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Chapter V

Macrophage Plasticity and Polarization: Cell Signaling Mechanisms and Roles in Immunity

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Abstract

Macrophages are hematopoietic cells that populate every tissue. The characteristics shared by most tissue macrophages include a high phagocytic function and a degradative potential that allows them to clear foreign and damaged cells (Gordon and Taylor, 2005). In response to tissue infection, macrophages also participate in the induction of innate immunity, which plays a critical role in the killing of microorganisms and their pathogenic factors (Gordon and Taylor, 2005). Antigen-presenting cells, such as monocytes/macrophages, play major roles as sentinels of the first line of defense or as mediators that shape the adaptive immune response. Once activated by microbial products, macrophages acquire microbicidal competence that usually leads to effective immunity (Benoit *et al*., 2008)**.** The adaptive responses to environmental signals are now recognized for both the mature and immature elements in the myelomonocytic differentiation pathway (Biswas and Mantovani, 2010). The orchestration of the immune response by macrophages (one of the first cells to recognize and respond to an infection or an inflammatory process) is complex and dynamic; macrophages interact with different immune cell populations, such as dendritic cells (DCs), neutrophils and lymphocytes, to recognize antigens and to initiate an adequate immune response at a systemic level or at the site of injury or infection. In this dynamic process, many signaling events are initiated that change the cell phenotype to one that better responds to the environment. In this chapter, the signaling events associated with macrophage adaptation and response to environmental signals will be presented, with a focus on the different phenotypes that these cells can assume in different situations.

Introduction

Macrophages are effector cells that have a large range of functions depending on the environmental stimuli, and they play a crucial role in the innate immune response by producing several mediators. The largest family of substances produced by macrophages are the cytokines (growth factors, interleukins, interferons, and chemokines); however, these cells also synthesize lipid mediators, such as prostaglandins, leukotrienes, and lipoxins, which are derived from arachidonic acid metabolism. The capacity of macrophages to produce this large range of immune regulator molecules makes macrophages one of the most important cells involved in regulating immunity against pathogens or in tissue remodeling. Macrophages are remarkable for the diverse activities in which they engage. Many of these activities appear to be opposing in nature, such as pro-inflammatory vs. anti-inflammatory activities, immunogenic vs. tolerogenic activities, and tissue destructive vs. tissue restorative activities (Stout *et al*., 2005).

The heterogeneous roles that macrophage play in immunity depend on the environmental stimuli. Macrophages recognize threats through receptors that induce specialized activation programs; the receptors signal to modify the functional properties of the cells, either directly or indirectly, by inducing gene and protein expression patterns (Martinez, 2011). The cascades that initiate the full classical or alternative macrophage programs begin in the membrane with a priming event that involves the stimulation of the cytokine receptors. The signal propagates from the membrane to intracellular proteins and eventually to the nucleus where specific gene expression programs are induced (Martinez, 2011). This characteristic of changing the cell phenotype and consequently its function depending on specific stimuli makes macrophage a heterogeneous lineage. The phenomenon of macrophage regulation and activation by environmental stimuli is called plasticity, and the property of changing the phenotype is called polarization.

Cytokines Produced by Immune Cells Give Rise to Macrophages with Distinct Phenotypes and Physiologies: M1 or M2 Polarization

Because macrophages are exposed to a multiplicity of signals with different intensities and temporal patterns *in vivo*, the polarization of these cells should be viewed as an operationally useful and simplified conceptual framework describing a continuum of diverse functional states (Mantovani *et al*., 2004). Classically, these cells undergo specific activation programs during T_H1 or T_H2 immune responses. When activated by T_H1 cytokines and bacterial toxins, macrophages polarize to the M1 phenotype, triggering a classical pro-inflammatory response. On the other hand, when activated by T_H2 cytokines or hormones, the cells polarize to the M2 phenotype, an alternative route characterized by anti-inflammatory properties. Specific gene regions are positively regulated in the M1 and negatively regulated in the M2 phenotype, i.e., alternative activation signals trigger the anti-inflammatory or resolution response in which inflammatory mediators are negatively regulated and are not induced. This plasticity allows macrophages to promote the orientation of adaptive responses

in a type I or type II direction and to express specialized and polarized effector functions. Polarized macrophages differ in terms of receptor expression, cytokine production, effector functions and chemokine repertoires (Mantovani *et al*., 2004), which determine the course of immunity in health or determine the resolution or progression of a disease.

M1 and M2 macrophages differ in relation to receptor expression, effector functions and cytokine production. The term "classically activated" has been used to describe the effector macrophages that are produced during cell-mediated immune responses. Mirroring T_H1-T_H2 polarization, two distinct states of polarized activation for macrophages have been recognized: the classically activated (M1) macrophage phenotype and the alternatively activated (M2) macrophage phenotype (Biswas and Montovani, 2010). Bacterial moieties, such as LPS and the T_H1 cytokine interferon-γ (IFN-γ), polarize macrophages toward the M1 phenotype. In contrast, M2 polarization was originally described as a response to the T_H2 cytokine IL-4. M2 macrophages exhibit more phagocytic activity, the high expression of scavenging, mannose and galactose receptors, the production of ornithine and polyamines through the arginase pathway, and a phenotype characterized by the low expression of IL-12 and the high expression of IL-10, the IL-1 decoy receptor and IL-1R. In general, these cells participate in polarized T_H2 responses, help to dampen inflammation, promote tissue remodeling and tumor progression, and exhibit immunoregulatory functions.

Table 1. Macrophage function and plasticity. Interleukins, cytokines and microbial products trigger the macrophage phenotype toward M1 or M2 cells. The panels list the markers for the M1 and M2 macrophage phenotypes

	M1 cell phenotype	M2 cell phenotype
Activators	IFN-γ	IL-4
	TNF-α	IL-13
	LPS	IL-10
Cytocine produced	IL-12	IL-10
	IL-1B	IL-1RA
	TNF-α	
Pattern-recognition receptors (PRRs)	Toll-like receptors NOD receptors	Mannose receptors FC γ receptors
Markers	iNOS	Arginase PPARs
Function	Anti-microbicidal Anti-tumoricidal Inflammatory response	Tissue repair Anti-inflammatory response

M1 and M2 Macrophages Possess Distinct Repertoires of Pathogen-Associated Molecular Patterns that Recognize a Wide Range of Pathogen Molecules

The recognition of microbial pathogens is essential for the initiation of innate immune responses, such as inflammation, and is mediated by patter-recognition receptors (PRRs) that

recognize molecular structures known as pathogen-associated molecular patterns (PAMPs) that are broadly shared by pathogens. Several families of PRRs, including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), DNA receptors (cytosolic sensors for DNA), mannose receptors, and scavenger receptors are known to play a crucial role in host defense (Kumar *et al*., 2011; Plüddemann *et al*., 2011) by activating macrophage cells to present antigens and produce cytokines.

In the early acute phase of an infection, macrophages are activated PAMPs allowing them to recognize, engulf, and kill invading pathogens. Following PAMP recognition, PRRs initiate a series of signaling programs that execute the first line of host defensive responses necessary for killing infectious microbes. Intact microbial pathogens are usually composed of a number of PAMPs, which activate multiple PRRs. Because different PRRs recognize several PAMPs, it is not surprising that the engagement of these specific receptors triggers distinct immune responses (Kumar *et al*., 2011; Plûddemann *et al*., 2011). PRRs are distinctly regulated in M1 and M2 macrophages. Higher levels of TLRs and NLRs are expressed in M1 macrophages, whereas mannose and scavenger receptors are PRR markers of the alternatively activated M2 macrophages (Goerdt *et al*., 1999).

M1 Macrophage Polarization and Orchestration of T_H1 Mediated Immunity

T_H1 cell production drives the classical M1 polarization of macrophages. These cells are characterized by their ability to release large amounts of proinflammatory cytokines (IL-1, IL-12, IL-17, IL-23 and tumor necrosis factor (TNF)), reactive nitrogen intermediates and reactive oxygen intermediates, and by the higher expression of the major histocompatibility complex class II (MHC II) and costimulatory molecules, efficient antigen presentation, and microbicidal or tumoricidal activity. M1 macrophages are part of a polarized T_H1 response, and they mediate resistance to intracellular pathogens and tumors and elicit-disruptive reactions. M1 macrophages, through their expression of cytokines and chemokines, drive the polarization and recruitment of T_H1 cells thereby amplifying a type 1 response (Biswas and Montovani, 2010).

The molecular mechanisms that lead to classical macrophage activation involve a combination of transcription factors that regulate the expression of pro-inflammatory genes. These transcription factors include signal transducing and activator transcription (STAT) molecules (especially STAT-1, which is activated following IFN-γ receptor ligation), and nuclear factor-kB (NF-kB) mitogen-activated protein kinases (MAPKs), which are activated in response to TLRs or TNF receptor ligation (Mosser and Edwards, 2008). To better elucidate the mechanisms by which cytokines and molecules induce the activation of signaling pathways that lead to macrophage activation, in the following sections, we will describe the major cytokines that engage in these processes. In addition, the molecular mechanisms that lead to macrophage activation will be presented.

The Response of Macrophages to Cytokines Produced by Lymphocytes, NK Cells and Dendritic Cells Are Essential for an Adequate T_H1-Derived Immunity

The Interferon Family

IFNs have been classified into three types that are structurally unrelated, bind to different receptors, and are encoded by separate chromosomal loci (Shroder *et al*., 2004). In humans, type I IFNs include 13 forms of IFN-α, one form of IFN-β, one form of IFN-ω, one form of IFN-ε, and one form of IFN-κ. Type II IFNs include IFN-γ, and type III include IFN-λ. IFN was originally identified as a substance "that interferes" with viral replication. However, the existence of three types of IFN that use three different receptors raised the possibility that these different types of IFNs play different roles in the host defense against viruses and other pathogens. In contrast to type I IFN, which appears to be related to the immunity against viruses, IFN-γ was shown to be essential for the protective immunity against many intramacrophagic bacteria, fungi, and parasites (Zhang *et al*., 2008**)**. This feature is unique among the IFNs.

