macrophages associated with breast carcinoma. The Lancet. 1993;**342**(8864):148-149

[23] Shojaei F et al. Role of myeloid cells in tumor angiogenesis and growth. Trends in Cell Biology. 2008;**18**(8):372-378

[24] Kessenbrock K, Plaks V, Werb ZJC. Matrix metalloproteinases: Regulators of the tumor microenvironment. Cell. 2010;**141**(1):52-67

[25] Mantovani A, Sica A. Macrophages, innate immunity and cancer: Balance, tolerance, and diversity. Current Opinion in Immunology. 2010;**22**(2):231-237

[26] Cortez-Retamozo V et al. Origins of tumor-associated macrophages and neutrophils. Proceedings of the National Academy of Sciences. 2012;**109**(7):2491-2496

[27] Franklin RA et al. The cellular and molecular origin of tumor-associated macrophages. Science. 2014;**344**(6186):921-925

[28] van de Laar L et al. Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages. Immunity. 2016;**44**(4):755-768

[29] Mantovani A et al. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends in Immunology. 2002;**23**(11):549-555

[30] Gordon S. Alternative activation of macrophages. Nature Reviews Immunology. 2003;**3**(1):23

[31] Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nature Reviews Immunology. 2005;**5**(12):953

[32] Mantovani A, Sica A, Locati MJI. Macrophage polarization comes of age. Immunity. 2005;**23**(4):344-346

[33] Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: An immunologic functional perspective. Annual Review of Immunology. 2009;**27**:451-483

[34] Rhee I. Diverse macrophages polarization in tumor microenvironment. Archives of Pharmacal Research. 2016;**39**(11):1588-1596

[35] Mantovani A, Sica A, Locati M. New vistas on macrophage differentiation and activation. European Journal of Immunology. 2007;**37**(1):14-16

[36] Yeung OW et al. Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma. Journal of Hepatology. 2015;**62**(3):607-616

[37] Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: Cancer as a paradigm. Nature Immunology. 2010;**11**(10):889

[38] Mantovani A et al. The chemokine system in diverse forms of macrophage activation and polarization. Trends in Immunology. 2004;**25**(12):677-686

[39] Kumar A et al. JNK pathway signalling: A novel and smarter therapeutic targets for various biological diseases. Future Medicinal Chemistry. 2015;**7**(15):2065-2086

[40] Zhou D et al. Macrophage polarization and function with emphasis on the evolving roles of coordinated regulation of cellular signalling pathways. Cellular Signalling. 2014;**26**(2):192-197

[41] Weisberg SP et al. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of Clinical Investigation. 2003;**112**(12):1796-1808

[42] Luyendyk JP et al. Genetic analysis of the role of the PI3K-Akt pathway in lipopolysaccharide-induced cytokine and tissue factor gene expression in monocytes/macrophages. The Journal of Immunology. 2008;**180**(6):4218-4226

[43] Lancaster GI, Febbraio MA. The immunomodulating role of exercise in metabolic disease[J]. Trends in Immunology, 2014;**35**(6):262-269

[44] Covarrubias AJ, Aksoylar HI, Horng T. Control of macrophage metabolism and activation by mTOR and Akt signalling. Seminars in Immunology. Elsevier: Academic Press; 2015;**27**(4):286-296

[45] Fukao T, Koyasu S. PI3K and negative regulation of TLR signalling. Trends in Immunology. 2003;**24**(7):358-363

[46] Troutman TD, Bazan JF, Pasare C. Toll-like receptors, signalling adapters and regulation of the pro-inflammatory response by PI3K. Cell Cycle. 2012;**11**(19):3559-3567

[47] Polumuri SK, Toshchakov VY, Vogel SN. Role of phosphatidylinositol-3 kinase in transcriptional regulation of TLR-induced IL-12 and IL-10 by Fcγ receptor ligation in murine macrophages. The Journal of Immunology. 2007;**179**(1):236-246

[48] Vergadi E et al. Akt signalling pathway in macrophage activation and M1/M2 polarization. The Journal of Immunology. 2017;**198**(3):1006-1014

[49] O'Shea JJ, Plenge RJI. JAK and STAT signalling molecules in immunoregulation and immune-mediated disease. Immunity. 2012;**36**(4):542-550

[50] Hu X et al. Crosstalk among Jak-STAT, Toll-like receptor, and ITAM-dependent pathways in macrophage activation. Journal of Leukocyte Biology. 2007;**82**(2):237-243

[51] Satoh T et al. Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. Nature. 2013;**495**(7442):524

[52] Bhattacharjee A et al. IL-4 and IL-13 employ discrete signalling pathways for target gene expression in alternatively activated monocytes/macrophages. Free Radical Biology and Medicine. 2013;**54**:1-16

