Advances in the Study of the Molecular Mechanisms of Macrophage Polarization in Mycobacterium Tuberculosis Infection

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Abstract: Mycobacterium tuberculosis (Mtb) infection-related tuberculosis is still a significant worldwide public health concern. The host immune response is insufficient to completely eliminate the pathogen, resulting in the formation of complex, dynamically evolving tuberculous granulomas, which are its main pathological feature. Granulomas serve as both a host defense structure limiting Mtb spread and a microenvironment for bacterial immune evasion and latent survival. In this process, macrophages, as the primary host cells for Mtb, play a crucial role, with the dynamic balance of their M1/M2 polarization determining the formation, maintenance, and outcome of granulomas. This article systematically reviews the multiple molecular mechanisms regulating macrophage polarization in tuberculous granulomas, focusing on the roles of host transcription factors, non-coding RNAs, metabolites, and Mtb effector proteins, aiming to provide new insights for host-guided therapy of tuberculosis.

Keywords: Mycobacterium Tuberculosis; Granuloma; Macrophage Polarization; M1/M2

1. Introduction

Mtb-induced early inflammatory responses drive granuloma formation by recruiting immune cells (such as macrophages, neutrophils, NK cells, DCs, B cells, T cells, etc.) to the site of infection. As Mtb's primary host cells, macrophages are essential for immune surveillance. Activated macrophages can differentiate into two distinct phenotypes: classical activation (M1) and alternative activation (M2) [1, 2]. M1 macrophages dominate macrophage polarization in the early stages of tuberculous granuloma formation, which greatly promotes granuloma formation as evidenced by an increase in granuloma-like structures, an increase in macrophage volume, and an effective decrease in Mtb load. But when the infection worsens, M1 macrophage-related marker expression levels gradually decline while M2 marker expression levels gradually rise, showing the opposite impact to M1 [3]. Consequently, non-granulomatous lung tissue exhibits M1 and M2 polarized macrophages, but M2 macrophages predominate in necrotizing and non-necrotizing granulomas as well as the late stage of tuberculous granuloma. Notably, M2 macrophage markers are more abundant in the internal region of tuberculous granuloma, suggesting that the M1/M2 phenotype balance may determine the bacterial control capacity of specific areas within the granuloma. To achieve optimal immune protection against Mtb, a delicate balance must be maintained between pro-inflammatory and anti-inflammatory monocytes and macrophages. The dynamic balance of macrophage polarization is finely regulated by multiple factors in the infection microenvironment, including host-derived factors (cytokines, transcription factors, non-coding RNAs and metabolites, etc.) and pathogen-related proteins. This signaling network ultimately determines the direction of macrophage differentiation toward the M1 or M2 phenotype by activating key pathways such as IRF/STAT and NF-κB.

2. The Regulatory Mechanism of Host Factors on Macrophage Polarization

2.1 The Key Role of Transcription Factors

At the host level, TLR-mediated microbial stimulation can activate transcription factors such as NF-κB and AP-1, upregulate pro-inflammatory cytokines such as IFN-γ and TNF-α, and promote M1 macrophage polarization, characterized by high iNOS expression. Meanwhile, cytokines such as IL-4 and IL-13 affect M2 polarization-related

molecules (mannose receptor, Ym1, Fizz1) by inducing STAT6 phosphorylation and upregulating arginase expression. Therefore, in the Th1 immune response, macrophages polarize towards the M1 type and release pro-inflammatory mediators [1, 2, 4]; in the Th2 immune response, macrophages polarize towards the M2 type and participate in inflammation resolution and tissue repair [1, 4].

In addition to classical transcription factors (STAT1, STAT5, NF-κB, IRF5, IRF3, HIF-1α promote M1 polarization; PPARγ, cMyc, IRF4, STAT6, STAT3, cMaf, HIF-2α promote M2 polarization), transcription factors such as BHLHE40 and ATF2 also play important regulatory roles [5, 6]. Hendrix et al. [6] discovered that the lack of Bhlhe40 resulted in an increase in anti-inflammatory chemicals and a decrease in pro-inflammatory signals in mouse bone marrow-derived myeloid cells grown in the presence of GM-CSF. Pro-inflammatory signals may be partially restored in Bhlhe40−/− myeloid cells when IL-10 is absent, suggesting that BHLHE40 stimulates pro-inflammatory responses via both IL-10-dependent and non-IL-10-dependent mechanisms [6]. Compared with wild-type infected mice, macrophages and neutrophils in the lungs of Bhlhe40−/− infected mice were defective in the production of inducible NO synthase, further supporting the role of BHLHE40 in promoting pro-inflammatory responses in innate immune cells [6]. Mechanistic studies have shown that BHLHE40 can promote HIF1α mRNA and protein expression through the LPS/TLR4/NF-κB pathway, drive macrophage metabolism to glycolysis, and support M1 polarization [5, 7, 8]. In summary, BHLHE40 promotes M1 polarization through the LPS/TLR4/NF-κB pathway on the one hand, and inhibits M2 polarization by inhibiting IL-10/IL-10R signaling on the other hand, thereby enhancing macrophage inflammatory responses. Rajabalee et al. showed in a genetic perturbation experiment using a THP-1 monocyte/macrophage model that high expression of ATF2 can induce monocytes to differentiate into M1-like macrophages. When stimulated with IFN-γ/LPS or Mtb, ATF2 upregulation can affect macrophage metabolic reprogramming and enhance control over intracellular bacterial replication [9]. Consistent with this, ATF2 overexpression can promote macrophage expression of inflammatory mediators.