IFN-γ was named the "immune IFN" because it is produced by lymphocytes in response to various immune stimuli. The major role of IFN-γ in immunity is due to its ability to act as the "macrophage activating factor" (Zhang *et al*., 2008; Vilcek, 2006). Because of its unique properties in activating the immune response to several intracellular pathogens, in the following sections, we will demonstrate how IFN-γ orchestrates the immune protective response and will focus on macrophage activation.

IFN Signaling

The action of IFN-γ occurs via the interaction of the cytokine with its receptor leading to the activation of Janus kinases (JAK1 and JAK2) and STAT1, which alter the transcriptional activity of the cell. The genes induced by IFN-γ encode many different molecules involved in increasing the acquired immune responses and effector functions of macrophages, which are described below.

The receptor for IFN-γ is composed of two structurally homologous polypeptides that belong to the family of type II cytokine receptors IFN-γR1 and IFN-γR2, which are associated with JAK1 and JAK2 kinases. Following ligand binding, the oligomerization of the IFN-γ receptor leads to the activation of receptor-associated JAK1 and JAK2 and the phosphorylation of a receptor tyrosine residue (Tyr440) that serves as a docking-site for STAT-1. The activation of STAT-1 by phosphorylation on Tyr701 leads to its dimerization, translocation to the nucleus, and binding to the regulatory DNA element the termed gamma-activated sequence (GAS), which activates the transcription of STAT-1 target genes. (Hu and Ivashkiv, 2009; Valledor *et al*., 2008).

IFN-γ stimulation also generates Ser727-phosphorylated STAT-1, which is important for enhancing the transcriptional activity of STAT-1. However, Ser727 phosphorylation has no impact on the homodimer formation of STAT-1, Tyr701 phosphorylation and nuclear

translocation. Therefore, STAT-1 requires the JAK-mediated Tyr701 phosphorylation and the MAPK-mediated Ser727-phosphorylated status to achieve its full transcriptional activity.

MAPKs are evolutionarily conserved serine/threonine kinases involved in the transduction of externally derived signals. The MAPKs cross-talk with JAK-STAT-1 or contribute to the polarization of macrophage M1 cells in a STAT-1 independent manner. These kinases include ERK1 and -2, JNK1, JNK2 and p38. The signaling pathway of each MAPK plays a selective role in macrophage activation in the response to IFN-γ. MEK1/2 plays an important role in the activation of STAT-1, and inhibition of MEK1/2 prevents the activation of STAT-1 in response to IFN-γ (Chung *et al*., 2011). The p38 kinase participates mainly in the regulation of the expression of genes required for the innate immune response, including iNOS, TNF-α and the chemokines responsible for T cell recruitment to injury sites (CCL5, CXCL9 and CXCL10), whereas JNK-1 acts on genes involved in antigen presentation by inducing the expression of MHC class II molecules (Valledor *et al*., 2008).

IFN-γ and Macrophage Cell Biology

The polarization of macrophages into the M1 lineage is an extraordinary dynamic process that depends on an orchestration of T_H1-derived cells. IFN-γ is produced by innate or adaptive immune cells, and natural killer (NK) cells are an important innate early source of this cytokine. NK cells respond to stress and infections by producing IFN-γ, which primes macrophages to secrete pro-inflammatory cytokines and to produce increased amounts of superoxide anions and oxygen and nitrogen radicals to increase their killing ability. Therefore, innate immune mediators allow macrophages to provide better resistance against infectious agents. The production of IFN-γ by NK cells is generally transient and, therefore, cannot sustain a population of activated macrophages. Consequently, an adaptive immune response is usually necessary to maintain classically activated macrophages and to confer a stable host defense against many intracellular microorganisms. Typically, this defense is provided by the sustained production of IFN-γ by T_H1 cells. These T cells are antigen specific, but the microbicidal and tumoricidal macrophages that they induce can kill indiscriminately (Mosser and Edwards, 2008).

The Synergistic Effect of IFN-γ and IL-12 Is Critical for an Adequate Response against Infections

IFN-γ induces the first wave of classical macrophage activation that stimulates IL-12 production, a cytokine crucial for the induction of T_H1 responses. In turn, the IL-12 that is produced by the macrophages stimulates NK and T_H1 cells to produce more IFN-γ. The IL-12 is the main mediator of the initial natural immune response against intracellular microorganisms and is a sequential inducer of cell-mediated immunity, the acquired immune response to these microorganisms. The most striking feature of this interleukin is to stimulate the production of IFN-γ by T_H1 cells and NK cells and to support the differentiation of T helper T cells to naive CD4+ T_H1 cells. This synergistic process of amplifying the activation of T_H1 cells and NK cells that result in the orchestration of a T_H1-type immune response

shows the extreme importance of the polarization of macrophages into M1 cells during the early stages of an intracellular pathogenic infection.

IFN-γ Stresses the Microbicidal Function of Macrophages

One of the most important effects of IFN-γ on macrophages is the activation of microbicidal effector functions. IFN-γ-activated macrophages display increased pinocytosis and receptor-mediated phagocytosis and enhanced microbial killing ability. The IFN-γ-activated microbicidal activity includes the induction of the NADPH-dependent phagocyte oxidase (NADPH oxidase) system ("respiratory burst") that primes NO production (Cassatella *et al*., 1990) and the up-regulation of lysosomal enzymes, which promotes microbe destruction. (Fang *et al*., 2004**).**

IFN-γ dependent reactive nitrogen intermediates (RNI) production is associated with the increased ability of phagocytic cells to kill ingested pathogens. IFN-γ induces RNI production by up-regulating the expression of the substrate, cofactor, and catalyst required for NO generation. IFN-γ up-regulates argininosuccinate synthetase (produces the L-arginine substrate), GTP-cyclohydroxylase I (supplies the tetrahydrobiopterin cofactor required for NO production), and the iNOS enzyme (NOS 2). The maximal induction of iNOS transcription requires the "priming" and "triggering" of stimuli, such as priming with IFN-γ (Schroder *et al*., 2004).

IFN-γ Stimulates MHC Class II Antigen Presentation

The class II pathway is constitutively active only in professional antigen-presenting cells (dendritic cells and their immediate precursors and B cells); however, similar to class I, the class II pathway is strongly inducible by IFN-γ on almost all cells (Bohem *et al*., 1997). In antigen-presenting cells and active macrophages, HLA class II genes govern the cellular apparatus responsible for the transcription of the cellular machinery that mediates antigen presentation (MHC class II molecules).

MHC class II molecules are cell-surface glycoproteins that are of central importance to the adaptive immune system because they present peptides, which are derived mainly from extracellular proteins, to the antigen receptor of CD4+ T cells. For the activation of HLA class II, IFN-γ stimulates the class II transactivator (*CIITA*) gene through the MAPK dependent pathway (JNK-1) (Valledor *et al*., 2008). The regulation of *CIITA* on macrophages stimulated by IFN-γ is essential to initiate an adaptive immune response and is known as a master regulator of MHC class II genes (Reith *et al*., 2005). *CIITA* activation by IFN-γ coincides with the initiation of a T_H1 response against intracellular bacterial and viral infections. The gene expression profile of HLA class II genes is governed exclusively by the master regulatory factor *CIITA*. This transactivator has a remarkable degree of specificity for HLA class II genes and for a limited number of other genes, most of which are involved in antigen processing (Kamper *et al*., 2011).

In addition to enhancing the antigen presentation by inducing the expression of MHC class II molecules, IFN-γ amplifies the recognition of antigen by increasing the expression of ligands that are recognized by T cells. IFN-γ up-regulates CD40, a classical receptor that

participates in the interaction of macrophages with dendritic cells and T cells to present antigens at transcriptional levels. This process is mediated by activation of the JAK-STAT-1 signaling pathway (Nguyen and Benveniste, 2000).

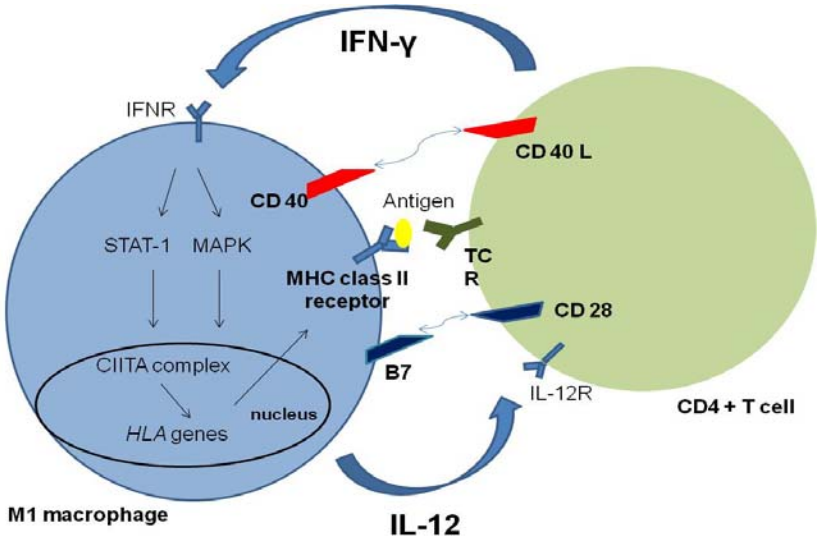

Figure 1. Antigen presentation by macrophages activated by IFN-γ and the synergistic activation of macrophages and lymphocytes by IFN-γ and IL-12.

Tumor Necrosis Factor-Alpha

Tumor necrosis factor-alpha (TNF-α) is a potent modulator of early inflammatory responses to a variety of physical, environmental, infectious and immunological stimuli during the acute inflammatory phase. Much of the biological effects exerted by TNF-α are due to its systemic action. For example, TNF-α acts on various organs to initiate inflammatory processes, it activates leucocytes and induces fever by acting in the hypothalamus and stimulating the production of IL-1 by endothelial cells and macrophages. In the liver, TNF-α induces the synthesis of acute phase proteins.