[53] Qin H et al. SOCS3 deficiency promotes M1 macrophage polarization

and inflammation. The Journal of Immunology. 2012;**189**(7):3439-3448

[54] Croker BA, Kiu H, Nicholson SE. SOCS regulation of the JAK/STAT signalling pathway. In: Seminars in Cell and Developmental Biology. Elsevier: Academic Press; 2008;**19**(4):414-422

[55] Kopan R, Ilagan MX. The canonical Notch signalling pathway: Unfolding the activation mechanism. Cell. 2009;**137**(2):216-233

[56] Fung E et al. Delta-like 4 induces notch signalling in macrophages: Implications for inflammation. Inflammation. 2007;**115**(23):2948-2956

[57] Liu J et al. Synergistic activation of interleukin-12 p35 gene transcription by interferon regulatory factor-1 and interferon consensus sequence-binding protein. Journal of Biological Chemistry. 2004;**279**(53):55609-55617

[58] Porta C et al. Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor κB. Proceedings of the National Academy of Sciences. 2009;**106**(35):14978-14983

[59] Bonizzi G, Karin M. The two NF-κB activation pathways and their role in innate and adaptive immunity. Trends in Immunology. 2004;**25**(6):280-288

[60] Takeda N et al. Differential activation and antagonistic function of HIF-α isoforms in macrophages are essential for NO homeostasis. Genes & Development. 2010;**24**(5):491-501

[61] Mantovani A, Allavena P, Sica A. Tumour-associated macrophages as a prototypic type II polarised phagocyte population: Role in tumour progression. European Journal of Cancer. 2004;**40**(11):1660-1667

[62] Condeelis J, Pollard JWJC. Macrophages: Obligate partners for tumor cell migration, invasion, and metastasis. Cell. 2006;**124**(2):263-266

[63] Bie B et al. Baicalein, a natural anti-cancer compound, alters microRNA expression profiles in Bel-7402 human hepatocellular carcinoma cells. Cellular Physiology and Biochemistry. 2017;**41**(4):1519-1531

[64] Wang W et al. Baicalein attenuates renal fibrosis by inhibiting inflammation via down-regulating NF-κB and MAPK signal pathways. Journal of Molecular Histology. 2015;**46**(3):283-290

[65] Zhao X et al. Baicalein suppress EMT of breast cancer by mediating tumor-associated macrophages polarization. American Journal of Cancer Research. 2018;**8**(8):1528

[66] Liu J et al. Saponins of Panax notoginseng: Chemistry, cellular targets and therapeutic opportunities in cardiovascular diseases. Expert Opinion on investigational Drugs. 2014;**23**(4):523-539

[67] Aplin JD. MUC-1 glycosylation in endometrium: Possible roles of the apical glycocalyx at implantation. Human Reproduction. 1999;**14**(suppl_2):17-25

[68] Wang CZ, Anderson S, Yuan CS. Phytochemistry and anticancer potential of notoginseng. The American Journal of Chinese Medicine. 2016;**44**(01):23-34

[69] Kim B et al. Panax notoginseng inhibits tumor growth through activating macrophage to M1 polarization. American Journal of Chinese Medicine. 2018;**46**(06):1369-1385. DOI: 10.1142/s0192415x18500726

[70] Zhang ZR et al. Osthole: A review on its bioactivities, pharmacological properties, and potential as alternative medicine. Evidence-Based

Complementary and Alternative Medicine. 2015;**2015**

[71] Lin ZK et al. Osthole inhibits the tumorigenesis of hepatocellular carcinoma cells. Oncology Reports. 2017;**37**(3):1611-1618

[72] Liu PY et al. Osthole induces human nasopharyngeal cancer cells apoptosis through Fas–Fas ligand and mitochondrial pathway. Environmental Toxicology. 2018;**33**(4):446-453

[73] Xu XM et al. Osthole suppresses migration and invasion of A549 human lung cancer cells through inhibition of matrix metalloproteinase-2 and matrix metallopeptidase-9 in vitro. Molecular Medicine Reports. 2012;**6**(5):1018-1022

[74] Wang B et al. Osthole inhibits pancreatic cancer progression by directly exerting negative effects on cancer cells and attenuating tumor-infiltrating M2 macrophages. Journal of Pharmacological Sciences. 2018;**137**(3):290-298. DOI: 10.1016/j.jphs.2018.07.007

[75] Wang JB et al. Hepatotoxicity or hepatoprotection? Pattern recognition for the paradoxical effect of the Chinese herb *Rheum palmatum* L. in treating rat liver injury. PloS One. 2011;**6**(9):e24498

[76] Zhao XA et al. Curcumin reduces Ly6C(hi) monocyte infiltration to protect against liver fibrosis by inhibiting Kupffer cells activation to reduce chemokines secretion. Biomedicine and Pharmacotherapy. 2018;**106**:868-878. DOI: 10.1016/j.biopha.2018.07.028

