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Macrophage Polarization in Chagas Disease

Nagela Ghabdan Zanluqui[1], Pryscilla Fanini Wowk[1] and Phileno Pinge-Filho[2*]

[1]Instituto Carlos Chagas, ICC-Fiocruz-PR, Rua Prof. Algacyr Munhoz Máder 3775, Bloco C CIC, 81350-010, Curitiba, Paraná, Brazil

[2]Laboratório de Imunopatologia Experimental, Departamento de Ciências Patológicas Centro de Ciências Biológicas, Universidade Estadual de Londrina, 86051-970, Londrina, Paraná, Brazil

*Corresponding author: Pinge-Filho P, Departamento de Ciências Patológicas, Centro de Ciências Biológicas, Universidade Estadual de Londrina, 86051-970, Londrina, Paraná, Brazil, Tel: + 55 43 3371-4978; Fax: + 55 43 3371-4465; E-mail: pingefilho@uel.br

Abstract

Macrophages are terminally differentiated cells of the mononuclear phagocytic system, which play an indispensable role in the maintenance of homeostasis and defense. Macrophages can be phenotypically polarized by the microenvironment to mount specific functional responses. Polarized macrophages can be broadly classified into two main groups: classically activated macrophages (M1), whose prototypical activating stimuli are IFN-γ and LPS, and alternatively activated macrophages (M2), further subdivided in M2a (after exposure to IL-4 or IL-13), M2b (immune complexes in combination with IL-1β or LPS) and M2c (IL-10, TGF-β or glucocorticoids). M1 exhibit potent microbicidal properties and promote strong IL-12-mediated Th1 responses, while M2 macrophages support Th2-associated effector functions. Here we review the main functions of polarized macrophages in Chagas disease and discuss their potential value in evaluating disease severity.

Keywords: Macrophage polarization; *Trypanosoma cruzi*; Therapies

Introduction

Chagas disease is caused by the protozoan *Trypanosoma cruzi*, which affects approximately eight million individuals in Latin America, out of which 30-40% either already have or will develop cardiomyopathy, digestive mega syndromes, or both [1]. More recently, another major concern has been the emergence of Chagas disease in non-endemic areas such as North America, Europe and the Western Pacific Region, due to immigration of infected individuals [2,3]. The disease is characterized by two clinical phases: a short, acute phase defined by patent parasitemia, and a long, progressive, chronic phase.

The parasite load in the acute phase of *T. cruzi* infection influences the activation of host immune system and the development of Chagas disease pathology in the late chronic phase [4]. Although the exact mechanisms that mediate control of parasites in humans, have not been elucidated [5], it is believed that parasites rely greatly on the function of innate immune cells such as natural killer (NK) cells, neutrophils, and macrophages [6]. *T. cruzi* induces monocytes and macrophages to produce various endogenous mediators, including cytokines, nitric oxide (NO), and prostaglandin E_2 (PGE_2) [7-9].

Monocyte populations are heterogeneous and can polarize depending on the micro-environmental stimuli. Classically activated monocytes differentiate into M1 macrophages after stimulation by microbial agents such as lipopolysaccharides (LPS) and Th1 pro-inflammatory cytokines such as IFN-γ, whereas alternatively activated or M2 macrophages are induced by other stimuli, including transforming growth factor beta (TGF-β), interleukin-4 (IL-4), interleukin-10 (IL-10), and interleukin-13 [10,11].

The two polarized macrophage populations are also functionally different: M1 cells have inflammatory functions, produce high levels of pro-inflammatory cytokines, produce reactive nitrogen and oxygen intermediates, and have bactericidal activity [12]. M2 cells have immunoregulatory functions, help in clearance of parasite, have increased phagocytic activity, and are involved in matrix remodeling, angiogenesis, wound healing [13], synovitis, and cartilage damage following hemarthrosis [14]. M2 macrophages are further characterized by the functional expression of their alternative activation markers. M2 macrophages have at least three subsets: M2a, induced by IL-4 or IL-13; M2b, induced by immune complexes and agonists of TLRs or IL-1 receptors; and M2c, induced by IL-10 and glucocorticoid hormones [15]. However, the distinct expression patterns of surface markers that clearly define macrophage subsets are still unclear, particularly in the case of human macrophages [16]. Recently, nomenclature and experimental guidelines were proposed to attain consensus between researchers regarding macrophage immunobiology [17].