TNF-α exerts an extreme spectrum of bioactivities and most cells show at least some TNF-α responsiveness. This characteristic makes TNF-α one of the "master regulators" of pro-inflammatory cytokine production. In addition to being major producers of TNF-α, macrophage cells are responsive to this inflammatory cytokine (Parameswaran and Patial, 2010). Later, we will highlight the main effects of TNF-α on the biology of macrophages and present the mechanisms related to the production and action of TNF-α in these cells.

Role of TNF-α in Macrophage Activation

Studies using neutralizing anti-TNF antibodies demonstrated that the host defense against pathogens is severely impaired in the absence of TNF. In addition, it has been demonstrated that diminished TNF production by M1 cells increases the susceptibility to intracellular

infections. A mechanism that impairs the immune response is the low production of NOS in the absence of TNF or the impairment of TNFR1 signal transduction (Bosschaerts *et al*., 2011; Magez *et al*., 2007). The effect of TNF in M1 activation appears to be mediated by iNOS expression, which results in the production of NOS and, consequently, enhances the killing ability of macrophages (Silva *et al*., 1995).

TNF Receptors and Signaling That Leads to M1 Polarization

The members of the TNF ligand family exert their biological functions via the interaction with their cognate membrane receptors comprising the TNF receptor (TNF-R) family. TNF-R1 is constitutively expressed in most tissues, whereas TNF-R2 expression is highly regulated and is typically found in cells of the immune system. In the vast majority of cells, TNF-R1 appears to be the key mediator of TNF signaling (Wajant *et al*., 2003).

The activation of both TNF receptors leads to the activation of two major transcription factors, AP-1 and NF-kB, which induce the transcription of genes involved in pro-inflammatory responses (Wajant *et al*., 2003; Baud and Karin, 2001). The activation of TNFR1 and TNFR2 by TNF-α induces receptor trimerization and the recruitment of several signaling proteins to the cytoplasmic domains of each receptor. TNFR1 mediates inflammatory responses through the recruitment of TNF-receptor-associated factor 2 (TRAF2) and receptor-interacting protein 1 (RIP1) forming a TRADD-RIP1-TRAF2 complex. This complex is internalized and released from TNF-R1 and is responsible for activating the NF-kB and MAPK signaling pathways (Parameswaran and Patial, 2010; Baud and Karin, 2001; Hsu *et al*., 1995).

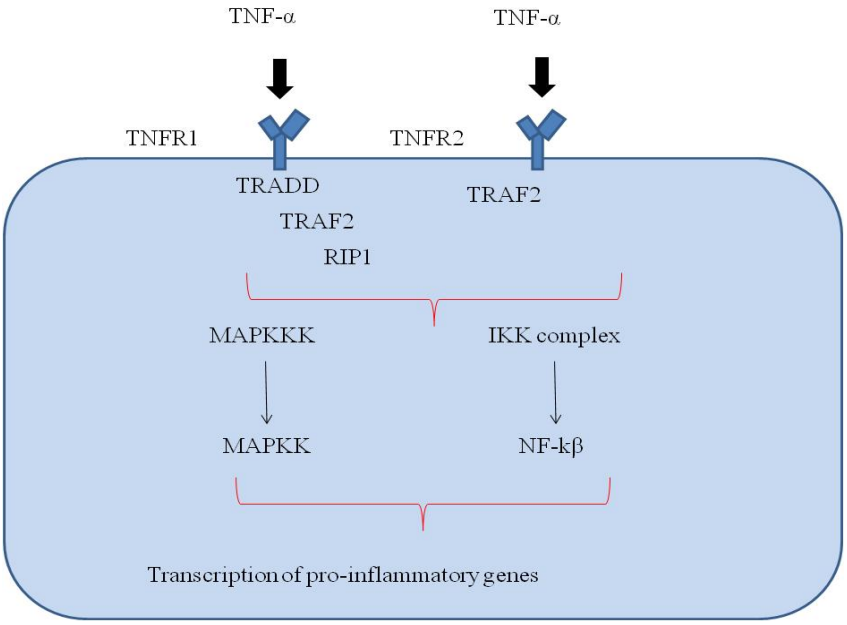

Figure 2. TNF-α signaling in macrophages.

Toll-Like Receptors

Toll-like receptors (TLRs) are the most characterized of the PRRs and are among the most potent activators of macrophage inflammatory responses. The activation of TLRs leads to the production of TNF, IL-6, IL-12, IL1, IFN-α and IFN-β and induces the expression of co-stimulatory molecules and those with MHC.

TLRs are type I transmembrane proteins and comprise an ectodomain, which contains leucine-rich repeats that mediate the recognition of PAMPs, a transmembrane region, and cytosolic Toll-IL-1 receptor (TIR) domains that activate downstream signaling pathways. These receptors are expressed either on the cell surface or are associated with intracellular vesicles. To date, 10 and 12 functional TLRs have been identified in human and mouse, respectively. Each TLR detects distinct PAMPs derived from viruses, bacteria, mycobacteria, fungi, and parasites including lipoproteins (recognized by TLR1, TLR2, and TLR6), double-stranded (ds) RNA (TLR3), lipopolysaccharide (LPS) (TLR4), flagellin (TLR5), single-stranded (ss) RNA (TLR7 and TLR8), and DNA (TLR9) (Kumar *et al*., 2009; Mäkelä *et al*., 2009; Kawai and Akira, 2006;).

TLR-dependent cytokine gene expression results from the enhanced activation and cooperation among several adaptor molecules, transcription factors and kinases. Following the recognition of the respective PAMPs, TLRs recruit a specific set of adaptor molecules that harbor a TIR domain (five adaptor molecules). All TLRs use the adaptor MyD88 except TLR3, which uses the TIR domain-containing adaptor protein-inducing IFN-β (TIRF) (Kawai and Akira, 2011; Mäkelä *et al*., 2009). Because of the complexity of the pathway, the TLR signaling pathway is categorized into MyD88-dependent and TRIF-dependent pathways.

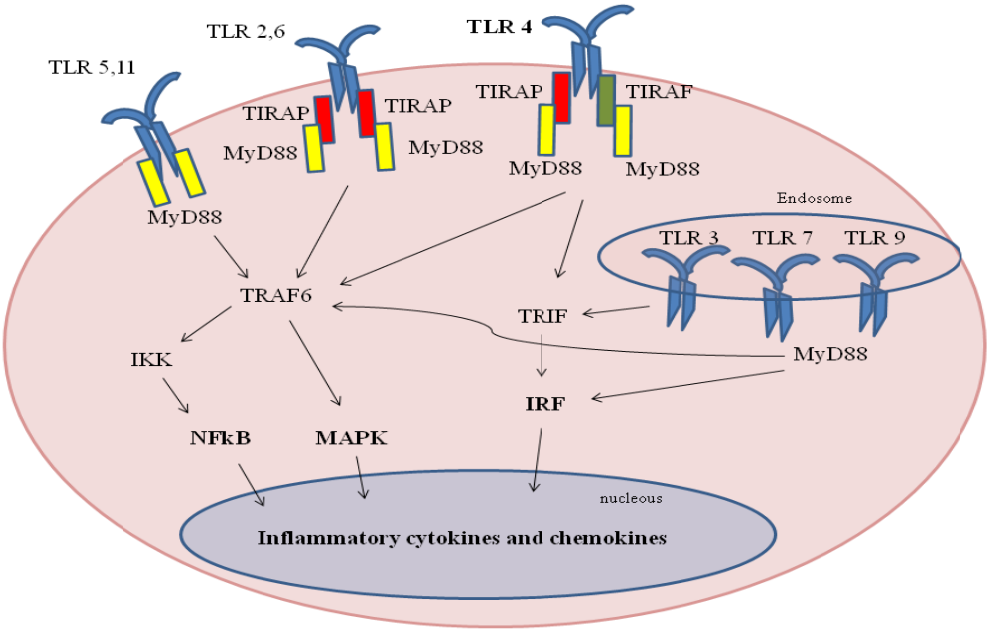

Figure 3. Toll-like receptor signaling.

In general, TLR signaling impairment leads to the immune deregulation state in bacterial, fungal, protozoan and viral infections. Several studies demonstrate that TLR activation impairment using specific inhibitors of MAPKs or transcription factors that mediate TLR signaling affects the activation state of macrophages. For example, macrophages that do not express TLR-4 (TLR4-/-) or the MyD88 adaptor (MyD88-/-) exhibit diminished phagocytic activity; lower levels of CXCL1, IL-1β and TNF-α; and lower expression of MHC (Romero *et al.,* 2011; Gasparoto *et al*., 2010; Shen *et al.,* 2010).

An interesting feature of TLRs in macrophage biology is the complex regulation of macrophage activation. The wide spectrum of microbial molecules that are recognized by the different TLRs allows the simultaneous stimulation of two or more TLRs. This concomitant activation results in synergistic, antagonistic or additive effects on macrophage biology and, consequently, the orchestration of the immune response (Kumar *et al*., 2009; Mäkelä *et al*., 2009). This concomitant activation is an interesting capability of macrophages in host defense because it allows these cells to recognize a wide spectrum of pathogen-associated molecules.

NOD-Like Receptors

NOD-Like Receptor (NLR) signaling is very similar to TLR signaling, and both pathways share downstream targets. The stimulation of the intracellular NLRs activates downstream signaling pathways, resulting in the production of proinflammatory mediators that defend the host against infection. NLRs detect microbial components in the cytosol and play a pivotal role in the recognition of intracellular PAMPs, mediating protective immune responses elicited by intracellular pathogens or endogenous danger signals. In addition, NLRs act in synergy with various TLRs to enhance immune responses (Qiu *et al*., 2011). NLR signaling recruits adaptor proteins, such as MyD88 and TRIF, which activate the MAPK and NF-κB signaling pathways (Chen *et al*., 2009).