[77] Wang Y et al. Smiglaside A ameliorates LPS-induced acute lung injury by modulating macrophage polarization via AMPK-PPARgamma pathway. Biochemical Pharmacology. 2018;**156**:385-395. DOI: 10.1016/j.bcp.2018.09.002

[78] Yang J et al. Ginsenoside Rg3 attenuates lipopolysaccharide-induced acute lung injury via MerTK-dependent activation of the PI3K/AKT/mTOR pathway. Frontiers in Pharmacology. 2018;**9**:850. DOI: 10.3389/fphar.2018.00850

[79] Lee TM et al. Preconditioned adipose-derived stem cells ameliorate cardiac fibrosis by regulating macrophage polarization in infarcted rat hearts through the PI3K/STAT3 pathway. Laboratory Investigation. 2019:1

[80] Liu Y et al. Berberine inhibits macrophage M1 polarization via AKT1/SOCS1/NF-kappaB signalling pathway to protect against DSS-induced colitis. International Immunopharmacology. 2018;**57**:121-131. DOI: 10.1016/j.intimp.2018.01.049

[81] Ye L et al. Inhibition of M1 macrophage activation in adipose tissue by berberine improves insulin resistance. Life Sciences. 2016;**166**: 82-91. DOI: 10.1016/j.lfs.2016.09.025

[82] Wang K et al. Bergenin, acting as an agonist of PPARgamma, ameliorates experimental colitis in mice through improving expression of SIRT1, and therefore inhibiting NF-kappaB-mediated macrophage activation. Frontiers in Pharmacology. 2017;**8**:981. DOI: 10.3389/fphar.2017.00981

[83] Luo D et al. Natural product celastrol suppressed macrophage M1 polarization against inflammation in diet-induced obese mice via regulating Nrf2/HO-1, MAP kinase and NF-kappaB pathways. Aging (Albany NY). 2017;**9**(10):2069

[84] Jiang M et al. Celastrol treatment protects against acute ischemic stroke-induced brain injury by promoting an IL-33/ST2 axis-mediated microglia/macrophage M2 polarization. Journal of Neuroinflammation. 2018;**15**(1):78. DOI: 10.1186/s12974-018-1124-6

[85] Li YQ et al. Corilagin counteracts IL-13Ralpha1 signalling pathway in

macrophages to mitigate schistosome egg-induced hepatic fibrosis. Frontiers in Cellular and Infection Microbiology. 2017;**7**:443. DOI: 10.3389/fcimb.2017.00443

[86] Li J et al. Crocin alleviates coronary atherosclerosis via inhibiting lipid synthesis and inducing M2 macrophage polarization. International Immunopharmacology. 2018;**55**:120-127. DOI: 10.1016/j.intimp.2017.11.037

[87] Yang Z et al. Curcumin-mediated bone marrow mesenchymal stem cell sheets create a favorable immune microenvironment for adult full-thickness cutaneous wound healing. Stem Cell Research and Therapy. 2018;**9**(1):21. DOI: 10.1186/s13287-018-0768-6

[88] Kou Y et al. Connexin 43 upregulation by dioscin inhibits melanoma progression via suppressing malignancy and inducing M1 polarization. International Journal of Cancer. 2017;**141**(8):1690-1703. DOI: 10.1002/ijc.30872

[89] Wang S et al. Diosgenin glucoside provides neuroprotection by regulating microglial M1 polarization. International Immunopharmacology. 2017;**50**:22-29. DOI: 10.1016/j.intimp.2017.06.008

[90] Iwanowycz S et al. Emodin inhibits breast cancer growth by blocking the tumor-promoting feedforward loop between cancer cells and macrophages. Molecular Cancer Therapeutics. 2016;**15**(8):1931-1942. DOI: 10.1158/1535-7163.Mct-15-0987

[91] Sun LX et al. The improvement of M1 polarization in macrophages by glycopeptide derived from *Ganoderma lucidum*. Immunologic Research. 2017;**65**(3):658-665. DOI: 10.1007/s12026-017-8893-3

[92] Jia J et al. BCL6 mediates the effects of gastrodin on promoting M2-like macrophage polarization and protecting against oxidative stress-induced apoptosis and cell death in macrophages. Biochemical and Biophysical Research Communications. 2017;**486**(2):458-464. DOI: 10.1016/j.bbrc.2017.03.062

[93] Shu ZM et al. Ginkgolide B protects against ischemic stroke via modulating microglia polarization in mice. CNS Neuroscience and Therapeutics. 2016;**22**(9):729-739. DOI: 10.1111/cns.12577

[94] Zhang X et al. Ginsenoside Rb1 enhances atherosclerotic plaque stability by skewing macrophages to the M2 phenotype. Journal of Cellular and Molecular Medicine. 2018;**22**(1):409-416. DOI: 10.1111/jcmm.13329