The role of macrophage polarization in parasitic diseases is far from being well defined. Several pathogens exploit regulatory responses to facilitate immune escape and enhance their own survival in the host. For example, *Leishmania* binds and triggers FcγR signaling during entry into the host, resulting in the development of macrophages, which are permissive for its intracellular growth [18]. It is noteworthy that cell activation is critical for the induction of an effective immune response against pathogens or tumors, as inappropriate and sustained activation/polarization of macrophages can lead to tissue damage, immune dysfunction, and disease [18]. Given their capacity to suppress adaptive immune responses, it is important to understand how M1-M2 macrophages contribute to dysfunctional immune responses in infections such as Chagas disease. This review highlights the current understanding of the interplay between *T. cruzi* infection and macrophage polarization.

Macrophage polarization

M1-M2 nomenclature derives from the Th1 and Th2 cytokines: In 1986 Mosmann, Coffman et al. reported that murine T-lymphocytes

could be divided into Th1 and Th2 cells, based on their respective cytokine production profiles: IFN-γ and IL-4 [19]. The concept of macrophage polarization was first introduced in 1992 with the discovery that IL-4 inhibits the respiratory burst of macrophages while enhancing expression of MHC-II and mannose receptors (CD206) on their cell surface [20]. Since then, two opposite and competing macrophages phenotypes were defined, often referred to as classically activated macrophages (M1 macrophages) and alternatively activated macrophages (M2 macrophages) [21,22].
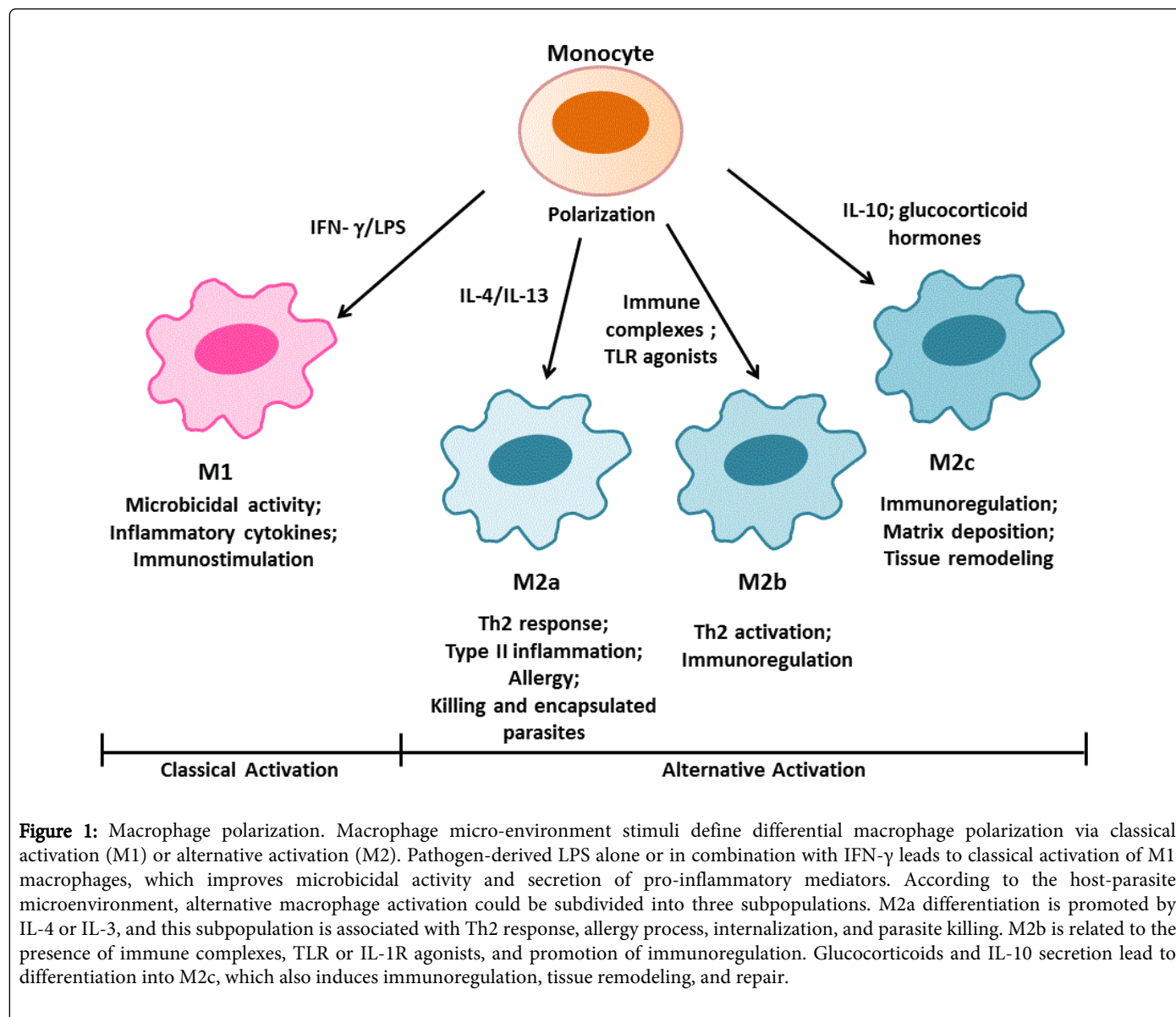