NOD1 and NOD2 recognize two different fragments of the bacterial cell wall component peptidoglycan, iEDAP (g-D-glutamyl-meso-diaminopimelic acid) and MDP (MurNAc-L-Ala-D-isoGln), respectively. NOD1 is described as a specific sensor of Gram-negative bacteria, whereas NOD2 is a more general detector of intracellular bacteria because its MDP ligand forms an integral part of the Gram-negative and Gram-positive bacterial peptidoglycan. To date, a crucial role for NOD2 in bacterial clearance has been demonstrated only for intragastric infections (Kersse *et al*., 2011).

One of the remarkable characteristics of NLRs in macrophage polarization is that they act synergistically with TLRs and induce caspase-1 activation through the assembly of large protein complexes named inflammasomes (Kanneganti *et al*., 2007). The inflammasome is responsible for the activation of inflammatory processes and has been shown to induce cell pyroptosis, a process of programmed cell death that is distinct from apoptosis. Once active, inflammasomes perform a variety of processes in response to the initial inflammatory signal. These processes include the proteolytic cleavage of pro-IL-1B into active IL1B, the cleavage of pro-IL-18 into IL-18 to induce IFN-γ secretion and natural killer cell activation (Martion *et al*., 2002; Gu *et al*., 1997).

T_H2 Response: M2 Macrophage Polarization and Orchestration of T_H2 Mediated Immunity

M2 macrophages exhibit increased phagocytic activity compared with M1 macrophages; high expression of scavenging, mannose and galactose receptors; and an enhanced arginase pathway; furthermore, they express low levels of IL-12 and high levels of IL-10. In general, these cells participate in polarized T_H2 responses, help with extracellular parasite clearance, dampen inflammation, promote tissue remodeling and tumor progression and have immunoregulatory functions (Biswas and Montovani, 2010; Mosser and Edwards, 2008).

Interleukins 4 and 13

IL-4 and IL-13 are the major stimuli for the alternative activation of macrophages. IL-4 and IL-13 are actively produced by cells from the innate and acquired immune system. Several cell types produce IL-4 and IL-13 including conventional CD4+ T_H2 and CD8+ T cells, basophils, mast cells, and eosinophils. The production of these interleukins may not be restricted to the primary inflammatory locus. In contrast to IFN-γ, IL-4 and IL-13 mediate immune responses typically characterized by eosinophilia, basophilia, mastocytosis, enhanced B cell class switching, and antibody production, which result in the plasma accumulation of IgE and IgG1 (Martinez *et al*., 2009).

IL-4 and IL-13 mediate the repression of proinflammatory cytokines and the induction of an anti-inflammatory milieu. In addition, these interleukins antagonize the secretion of IL-12, IL-1β, TNF, and IL-8 induced by classical activation and other proinflammatory factors. In macrophages, IL-4 and IL-13 signaling pathways induce the expression of major molecules responsible for M2 polarization, such as arginase-1, the mannose receptor, PPARγ (peroxisome proliferator-activated receptor), and the IL-10 and IL-1 receptor antagonist (Nelson *et al*., 2011; Martinez *et al*., 2009; Mosser, 2003) and growth factors, such as TGF-β which participates in the tissue remodeling process.

Interleukin-4 and Interleukin-13 Signaling

Differences in the expression of type I or type II receptors on different cell types dictate their sensitivity to IL-4 and IL-13. The IL-4 receptor (IL-4R) is a type I (heterodimers composed of the IL-4Rα chain (p140) and the IL-2Rγ chain) and a type II receptor, whereas the IL-13 receptor (IL-13R) is only a type II receptor. An interesting characteristic of the IL-4 and IL-13 pathways is that both induce similar phenotypes in macrophages. The similarities in IL-4R and IL-13R are responsible for this convergent signaling. The functional IL-13R is a heterodimer of IL-4Rα and IL-13Rα1 chains, and this feature allows IL-4 and IL-13 to bind to IL-13R and exert similar effects on macrophages and other target cells (Martinez *et al*., 2009; Moy *et al*., 2001; Nelms *et al*., 1999).

IL-4 and IL-13 activate gene expression by binding to IL-4R and IL-13R complexes on the surface of IL-4-IL13-responsive target cells. The dimerization of type I and type II receptors activates the JAK-STAT signaling pathway, specifically STAT-6. Activated STAT6

then homodimerizes and translocates to the nucleus where it binds with a high affinity to STAT-binding elements (SBE) in the promoters of various IL-4-IL-13 responsive genes (Dickensen *et al*., 1999; Nelms *et al*., 1999).

IL-4 and IL-13 also activate the PI3K pathway. The insulin substrate receptor (IRS) interacts with JAK in the IL-4R and IL-13R domains and activates PI3K. The activation of PI3K, in addition to exerting a known role in regulating glucose metabolism in macrophages, mediates fluid-phase pinocytosis and endocytosis, important processes in the capture of antigens (Araki *et al*., 1996).

Interleukin-10

IL-10 exerts a potent anti-inflammatory effect in immunity by modulating macrophage functions. In the past, IL-10 was thought to "deactivate" macrophage cells because when stimulated with IL-10, macrophages exhibited lower microbicidal activities. It is now known that IL-10 does not deactivate macrophage cells but directs macrophages into the active M2 phenotype.

The mechanisms by which IL-10 induces the alternative activation of macrophages follow the same principles as IL-4 and IL-13, inducing the expression of anti-inflammatory molecules and transcriptional repressors that in turn mediate the repression of cytokines and inflammatory gene promoters.

IL-10 Signaling Pathway

Similar to IL-4R and IL-13R, the IL-10 receptor (IL-10R) is member of the type II cytokine receptor family and consists of two chains both of which are associated with the Janus family of protein in the JAK-STAT signaling pathway. In the case of IL-10, JAK activation leads to the phosphorylation and thus the activation of STAT-3 (Moore *et al*., 2001).

The effects of IL-10 in suppressing M1 macrophage activity and directing the cells to the M2 phenotype is mediated by two major regulators, STAT-3 and PI3K-Akt-glycogen synthase kinase 3 (GSK3). In fact, inhibiting STAT-3 or PI3K-Akt signaling alone does not completely impair the IL-10 response. These observations suggest that both pathways selectively possess target genes, which contributes to the activity of IL-10 in macrophages.

Mannose Receptors

Mannose Receptor (MR) is an important phagocytic receptor that mediates the binding and ingestion of micro-organisms containing surface mannose residues and soluble mannose-containing glycoproteins. Macrophages that present an alternative activation phenotype are characterized by high macrophage MR expression due to their response to IL-4. On the other hand, INF-stimulated macrophages exhibit low MR expression (Stain *et al*., 1992).

A mechanism of the MR inhibition of inflammatory interleukins and cytokines occurs by blocking TLR4-signaling that induces IL-12 and TNF production by the up-regulation of

inhibitors of TLR signaling (Pathak *et al*., 2005). Another remarkable feature that can differentiate between M1 and M2 cells is their capacity to kill intracellular pathogens. MR induces the expression of arginase-1, which in turn diminishes the NO-mediated defense (Garrido *et al*., 2011). In addition, the activation of MR delays phagosome-lysosome fusion, a process that is critical for killing engulfed microorganisms (Sweet *et al*., 2010).

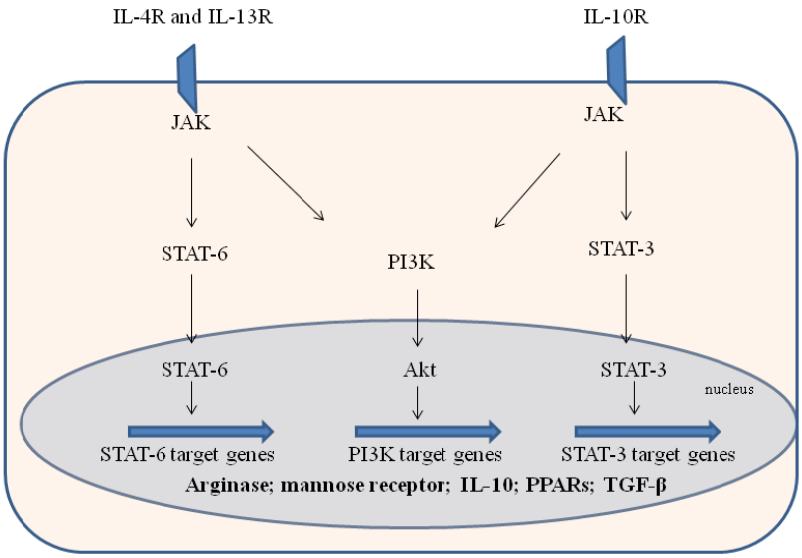

Figure 4. STAT-6, STAT-3 and phosphatidylinositol 3-kinase signaling are required for the expression of IL-4, IL-13 and IL-10 target genes.

Peroxisome proliferator-activated receptors, a Family of Metabolic Nuclear Receptors That Enhances M2 Alternative Activation in Macrophages in Synergy with STAT-6 Signaling

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily, which function as transcription factors regulating the expression of genes. There are three PPAR isoforms, PPARα, PPARβ/δ, and PPARγ, also known as NR1C1, NR1C2, and NR1C3, respectively, and they differ in their tissue distribution and function (Auwerx *et al*., 2006).

PPARα and γ exert anti-inflammatory activities by antagonizing pro-inflammatory signaling pathways in part through their interaction with other transcription factors including NFκB and signal transducers and activators of transcription (STATs). In addition, PPARs act in synergy with anti-inflammatory molecules. IL-4 induces fatty acid metabolism. The oxidative metabolism of fatty acid generates substrates that are activators of PPARs. Immune regulation through the activation of PPARs occurs in response to long-chain unsaturated fatty acids generated from the cyclooxygenase and lipoxygenase pathways. In this way, PPAR

functions as a fatty acid sensor to facilitate the acquisition of the alternative activated phenotype by macrophages (Glass and Saijo, 2010; Chan *et al*., 2009; Shimizu, 2009).

IL-4 induces the production of PPAR ligands in macrophages. PPARγ regulates primarily metabolic programs in alternatively activated macrophages, whereas PPARδ is required for the full expression of their immune phenotype including the expression of pattern-recognition receptors and costimulatory molecules and the suppression of the inflammatory response of M1 (Chawla, 2010; Martinez *et al*., 2009). PPARs are related to the acquisition and long-term maintenance of the M2 phenotype.