[95] Tang Y et al. Isoliquiritigenin attenuates LPS-induced AKI by suppression of inflammation involving NF-κB pathway. American Journal of Translational Research. 2018;**10**(12):4141

[96] Woo JH et al. Effect of kumatakenin isolated from cloves on the apoptosis of cancer cells and the alternative activation of tumor-associated macrophages. Journal of Agricultural and Food Chemistry. 2017;**65**(36):7893-7899. DOI: 10.1021/acs.jafc.7b01543

[97] Tai Y, Qiu Y, Bao Z. Magnesium lithospermate B suppresses lipopolysaccharide-induced neuroinflammation in BV2 microglial cells and attenuates neurodegeneration in lipopolysaccharide-injected mice. Journal of Molecular Neuroscience. 2018;**64**(1):80-92. DOI: 10.1007/s12031-017-1007-9

[98] Chung HS, Lee BS, Ma JY. Ethanol extract of Mylabris phalerata inhibits M2 polarization induced by recombinant IL-4 and IL-13 in murine macrophages. Evidence-based Complementary and Alternative

Medicine. 2017;**2017**:4218468. DOI: 10.1155/2017/4218468

[99] Luo XQ et al. Paeoniflorin exerts neuroprotective effects by modulating the M1/M2 subset polarization of microglia/macrophages in the hippocampal CA1 region of vascular dementia rats via cannabinoid receptor 2. Chinese Medicine. 2018;**13**:14. DOI: 10.1186/s13020-018-0173-1

[100] Choi JG et al. Protective effect of panax notoginseng root water extract against influenza A virus infection by enhancing antiviral interferon-mediated immune responses and natural killer cell activity. Frontiers in Immunology. 2017;**8**:1542. DOI: 10.3389/fimmu.2017.01542

[101] Zhu Y et al. The pentacyclic triterpene Lupeol switches M1 macrophages to M2 and ameliorates experimental inflammatory bowel disease. International Immunopharmacology. 2016;**30**:74-84. DOI: 10.1016/j.intimp.2015.11.031

[102] Huang WC et al. Modulation of macrophage polarization and lung cancer cell stemness by MUC1 and development of a related small-molecule inhibitor pterostilbene. Oncotarget. 2016;7(26):39363-39375. DOI: 10.18632/oncotarget.8101

[103] Xu X et al. Punicalagin, a PTP1B inhibitor, induces M2c phenotype polarization via up-regulation of HO-1 in murine macrophages. Free Radical Biology and Medicine. 2017;**110**:408-420. DOI: 10.1016/j.freeradbiomed.2017.06.014

[104] Chen L et al. Triterpenoid herbal saponins enhance beneficial bacteria, decrease sulfate-reducing bacteria, modulate inflammatory intestinal microenvironment and exert cancer preventive effects in ApcMin/+ mice. Oncotarget. 2016;7(21):31226-31242. DOI: 10.18632/oncotarget.8886

[105] Gao S et al. Tanshinone IIA alleviates inflammatory response and directs macrophage polarization in lipopolysaccharide-stimulated RAW264. Cell. 2018;**7**:1-12

[106] Lim SM et al. Timosaponin AIII and its metabolite sarsasapogenin ameliorate colitis in mice by inhibiting NF-kappaB and MAPK activation and restoring Th17/Treg cell balance. International Immunopharmacology. 2015;**25**(2):493-503. DOI: 10.1016/j.intimp.2015.02.016

[107] Jiandong L et al. Trichosanthes kirilowii lectin ameliorates streptozocin-induced kidney injury via modulation of the balance between M1/M2 phenotype macrophage. Biomedicine and Pharmacotherapy. 2018;**109**:93-102. DOI: 10.1016/j.biopha.2018.10.060

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Macrophages are the sentinels of the immune system whose role has evolved beyond providing aseptic conditions to homeostasis, immune regulation, development, and behaviour. These cells have varied ontogenetic origins which reflects in their phenotypic and functional heterogeneity. Macrophage functions are fine-tuned by exogenous and endogenous signals and once tweaked, the information is included in their genetic makeup, albeit not indefinitely. Subversion of the macrophage functions is the hallmark of many pathogenic organisms and modulation of macrophage activity is pivotal to many therapeutic strategies. Fascinating and rapid developments in this field have necessitated the maintenance of currency of knowledge. This book provides a current account of information on varied topics in macrophage biology. Literature surveys have been presented in a captivating and lucid language. The contributing authors have also provided brief accounts of their own research. Every chapter provides a future perspective of what more could be achieved in the context of the current knowledge. The book will be of interest to students and researchers in microbiology, immunobiology, translational research, pathology, and related fields.

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