Figure 1: Macrophage polarization. Macrophage micro-environment stimuli define differential macrophage polarization via classical activation (M1) or alternative activation (M2). Pathogen-derived LPS alone or in combination with IFN-γ leads to classical activation of M1 macrophages, which improves microbicidal activity and secretion of pro-inflammatory mediators. According to the host-parasite microenvironment, alternative macrophage activation could be subdivided into three subpopulations. M2a differentiation is promoted by IL-4 or IL-3, and this subpopulation is associated with Th2 response, allergy process, internalization, and parasite killing. M2b is related to the presence of immune complexes, TLR or IL-1R agonists, and promotion of immunoregulation. Glucocorticoids and IL-10 secretion lead to differentiation into M2c, which also induces immunoregulation, tissue remodeling, and repair.

In fact, macrophages can secrete either IL-12 or IL-10, cross regulatory cytokines crucial for the elicitation of IFN-γ production and development of Th1 cells or IL-4/IL-13 secretion and development of Th2 cells proliferation, respectively [22,23].

This classification was further extended by Mantovani and collaborators [24], M1 polarization included, the classical activation, obtained by stimulation with pathogen-derived LPS alone or in combination with IFN-γ, which improve microbicidal activity and pro-inflammatory mediator secretion, while M2 polarization, mainly associated with tissue repair, was further subdivided in M2a or alternatively activated macrophages; M2b, corresponding to type II activated macrophages; and M2c, which includes heterogeneous macrophage deactivation stimuli (Figure 1). Polarization states and functional properties of macrophages largely depend on environmental conditions, such as hypoxia, cytokines, pathogen-derived TLR-ligands, and lipid mediators [21].

Some molecular factors underlying macrophage polarization have been identified [25,26]. These include members of the IRF/Stat families, Myc, NF-κB hetero-and homo-dimers, KLF4, PPARγ, as well as miRNA, and epigenetic modifications [25-27].