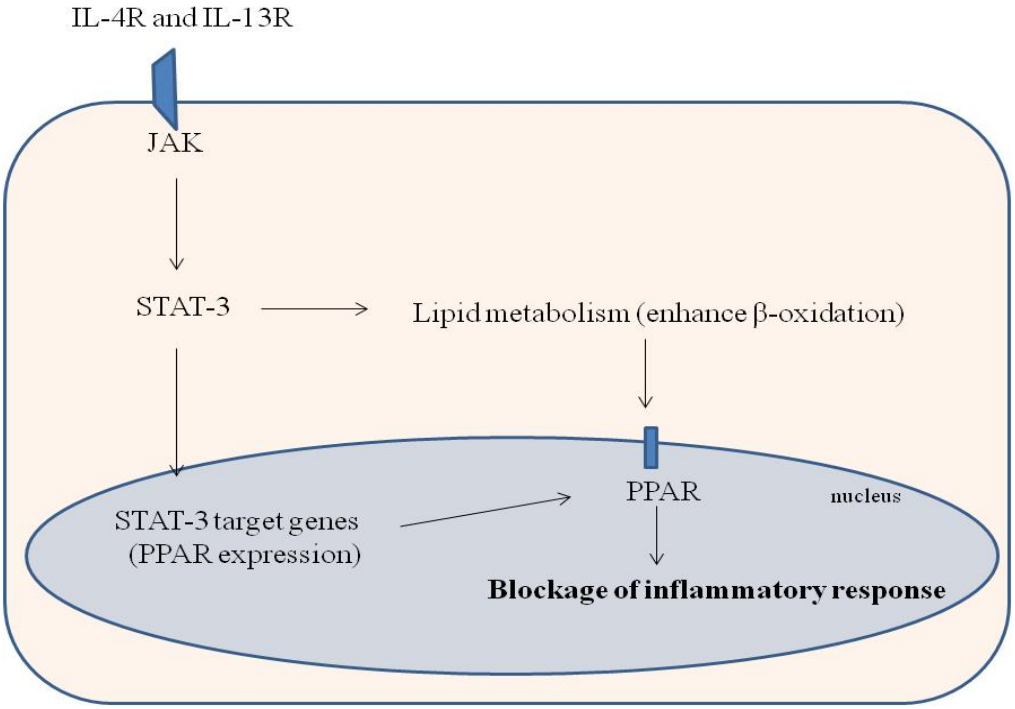

Figure 5. IL-4 and IL-13 induces production of PPAR ligands in macrophages which in turn enhances M2 alternative activation.

The Arginase Pathway in the Immune System: Metabolism and Implications in Infectious Disease and Cancer

Arginine is a crucial amino acid that modulates the cellular immune response. Arginine is also a common substrate for both inducible iNOS and arginase. The generation of nitric oxide from arginine is responsible for the efficient immune response and the cytotoxicity of host cells to kill the invading pathogens and tumor cells. On the other hand, the conversion of arginine to ornithine and urea via the arginase pathway can support the growth of bacterial and parasitic pathogens (Das *et al*., 2010) and the development of tumors.

Arginine Metabolism: A Brief Overview

The arginase enzyme metabolizes arginine to ornithine and urea. Ornithine can be metabolized further to polyamines (putrescine, spermidine, and spermine) via ornithine decarboxylase (ODC). Polyamines are small cationic molecules that participate in a variety of fundamental cellular functions (e.g., proliferation and cell membrane transport). The metabolism of ornithine via ornithine aminotransferase (OAT) generates proline, which is an essential component of collagen. In addition, arginine is a substrate for iNOS leading to NO and other reactive nitrogen intermediates (Munder et al., 2009; Morris *et al*., 2002).

Role of Arginine Metabolism in Infectious Disease and Cancer: Regulation of Arginase Activity

The arginase enzyme may be causally involved in disease pathogenesis because of the suppression of NO-mediated cytotoxicity via L-arginine consumption, the enhancement of collagen synthesis and fibrosis via proline generation and the enhancement of cellular proliferation via polyamine generation (Munder *et al*., 2009).

Arginase 1 (ARG1) is a prototypic alternative activation marker. The expression of ARG1 induces a shift in arginine metabolism from the IFN-γ-induced production of NO via iNOS toward the production of ornithine and polyamines, which are important for wound healing. These mechanisms of induction of the *arg1* gene or other factors that interfere with arginine metabolism are induced by several intracellular pathogens (bacteria, fungi and protozoans) as a mechanism of host immune evasion.

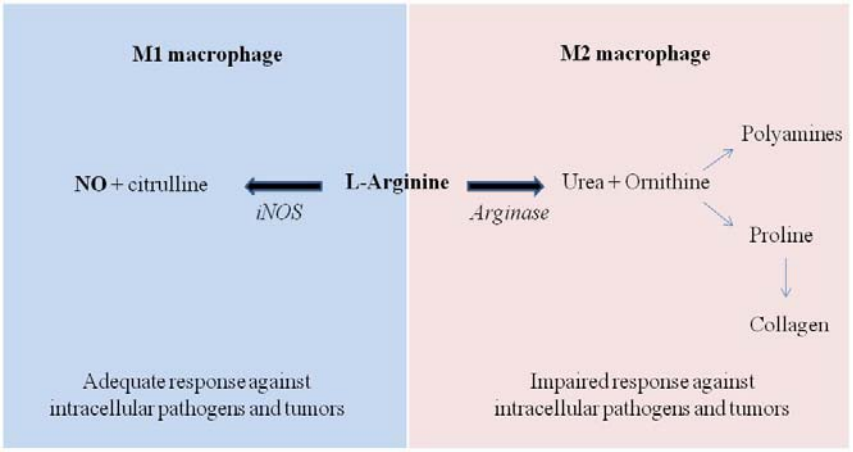

Figure 6. Arginase pathways in polarized macrophages. The enzyme arginase metabolizes arginine to ornithine and urea. Ornithine can be further metabolized to polyamines (putrescine, spermidine, and spermine) via ornithine decarboxylase (ODC). Polyamines are small cationic molecules, which participate in a variety of fundamental cellular functions (e.g., proliferation and cell membrane transport). The metabolism of ornithine via ornithine aminotransferase (OAT) generates proline, which is an essential component of collagen. In addition, arginine is a substrate for nitric oxide synthase (NOS) leading to NO and other reactive nitrogen intermediates (Munder *et al*., 2009; Morris *et al*., 2002).

The shift in arginine metabolism from the production of NOS toward polyamines has implications that favor pathogens. First, the enhanced arginase activity diminishes the killing capacity of macrophages by diminishing the respiratory burst, and the polyamines generated by arginase activity down-regulate pro-inflammatory cytokine release (Das *et al*., 2010; Lahiri *et al*., 2007; Zabaleta *et al*., 2004). Second, polyamines provide a substrate that can be utilized by the pathogen (Das *et al*., 2010; Kropf *et al*., 2005).

In tumor cells, the regulation of arginase metabolism is associated with pro-tumoral activity. The ornithine produced by M2 macrophages as a result of arginase activity, is exported and is taken up by tumor cells to serve as precursors for DNA synthesis, which is intense in tumor cells (Morris *et al*., 1998).

Arginase is up-regulated in M2 cells and is related to the induction of tumor growth. Macrophages surrounding the tumor microenvironment exhibit high arginase activity and low production of ROS, which is typical of M2 polarization. Interfering with arginase expression in mice by deleting the signaling molecules associated with enzyme expression is associated with reduced syngeneic tumor growth (Sharda *et al*., 2011).

Pathogen Defense

The goal of the M2 cells in orchestrating the T_H2 response is the elimination and control of infection by extracellular pathogens such as helminthes because these pathogens are too large to be phagocytosed. IL-4 and IL-13 link the innate response mediated by M2 cells with the acquired response inducing antibody production.

The main role of M2 cells in immunity against worms is their interaction with B cells to present antigens and to induce the production and release of IgE. M2 cells efficiently present antigen to B cells because they also express high levels of antibody receptors that recognize antibody-pathogen molecule complexes.

Memory CD4+ T cells induce M2 cells by secreting IL-4. In turn, these alternatively activated macrophages function as important effector cells of the protective memory response, contributing to parasite elimination. These macrophages impair larval parasite health and mobility through an arginase-dependent pathway against intestinal nematode parasites (Anthony *et al*., 2006).

Role of Macrophage Polarization in Cancer: A Shift in the Immune State Correlates with Disease Prognosis

Macrophages play key roles in cancer by regulating inflammatory processes. Inflammation is a known determinant in the initiation and promotion of cancer, and it contributes to both specific and innate tumor rejection (Siveen and Kuttan, 2009).

The microenvironment surrounding the tumor mass contains a characteristic inflammatory infiltrate associated with constant tissue remodeling. This infiltrate is characterized by a distinct immune cell phenotype, which produces a large range of

inflammatory and anti-inflammatory stimuli. Depending on the stimulus, the tumor can be rejected by the production of anti-tumor cytokines that directly destroy tumor cells, which is mediated by the T_H1 response (cytotoxicity and apoptosis), or the tumor proliferates and promotes disease progression by direct immune defense against the T_H2-type. In this context, the effects of polarized macrophages in cancer are relatively well established.

The M2 phenotype appears to be predominant in the tumor-associated macrophages (TAM) in the tumor environment. These cells have a poor antigen-presentation capability, produce factors that suppress T-cell proliferation and activity, and contribute to tumor biology (Guruvayoorappan, 2008). The tissue remodeling properties of these cells are closely related to the invasion and metastasis processes.