The observation that macrophage functional phenotypes can be manipulated has drawn attention towards macrophages as a potential therapeutic target for cancer therapy [28-30]. Thus, elucidation of the signaling pathways that regulate functional polarization of

macrophage in *T. cruzi* infection will aid in the designing of strategies for the modification of macrophage behavior.

Macrophage Polarization in *T. cruzi* infection

M1-M2 signatures: During the early stages of infection, *T. cruzi* induces an intense inflammatory response, which plays a crucial role in the pathogenesis of the disease [31]. During this stage the macrophages induce a cascade of cytokines: initially they produce IL-12, which act on NK cells to induce IFN-γ production, which in turn increases the production of IL-12, TNF-α and NO in macrophages, thus contributing to the killing of the parasite [32,33].
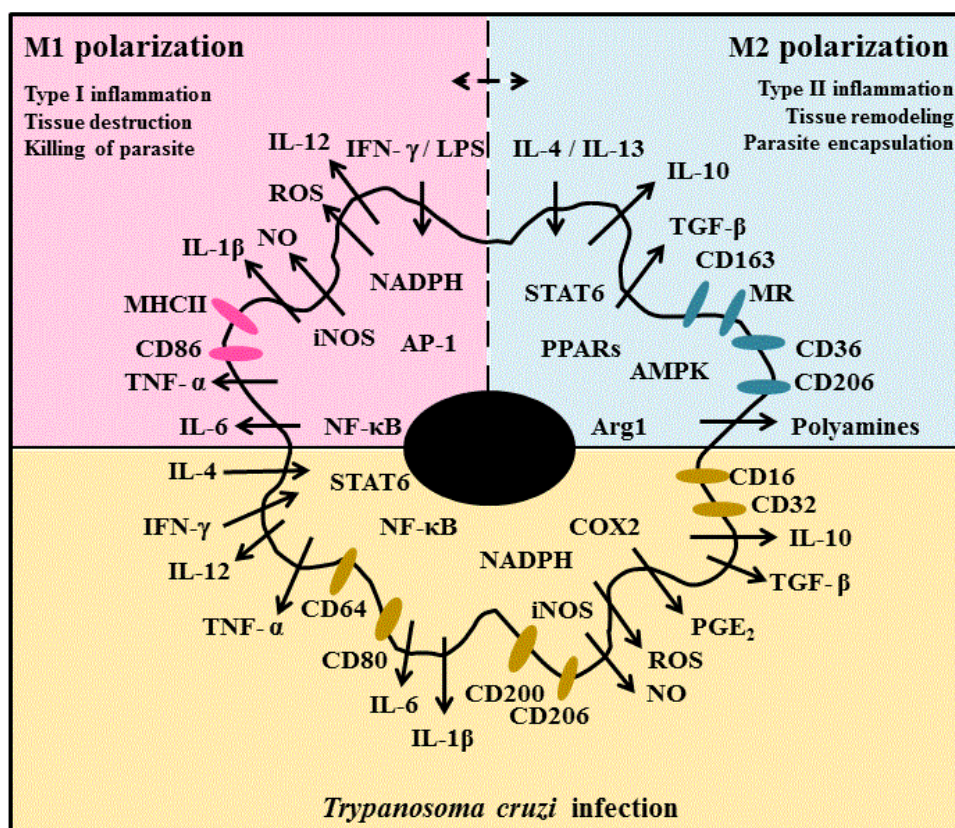

Figure 2: Macrophage polarization in the acute phase of *Trypanosoma cruzi* infection. M1 polarization is associated with Th1 cytokine secretion and increased expression of iNOS, NADPH, MHC-class II, CD86, AP-1, and NK-κB, in addition to that of inflammatory mediators such as TNF-α, IL-6, NO, ROS, IL-12, and IL-1β, resulting in tissue damage and parasite killing (Left panel). M2 macrophages are related to tissue remodeling, encapsulation of parasite, Th2 cytokine secretion, which improves the expression of CD36, CD163, MR (mannose receptor), AMPK, Arg1, PPARs, and STAT6, in addition to improving IL-10 and TGF-β expression and polyamine secretion (Right panel). *T. cruzi* macrophage infection increases the expression of CD64, CD80, NADPH, COX2, iNOS, NF-κB, STAT6, CD200, CD206, CD16, and CD32, as well as the secretion of TNF-α, IL-12, IL-1β, IL-6, NO, and ROS, which promotes parasite elimination, and secretion of IL-10, TGF-β, and PGE2, critical for immunoregulation and reduction of tissue damage resulting from excessive stimuli (Lower panel).

In fact, spleen or peritoneal macrophages harvested from mice in the acute phase of *T. cruzi* infection release large amounts of NO in the absence of any other stimuli and accumulate high levels of inducible nitric oxide synthase (iNOS) mRNA with secretion of TNF-α, IL-6 and IL-1-β in cultured macrophages [34,35]. This initial inflammatory response leads to M1 polarization.

The protective role of M1 macrophage has been exemplified in mice deficient for components of the IL-12 pathway [36]. Moreover, peritoneal macrophages from IL-12p40 gene knockout mice have a bias toward the M2 profile, spontaneously secreting large amounts of TGF-β and responding to rIFN-γ with weak NO production in *T. cruzi* infected peritoneal macrophages [37]. This host response is associated with the control of acute infection.

At the same time, macrophages and NK cells synthesize regulatory cytokines such as IL-10 and IL-4 to reduce the harmful effects associated with excessive stimulation of the immune system [38]. IL-4 in association with IL-10, modulates IFN-γ production and provides resistance against *T. cruzi* infection [39]. This new environmental change leads to M2 polarization. Thus, *T. cruzi* infection stimulates the activation of both M1 and M2 type macrophages during the early phases (Figure 2).

The persistence of inflammatory responses associated with tissue fibrosis and cell death is a hallmark of chronic Chagas disease. Macrophages present in chagasic hearts as well as those exposed *in vitro* to sera from *T. cruzi*-infected mice polarized toward a pro-inflammatory phenotype (CD64hi CD80hi) with extensive production of TNF-α/IFN-γ [40].

Interestingly, the multi-component DNA-prime/protein-boost vaccine (TcVac2) conferred protection against persistence of parasite and inflammation [41]. The vaccine induced a significant decline in the positive TNF-α/macrophage population in the heart and it was reflected by M2 polarization (CD200+, CD206+, CD16+, CD32+, IL-4, and IL-10 producing) upon *in vitro* incubation with sera from TcVac2-vaccinated infected mice [40].

PPARα and PPARγ ligands in M2 polarization

Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent nuclear transcription factors. PPARγ is a member of the nuclear hormone receptor family that has been implicated in inflammation and macrophage activation [42]. The importance of PPARγ in regulating the M1/M2 phenotypic switch has been confirmed by Amine Bouhlel and collaborators (2007). These authors demonstrated that activation of PPARγ potentiates the polarization of circulating monocytes into M2 macrophages [38]. 15-Deoxy-Δ12, 14 prostaglandin J2 81 (15dPGJ2), has high affinity for PPARγ and can exert its effects by either binding to PPARγ or through interaction with intracellular targets such as the NF-κB-signaling pathway or Erk MAP kinase cascade [38].

The role of PPARγ ligands in *T. cruzi* infection is poorly understood. Hovsepian and collaborators (2011) reported the first evidence that treatment with 15dPGJ2 increases the number of intracellular parasites and inhibits the expression and activity of different inflammatory enzymes such as NOS2, matrix metalloproteinase as well as the mRNA expression of pro-inflammatory cytokines (TNF-α and IL-6) in neonatal mouse cardiomyocytes after *T. cruzi* infection [43].