Tams Are Recruited by Tumors to Enhance Neoangiogenesis and Tumor Progression

Neoangiogenesis and neovasculogenesis induced by tumors are usually disorganized and prone to collapse resulting in areas of inadequate irrigation/perfusion, which leads to hypoxia. These conditions of low oxygen tension induce the recruitment of M2 cells, which in turn contributes to angiogenesis. Tumor cells express and release several growth factors, cytokines, and chemokines that recruit and activate M2 cells in the tumor micro-environment (Dirkx *et al*., 2006). The monocyte chemotactic protein (MCP) is the main determinant of the macrophage content in tumors. Once targeted to hypoxic sites, TAM functions are affected greatly by hypoxia-related factors. Tumors also produce large amounts of IL-4 and IL-10 that induce the local and recruited macrophages to differentiate into M2 cells (Wang and Joyce, 2010). In turn, the alternative activated macrophages produce vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and IL-6 to promote angiogenesis and tumor proliferation (Siveen and Kuttan, 2009).

Table 2. TAM markers and their role in tumor progression and consequently, in cancer disease progression. Observe that all molecules constitute M2 polarized macrophages and are induced by cytokines produced by tumor and immune cells

TAM-associated cytokines and markers	Effect on tumor cell and cancer biology
EGF	Growth
VEGF	Angiogenesis
FGF	Growth
IL-1ra	Diminish M1/T_H1 inflammatory response
MMP	Tissue invasion; Metastasis
Arginase-1	Growth; Decrease macrophage killing capacity

Invasive and metastatic behaviors are salient features of aggressive tumors. TAM secretes proinvasive factors that stimulate tumor cell mobility and adhesion in a number of tumor types. One known mechanism that induces metastasis is mediated by enzymes, such as matrix

metaloproteinase (MMP), which are responsible for destroying the basement membrane and extracellular matrix. The degradation of macromolecules, such as collagens, reduces the physical barriers that tumor cells have to overcome as they migrate toward tissues (Bitoux and Stamenkovic, 2008). Another interesting feature of MMP in tumor biology is that the fragments resulting from the degradation of the membrane and extracellular components exhibit bioactive functions. In breast cancer, laminin products bind and activate EGFR inducing tumor migration and growth (Schenk *et al*., 2003).

Immune Modulation Therapy of Therapeutic Targets Provide New Perspectives in Cancer Therapy

New molecular targeted therapies have been shown to interact with immunological pathways, and this interaction is critical for the response and clinical outcome. Immune modulatory mechanisms are related to an enhanced response in some chemotherapy scheme combinations and in studies employing new molecule candidates to be employed in cancer treatment (Tartour *et al* 2011; Noori *et al*., 2010; Weigert *et al* 2009).

In fact, the M2-like pro-tumoral phenotype of TAM observed in most cancers is reversible. Blocking IL-4 or IL-4Rα signaling diminishes lung metastasis, which correlates with the lower expression of M2 genes (such as *Arg1* and *Tgfb1*) but the higher expression of M1 genes (such as *Il6*, *Nos2* and *Il12a* (encoding IL-12p35) in TAM conferring an anti-tumor response activity (Biswas and Montovani, 2010).

Classically activated macrophages kill cancer cells and elicit tumor-destructive reactions. IFN-γ can "reeducate" TAM, thereby providing a proof of principle of its anti-tumor activity in humans. The identification of the molecular pathways responsible for the recruitment and skewing of macrophages in tumors provides a basis for ongoing therapeutic clinical trials (Biswas and Montovani, 2010).

Integration Cell Signaling Pathways of M1 and M2 Polarized Macrophages: Molecular Mechanisms Implicated in the Regulation of the Inflammatory Response and Macrophage Phenotype

Cytokines influence the synthesis and actions of other cytokines. The ability of one cytokine to simulate the production of a second one results in a cascade in which the second cytokine may exert the biological functions of the first. Two cytokines may antagonize the effects of each other, produce additive effects or act in a synergistic manner. These effects may be true for both T_H1 and T_H2 cytokines and may occur between them, exerting feedback stimulatory or inhibitory mechanisms.

Cross-Talk between M1 Signaling Pathways: TLR and IFNR Signaling Interaction

Priming macrophages with IFN-γ mediates changes from the resting to the pre-activation state that is highly receptive to a second activating signal. Synergism occurs at multiple levels ranging from signal recognition to the convergence of signals at the promoters of target genes. Several genes contain both STAT-1 and NF-kB binding sites in their promoters. For that reason, maximal transcription requires both signals (IFN and TLR) in several cases. This phenomenon is termed "priming" (Hu and Ivashkiv, 2009; Schroder *et al*., 2004).

IFN and TLR signaling pathways synergize to secrete nitric oxide and IL-12. As discussed in a previous section, the secretion of IL-12 activates T cells to produce more IFN-γ. Priming macrophages with IFN induces *tlr2*, *tlr4*, *tlr6* and *tlr9* mRNAs, with the consequent up-regulated expression of TLR2, TLR4, TLR6 and TLR9 resulting in the LPS-binding ability of macrophage.

MyD88 that is associated with TLR but not IFN signaling potentiates the effects of IFN on the target cell. This adaptor protein enhances the half-life of IFN-γ-induced-mRNA transcripts allowing IFN-γ to enhance the expression of genes encoding proinflammatory molecules (Sun and Ding, 2006).

Cross-Regulation of the JAK-STAT Pathway

Various combinations of JAKs (JAK1, JAK2, JAK3 and TYK2) and STATs (STAT-1, STAT-2, STAT-3, STAT-4, STAT-5a, STAT-5b and STAT-6) are activated to transduce cytokine signals; however, recent gene targeting studies have clarified the non-redundant and specific roles of each JAK and STAT member in different cytokines. This specificity and the activation of different STATs to a given stimulus (cytokine binding to its receptor) lead to the transcription of target genes, which often have an antagonistic role in the activation of macrophages into M1 or M2 cells. Among them, STAT-1 is relatively specific to IFNs, STAT-3 is activated by IL-6 and other gp130-related cytokines, STAT-4 is activated by IL-12, and STAT-6 is specifically activated by IL-4 and IL-13. STAT-5 is activated by various cytokines including IL-2, IL-3, erythropoietin and growth hormone (Mohr *et al*., 2011; Yoshimura, 2006). In general, STAT-1 has a critical role in M1 polarization, and STAT-6 is correlated to M2 polarization.

The JAK-STAT signaling pathway leads to macrophage polarization to M1 or M2. This regulation allows a feedback mechanism to control the immune response and attenuate the effects of high expression of pro-inflammatory or anti-inflammatory cytokines. Distinct cytokines have their activities mediated by JAK-STAT signaling, and this has important implications for the regulation of macrophage biology.

IFN-γ directly inhibits signaling pathways downstream of anti-inflammatory cytokines to antagonize their suppressive functions. IFN-γ antagonizes the effects of IL-10 by attenuating IL-10 production and suppressing IL-10 signaling by disrupting STAT-3 and/or STAT-6 activation (Dickensheets *et al*., 1999). In addition, IFN-γ does not suppress upstream IL-10 signaling directly but induces a shift in the balance of IL-10 STAT activation from STAT-3 to

STAT-1 resulting in the induction of STAT-1 target genes by IL-10 (Hu and Ivashkiv, 2009; Herrero *et al*., 2003).

SOCS Proteins Regulate the Inflammatory Response by Regulating STATs Signaling

After receptor cytokine stimulation and the initiation of signal transduction-associated proteins, suppressors of cytokine signaling (SOCS proteins) are induced. SOCS proteins exert inhibitory signaling effects on STAT levels as feedback inhibitory mechanisms to attenuate high inflammatory responses. SOCS-1 and SOCS-3 are associated with the termination of IFN-γ and TLR pro-inflammatory signals, respectively. A mechanism by which anti-inflammatory-associated cytokines (such as IL-4, IL-13 and IL-10) inhibit M1 polarization is the induction of SOCS protein expression. For example, IL-10 induces the high expression of SOCS-3 to attenuate the TLR signal (O'Shea and Murray, 2008; Shuai and Liu, 2003).

A wide range of pathogens, such as *Leishmania* parasites and some bacterias, also induce the up-regulation of SOCS proteins and, consequently, impair the classical activation-mediated response of macrophages (Vásquez *et al* 2006; Bertholet *et al*., 2003).

Phosphorylation and Dephosphorylation Reactions Regulate JAK and STAT Protein Activation

Because STAT proteins are activated by the kinase-mediated phosphorylation of tyrosine or serine residues (Shuai and Liu, 2003), several members of the tyrosine phosphatase family and serine/threonine phosphatase family are potent inhibitors of STATs (Shuai and Liu, 2003). In general, all STAT proteins can be regulated by phosphatases. Tyrosine phosphatases dephosphorylate active STATs in the cytoplasm and nucleus impairing gene transcription. JAKs are also deactivated by phosphatases (Baron and Davignon, 2008; Woetmann *et al*., 2003).

Regulation of Macrophage Activity by PI3K-Akt-Glycogen-Synthase Kinase 3 (GSK3) Signaling

Some studies demonstrate that IFN-γ selectively suppresses the IL-10 response without affecting the activation state of JAK-STAT-3 signaling pathways induced by IL-4. In this case, the inhibitory action of IFN-γ appears to be mediated by the suppression of PI3K-Akt signaling by the enzyme glycogen synthase kinase-3 (Antoniv and Ivashkiv, 2011). This pathway is another example of the convergence of the energetic cell metabolism state and the inflammatory response. The active GSK 3 enzyme inhibits Akt signaling. GSK 3 activation is induced by IFN-γ, and this suppresses the transcription of Akt-target genes induced by IL-4, IL-13 and IL-10.

Table 3. Regulation of macrophage activation by JAK-STAT signaling pathway

Cytokine	JAK-STAT transducer pathway	Macrophage polarization	Negative regulators
IFN-γ	STAT-1	M1	SOCS-1 SOCS-3 Phosphatases
IL-10	STAT-3	M2	SOCS-1 SOCS-3 Phosphatases
IL-12	STAT-4	M1	SOCS-1 SOCS-3 Phosphatases
IL-4 IL-13	STAT-6	M2	GSK-3 Phosphatases

Conclusion

The heterogeneous roles that macrophage play in immunity depend on the environmental stimuli. The signaling events associated with macrophage adaptation and response to environmental signals are critical to understand the mechanisms of several pathologies. In fact, immune regulatory mechanisms are related to an enhanced clinical response against tumors and chronic infections. Since polarized inflammation plays different roles during tumor progression, an interesting intervention would be the repolarization of macrophages, as this will turn macrophage cells phenotype into effective fighters against cancer and pathogens.

References

Antoniv, T. T. Ivashkiv, L. B. (2011). Interleukin-10-induced gene expression and suppressive function are selectively modulated by the PI3K-Akt-GSK3 pathway. *Immunology,* 132, 567-577.

Araki, N. Johnson, M. T. Swanson, J. A. (1996). A role for phosphoinositide 3-kinase in the completion of macropinocytosis and phagocytosis by macrophages. *The journal of cell biology*, 135, 1249-1260.

Baron, M. Davignon, J. L. (2008). Inhibition of IFN-gamma-induced STAT1 tyrosine phosphorylation by human CMV is mediated by SHP2. *Journal of immunology,* 181, 5530-5536.

Baud, V. Karin, M. (2001). Signal transduction by tumor necrosis factor and its relatives. *Trends in cell biology*, 11, 372-377.

Benoit, M. Desnues, B. Mege, J. L. (2008). Macrophage polarization in bacterial infections. *Journal of immunology*, 181, 3733-3739.

Bertholet, S. Dickensheets, H. L. Sheikh, F. Gam, A. A. Donnelly, R. P. Kenney, R. T. (2003). *Leishmania donovani*-induced expression of suppressor of cytokine signaling 3 in human macrophages: a novel mechanism for intracellular parasite suppression of activation. *Infection and immunity,* 71, 2095-2101.

Biswas, S. K and Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm, *Nature immunology*. 11, 889-896.

Boehm, U. Klamp, T. Groot, M. Howard, J. C. (1997). Cellular responses to interferon-gamma. *Annual review of immunology*, 15, 749-795.

Bosschaerts, T. Morias, Y. Stijlemans, B. Hérin, M. Porta, C. Sica, A. Mantovani, A. De Baetselier, P. Beschin, A. (2011). IL-10 limits production of pathogenic TNF by M1 myeloid cells through induction of nuclear NF-κB p50 member in *Trypanosoma congolense* infection-resistant C57BL/6 mice. *European journal of immunology*, 41, 3270-3280.

Cassatella, M. A. Bazzoni, F. Flynn, R. M. Dusi, S. Trinchieri, G. Rossi, F. (1990). Molecular basis of interferon-gamma and lipopolysaccharide enhancement of phagocyte respiratory burst capability. Studies on the gene expression of several NADPH oxidase components. *The journal of biological chemistry*, 265, 20241-20246.

Chan, M. M. Evans, K. W. Moore, A. R. Fong, D. (2010). Peroxisome proliferator-activated receptor (PPAR): balance for survival in parasitic infections. *Journal of biomedicine and biotechnology*, 2010:828951.

Chawla, A. (2010). Control of macrophage activation and function by PPARs. *Circulation research*, 106, 1559-1569.

Chen, G. Shaw, M. H. Kim, Y. G. Nuñez, G. (2009). NOD-like receptors: role in innate immunity and inflammatory disease. *Annual review of pathology,* 4, 365-398.

Chung, E. Y, Kim, B. H. Hong, J. T. Lee, C. K. Ahn, B. Nam, S. Y. Han, S. B. Kim, Y. (2011). Resveratrol down-regulates interferon-γ-inducible inflammatory genes in macrophages: molecular mechanism via decreased STAT-1 activation. *Journal of the nutritional biochemistry*, 22, 902-909.

Das, P. Lahiri, A. Lahiri, A. Chakravortty, D. (2010). Modulation of the arginase pathway in the context of microbial pathogenesis: a metabolic enzyme moonlighting as an immune modulator. *PLoS Pathogens,* 6:e1000899.

Dickensheets, H. L. Venkataraman, C. Schindler, U. Donnelly, R. P. (1999). Interferons inhibit activation of STAT6 by interleukin 4 in human monocytes by inducing SOCS-1 gene expression. *Proceedings of the National Academy of Science of the United States of America*, 96, 10800-10805.

Dickensheets, H. L. Venkataraman, C. Schindler, U. Donnelly, R. P. (1996). Interferons inhibit activation of STAT6 by interleukin 4 in human monocytes by inducing SOCS-1 gene expression. *Proceeding of the national academy of science of United States of America,* 96, 10800-10805.

Dirkx, A. E. Oude Egbrink, M. G. Wagstaff, J. Griffioen, A. W. (2006). Monocyte/macrophage infiltration in tumors: modulators of angiogenesis. *The journal of leukocyte biology*, 80, 1183-1196.

Fang, F. C. (2004). Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nature reviews. Microbiology*, 2, 820-832.

Garrido, V. V. Dulgerian, L. R. Stempin, C. C. Cerbán, F. M. (2011). The increase in mannose receptor recycling favors arginase induction and trypanosoma cruzi survival in macrophages. *International journal of biological sience*, 7, 1257-1272.

Gasparoto, T. H. Tessarolli, V. Garlet, T. P. Torres, S. A. Garlet, G. P. da Silva, J. S. Campanelli, A. P. (2010). Absence of functional TLR4 impairs response of macrophages after *Candida albicans* infection. *Medical Mycology*, 48, 1009-1017.

Glass, C. K. Saijo, K. (2010). Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. *Nature reviews. Immunology.* 10, 365-376.

Goerdt, S. Politz, O. Schledzewski, K. Birk, R. Gratchev, A. Guillot, P. Hakiy, N. Klemke, C. D. Dippel, E. Kodelja, V. Orfanos, C. E. (1999). Alternative versus classical activation of macrophages. *Pathobiology,* 67, 222-226.

Gordon, S. Taylor, P. R. (2005). Monocyte and macrophage heterogeneity. Nature reviews. *Immunology,* 5, 953-964.

Gu, Y. Kuida, K. Tsutsui, H. Ku, G. Hsiao, K. Fleming, M. A. Hayash N. Higashino, K. Okamura, H. Nakanishi, K. Kurimoto, M. Tanimoto, T. Flavell, R. A. Sato, V. Harding, M. W. Livingston, D. J. Su, M. S. (1997). Activation of interferon-gamma inducing factor mediated by interleukin-1beta converting enzyme. *Science,* 275, 206-209.

Guruvayoorappan, C. (2008). Tumor versus tumor-associated macrophages: how hot is the link? *Integrative cancer therapy*, 7, 90-95.

Herrero, C. Hu, X. Li, W. P. Samuels, S. Sharif, M. N. Kotenko, S. Ivashkiv, L. B. (2003). Reprogramming of IL-10 activity and signaling by IFN-gamma. *Journal of immunology*, 171, 5034-5041.

Hsu, H. Xiong, J. Goeddel, D. V. (1995). The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. *Cell,* 81, 495-504.

Hu, X. Ivashkiv, L. B. (2009). Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases. *Immunity,* 16, 539-550.

Kämper, N. Franken, S. Temme, S. Koch, S. Bieber, T. Koch, N. (2011). {gamma}-Interferon-regulated chaperone governs human lymphocyte antigen class II expression. *FASEB Jour*nal. doi: 10.1096/fj.11-189670.

Kanneganti, T. D. Lamkanfi, M. Núñez, G. (2007). Intracellular NOD-like receptors in host defense and disease. Immunity, 27, 549-559.

Kawai, T. and Akira, S. (2006). TLR signaling. *Cell death and differentiation,* 13, 816-825.

Kawai, T. Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity*, 34, 637-50.

Kersse, K. Bertrand, M. J. Lamkanfi, M. Vandenabeele, P. (2011). NOD-like receptors and the innate immune system: Coping with danger, damage and death. *Cytokine growth factor reviews*, 22, 257-276.

Kropf, P. Fuentes, J. M. Fähnrich, E. Arpa, L. Herath, S. Weber, V. Soler, G. Celada, A. Modolell, M. Müller, I. (2005). Arginase and polyamine synthesis are key factors in the regulation of experimental leishmaniasis in vivo. *FASEB Journal*, 19, 1000-1002.

Kumar, H. Kawai, T. Akira, S. (2009). Toll-like receptors and innate immunity. *Biochemical and biophysical research com*munication, 388, 621-625.

Kumar, H. Kawai, T. Akira, S. (2011). Pathogen recognition by the innate immune system. *International reviews of immunology*, 30, 16-34.

Lahiri, A. Das, P. Chakravortty, D. (2008). Arginase modulates Salmonella induced nitric oxide production in RAW264.7 macrophages and is required for *Salmonella* pathogenesis in mice model of infection. *Microbes and infections*, 2008, 10, 1166-1174.

Le Bitoux, M. A. Stamenkovic, I. (2008). Tumor-host interactions: the role of inflammation. *Histochemistry and cell biology*, 130, 1079-1090.

Magez, S. Radwanska, M. Drennan, M. Fick, L. Baral, T. N. Allie, N. Jacobs, M. Nedospasov, S. Brombacher, F. Ryffel, B. De Baetselier, P. (2007). Tumor necrosis factor (TNF) receptor-1 (TNFp55) signal transduction and macrophage-derived soluble TNF are crucial for nitric oxide-mediated *Trypanosoma congolense* parasite killing. *Journal of infectious diseases,* 196, 954-962.

Mäkelä, S. M. Strengell, M. Pietilä, T. E. Osterlund, P. Julkunen, I. (2009). Multiple signaling pathways contribute to synergistic TLR ligand-dependent cytokine gene expression in human monocyte-derived macrophages and dendritic cells. *The journal of leukocyte biology*, 85, 664-672.

Mantovani, A. Allavena, P. Sica, A. (2004). Tumour-associated macrophages as a prototypic type II polarised phagocyte population: role in tumour progression. *European journal of cancer*, 40, 1660-1667.

Martinez, F. O. (2011). Regulators of macrophage activation. *European journal of immunology*. 41, 1531-1534.