Different investigations using PPARα isoform ligands have shown a reduction in the symptoms of inflammation and disease in several models, including allergic airway disease, arthritis, and inflammatory bowel disease [38]. Penas et al. (2011) showed that treatment with either PPARα or PPARγ ligands drive M1-to-M2 polarization of macrophages in *T. cruzi*-infected mice [30].

Interestingly, PPAR agonists induce M1 macrophage polarization via cytosolic phospholipase A2 (cPLA2) and cyclooxygenase-2 (COX-2 inhibition, against *Leishmania mexicana* [44]. The potential use of PPAR agonists as modulators of overt inflammatory response during the course of Chagas disease can open a new avenue in the pharmacological approach to improve host health.

Manipulating macrophage metabolism is an attractive approach to controlling *T. cruzi* infection

When interacting with the host cell, *T. cruzi* stimulates the phosphorylation of several tyrosine residues, and this could be involved in the survival, multiplication, and pathogenicity of the parasite. Depending on the strain and developmental form of the parasite, distinct signaling pathways might be induced [42].

A recent study revealed for the first time the participation of mammalian target of mTOR in *T. cruzi* cell invasion [45]. Using an elegant study design, the authors demonstrated that the treatment of HeLa cells with the mTOR inhibitor, rapamycin, reduced lysosomal exocytosis and *T. cruzi* metacyclic trypomastigotes (MT) invasion. Downregulation of phosphatidylinositol 3-kinase and protein kinase C also impaired exocytosis and MT internalization. Thus, mammalian PI3/TOR kinase inhibitors can be a productive start point for anti-trypanosomatid drug discovery. The only problem was that *T. cruzi* was relatively insensitive to rapamycin inhibitor compared with some other trypanosomatid pathogens, such as *T. brucei* [43].

However, we know that the AMP-activated protein kinase (AMPK) regulates energy homeostasis and metabolic stress, acting like a cellular energy sensor. AMPK is activated by high AMP and low ATP via a complex mechanism, which involves allosteric regulation, promotion of phosphorylation, and inhibition of dephosphorylation. When AMP/ATP ratio is high, AMPK is activated, it protects the cell by switching off the ATP-consuming pathways (e.g. fatty acid synthesis and sterol synthesis) and switching on alternative pathways for ATP generation (e.g. fatty acid oxidation) [46]. AMPK activation in macrophages is associated with M2 polarization, which suppresses pro-inflammatory responses and promotes anti-inflammatory functional phenotype [30]. Interestingly, when macrophages were stimulated with IL-10 and TGF-β, it resulted in the rapid activation of AMPK, whereas stimulation of macrophages with LPS-induced cytokines resulted in AMPK inactivation. In addition, inhibition of AMPK increased the mRNA levels of TNF-α, IL-6, and COX-2 [30]. AMPK has also shown to inactivate the mammalian target of rapamycin (mTOR) pathway via phosphorylation and activation of the mTOR inhibitor, tuberous sclerosis complex-2 TSC2 [47].

AMPK and mTOR are critical regulators of host cell metabolism making them logical targets for manipulation by invading pathogens such as *T. cruzi*. It would be interesting to determine if *T. cruzi* induces AMPK to generate energy and nutrients for its growth in the host cell. We must remember that inhibiting AMPK or inducing mTOR can provide essential conditions for *T. cruzi* replication [48].

Conclusion

Control of the *T. cruzi* infections is critically dependent on cytokine-mediated macrophage activation leading to intracellular killing of the parasite. M1 polarization is closely linked to the elimination of parasites, and M2 polarization could be effective in preventing the progression of oxidative and inflammatory pathology in Chagas disease. AMPK and mTOR are rational targets for manipulation by invading pathogens such as *T. cruzi*. Manipulation of host metabolism using PPAR agonists seems an attractive approach to controlling *T. cruzi* infection as targeting the host rather than the pathogen can considerably reduce the ability of pathogens to develop drug resistance.

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