Martinez, F. O. Helming, L. Gordon, S. (2009). Alternative activation of macrophages: an immunologic functional perspective. *Annual review of immunology,* 27, 451-483.

Martinon, F. Burns, K. Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Molecular cell,* 10, 417-426.

Michalik, L. Auwerx, J. Berger, J. P. Chatterjee, V. K. Glass, C. K. Gonzalez, F. J. Grimaldi, P. A. Kadowaki, T. Lazar, M. A. O'Rahilly, S. Palmer, C. N. Plutzky, J. Reddy, J. K. Spiegelman, B. M. Staels, B. Wahli, W. (2006). International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacological reviews*, 58, 726-741.

Mohr, A. Chatain, N. Domoszlai, T. Rinis, N. Sommerauer, M. Vogt, M. Müller-Newen, G. (2011). Dynamics and non-canonical aspects of JAK/STAT signalling. *European journal of cell biology,* doi:10.1016/j.ejcb.2011.09.005.

Moore, K. W de Waal Malefyt, R. Coffman, R. L. O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annual review of immunology*, 19, 683-765.

Morris, S. M. (2002). Regulation of enzymes of the urea cycle and arginine metabolism. *Annual review of nutri*tion, 22, 87-105.

Morris, S. M. Jr. Kepka-Lenhart, D. Chen, L. C. (1998). Differential regulation of arginases and inducible nitric oxide synthase in murine macrophage cells. *The American journal of physiology*, 275:E740-7.

Mosser, D. M, Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. Nature reviews Immunology. 8, 958-969.

Mosser, D. M. (2003). The many faces of macrophage activation. *Journal of leukocyte biology,* 73, 209-212.

Moy, F. J. Diblasio, E. Wilhelm, J. Powers, R. (2001). Solution structure of human IL-13 and implication for receptor binding. *Journal of molecular biology,* 310, 219-230.

Munder, M. (2009). Arginase: an emerging key player in the mammalian immune system. *British journal of pharmacology*, 158, 638-651.

Nelms, K. Keegan, A. D. Zamorano, J. Ryan, J. J. Paul, W. E. (1999). The IL-4 receptor: signaling mechanisms and biologic functions. *Annual review of immunology*, 17, 701-738.

Nelson, S. M. Lei, X. Prabhu, K. S. (2011). Selenium levels affect the IL-4-induced expression of alternative activation markers in murine macrophages. *The journal of nutrition*, 141, 1754-1761.

Nguyen, V. T. Benveniste, E. N. (2000). Involvement of STAT-1 and ets family members in interferon-gamma induction of CD40 transcription in microglia/macrophages. *The journal of biological chemistry*, 275, 23674-23684.

Noori, S. Taghikhani, M. Hassan, Z. M. Allameha, A. Mostafaei, A. (2010). Tehranolide molecule modulates the immune response, reduce regulatory T cell and inhibits tumor growth in vivo. *Molecular immunology*, 47, 1579-1584.

O'Shea, J. J. Murray, P. J. (2008). Cytokine signaling modules in inflammatory responses. *Immunity*, 28, 477-487.

Parameswaran, N. Patial, S. (2010). Tumor necrosis factor-α signaling in macrophages. *Critical reviews in eukaryotic gene Expression*, 20, 87-103.

Pathak, S. K. Basu, S. Bhattacharyya, A. Pathak, S. Kundu, M. Basu, J. (2005). *Mycobacterium tuberculosis* lipoarabinomannan-mediated IRAK-M induction negatively regulates Toll-like receptor-dependent interleukin-12 p40 production in macrophages. *The journal of biological chemistry*, 280, 42794-42800.

Plüddemann, A. Mukhopadhyay, S. Gordon, S. (2011). Innate immunity to intracellular pathogens: macrophage receptors and responses to microbial entry. *Immunology reviews*, 240, 11-24.

Qiu, F. Maniar, A. Diaz, M. Q. Chapoval, A. I. Medvedev, A. E. (2011). Activation of cytokine-producing and antitumor activities of natural killer cells and macrophages by engagement of Toll-like and NOD-like receptors. *Innate Immunity*, 17, 375-387.

Reith, W. LeibundGut-Landmann, S. Waldburger, J. M. (2005). Regulation of MHC class II gene expression by the class II transactivator. *Nature reviews. Immunology*, 5, 793-806.

Romero, C. D. Varma, T. K. Hobbs, J. B. Reyes, A. Driver, B. Sherwood, E. R. (2011). The Toll-like receptor 4 agonist monophosphoryl lipid a augments innate host resistance to systemic bacterial infection. *Infection and immunity*, 79, 3576-3587.

Schenk, S. Hintermann, E. Bilban, M. Koshikawa, N. Hojilla, C. Khokha, R. Quaranta, V. (2003). Binding to EGF receptor of a laminin-5 EGF-like fragment liberated during MMP-dependent mammary gland involution. *The journal of cell biology*,161, 197-209.

Schroder, K. Hertzog, P. J. Ravasi, T. Hume, D. A. (2004). Interferon-gamma: an overview of signals, mechanisms and functions. *Journal of leukocyte biology*, 75, 163-189.

Sharda, D. R. Yu, S. Ray, M. Squadrito, M. L. De Palma, M. Wynn, T. A. Morris, S. M. Jr. Hankey, P. A. (2011). Regulation of macrophage arginase expression and tumor growth by the Ron receptor tyrosine kinase. *Journal of immunology*, 187, 2181-2192.

Shen, Y. Kawamura, I. Nomura, T. Tsuchiya, K. Hara, H. Dewamitta, S. R. Sakai, S. Qu, H. Daim, S. Yamamoto, T. Mitsuyama, M. (2010). Toll-like receptor 2- and MyD88-dependent phosphatidylinositol 3-kinase and Rac1 activation facilitates the phagocytosis of *Listeria monocytogenes* by murine macrophages. *Infection and immunity*. 78, 2857-2867.

Shimizu, T. (2010). Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. *Annual review of pharmacology and toxicology*, 49, 123-50.

Shuai, K. Liu, B. (2003). Regulation of JAK-STAT signalling in the immune system. *Nature reviews Immunology*, 3, 900-911.

Silva, J. S. Vespa, G. N. Cardoso, M. A. Aliberti, J. C. Cunha, F. Q. (1995). Tumor necrosis factor alpha mediates resistance to *Trypanosoma cruzi* infection in mice by inducing nitric oxide production in infected gamma interferon-activated macrophages. *Infection and immunity*, 63, 4862-4867.

Siveen, K. S. Kuttan, G. (2009). Role of macrophages in tumour progression. *Immunology letters*, 123, 97-102.

Stein, M. Keshav, S. Harris, N. Gordon, S. (1992). Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *The journal of experimental medicine,* 176, 287-292.

Stout, R. D. Jiang, C. Matta, B. Tietzel, I. Watkins, S.K. Suttles, J. (2005). Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *Jorunal of immunology,* 175, 342-349.

Sun, D. Ding, A. (2006). MyD88-mediated stabilization of interferon-gamma-induced cytokine and chemokine mRNA. *Nature immunology*, 7, 375-381.

Sweet, L. Singh, P. P. Azad, A. K. Rajaram, M. V. Schlesinger, L. S. Schorey, J. S. (2010). Mannose receptor-dependent delay in phagosome maturation by *Mycobacterium avium* glycopeptidolipids. *Infection and Immunity*, 78, 518-526.

Tartour, E. Pere, H. Maillere, B. Terme, M. Merillon, N. Taieb, J. Sandoval, F. Quintin-Colonna, F. Lacerda, K. Karadimou, A. Badoual, C. Tedgui, A. Fridman, W. H. Oudard, S. (2011). Angiogenesis and immunity: a bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy. *Cancer metastasis reviews*, 30, 83-95.

Valledor, A. F. Sánchez-Tilló, E. Arpa, L. Park, J. M. Caelles, C. Lloberas, J. Celada, A. (2008). Selective roles of MAPKs during the macrophage response to IFN-gamma. *Journal of immunology*, 180, 4523-4529.

Vázquez, N. Greenwell-Wild, T. Rekka, S. Orenstein, J. M. Wahl, S. M. (2006). *Mycobacterium avium*-induced SOCS contributes to resistance to IFN-gamma-mediated mycobactericidal activity in human macrophages. *Journal of leukocyte biology*, 80, 1136-1144.

Vilcek, J. (2006). Fifty years of interferon research: aiming at a moving target. *Immunity,* 25, 343-348.

Wajant, H. Pfizenmaier, K. Scheurich, P. (2003). Tumor necrosis factor signaling. *Cell death and differentiation*, 10, 45-65.

Wang, H. W. Joyce, J. A. (2010). Alternative activation of tumor-associated macrophages by IL-4: priming for protumoral functions. *Cell Cycle,* 9, 4824-4835.

Weigert, A. Sekar, D. Brüne, B. (2009). Tumor-associated macrophages as targets for tumor immunotherapy. *Immunotherapy*, 1, 83-95.

Woetmann, A. Brockdorff, J. Lovato, P. Nielsen, M. Leick, V. Rieneck, K. Svejgaard, A. Geisler, C. Ødum, N. (2003). Protein phosphatase 2A (PP2A) regulates interleukin-4-mediated STAT6 signaling. *The journal of biological chemistry,* 278, 2787-2791.

Yoshimura, A. (2006). Signal transduction of inflammatory cytokines and tumor development. *Cancer science,* 97, 439-447.

Zabaleta, J. McGee, D. J. Zea, A. H. Hernández, C. P. Rodriguez, P. C. Sierra, R. A. Correa, P. Ochoa, A. C. (2004). *Helicobacter pylori* arginase inhibits T cell proliferation and reduces the expression of the TCR zeta-chain (CD3zeta). *Journal immunology*, 173, 586-593.

Zhang, S. Kim, C. C. Batra, S. McKerrow, J. H. Loke, P. (2010). Delineation of diverse macrophage activation programs in response to intracellular parasites and cytokines. *PLoS neglected tropical disease*, 30, e648.