2.2 Regulatory Role of Noncoding RNA

The function of circular RNA (circRNA) and long non-coding RNA (lncRNA) in controlling macrophage polarization in tuberculosis infection has garnered significant interest in recent years. Long noncoding RNAs (lncRNAs) are transcripts exceeding 200 nucleotides in length that do not encode proteins. Through a variety of mechanisms, including binding proteins, controlling gene expression, modifying epigenetics, and interacting with microRNA, it can perform regulatory activities [9]. Studies have demonstrated that LincRNA-Cox2 can promote macrophage polarization toward the pro-inflammatory M1 phenotype, which helps the host control bacterial growth [10]. Its mechanism involves the activation of NF-κB and STAT3 by LincRNA-Cox2, thereby limiting the survival of Mtb in human macrophages [10]. Among them, NF-κB mainly initiates the pro-inflammatory antibacterial response, while STAT3 helps to control inflammation, but overexpression weakens the antibacterial effect [11]. Referred to as the "anti-inflammatory STAT", STAT3 governs macrophage polarization once it is phosphorylated and activated through the JAK/STAT signaling cascade [11]. By interacting with miR125b-5p, which promotes the NF-κB pathway by suppressing A20 protein (NF-κB repressor), LncRNA XIST plays a significant role in Mtb infection in mouse and human macrophages. Knockdown of lncRNA XIST in RAW 264.7 cells and cultured human monocyte–derived macrophages substantially lowered Mtb survival; conversely, overexpression enhanced Mtb persistence. Therefore, LncRNA XIST may support M2 polarization through the miR-125b-5p/NF-κB axis, which is beneficial to bacterial survival. CircRNAs are a class of single-stranded closed circular RNAs that do not contain a 5' cap structure and a 3' poly(A) structure, and participate in a variety of pathophysiological processes. A substantial body of evidence suggests that circRNAs can act as competing endogenous RNAs, functioning as miRNA sponges to block the activity of target miRNAs [12]. CircRNAs are highly durable and resistant to RNase digestion in interstitial fluid, peripheral blood, and saliva because of their

covalently closed circular structure. As a result, they may be used as molecular markers for the diagnosis and prognosis of a number of disorders. CircTRAPPC6B, which is downregulated in tuberculosis patients, enhances IL-6 and IL-1β production by targeting miR-892c-3p, thereby driving macrophage repolarization from an M2-like to an M1-like phenotype [13]. By downregulating miR-224-5p, miR-324-5p, and miR-488-5p while upregulating CTLA4 expression, circRNA-0003528 (which is upregulated in tuberculosis patients) may promote the polarization of macrophages from the M1 to M2 phenotype. However, current research on non-coding RNAs is mostly focused on unidirectional regulation, and their interaction with other cell types in the complex microenvironment of granulomas has not yet been elucidated. Existing evidence is mostly derived from in vitro models, and there is an urgent need to verify the expression of non-coding RNAs and their regulatory role in immune microenvironment homeostasis in vivo. In the future, it is necessary to explore the feasibility of non-coding RNAs as diagnostic markers or therapeutic targets and to analyze their relationship with bacterial immune escape processes.

2.3 Regulatory Function of Metabolites

Additionally, host metabolites play a role in controlling macrophage polarization. Anti-inflammatory M2 polarization is known to be facilitated by PPARγ activation. In studying the role of cytochrome P450 (CYP) in M1 and M2 macrophages, Hee Young Cho et al. [14] demonstrated that CYP2C19 is predominantly expressed in M2 macrophages, where its intrinsic activity plays a crucial role in promoting M2 polarization during monocyte/macrophage differentiation. This occurs via activation of PPARγ signaling through the supply of the agonists 11,12-EET and 14,15-EET. This work demonstrated CYP2C19's regulatory function in M1/M2 differentiation, indicating that it might function as an immunological marker of M2 polarization. Furthermore, prior research has demonstrated that the bioactive lipid mediator sphingosine-1phosphate (S1P) influences macrophage polarization [15]. Recent studies have shown that S1PR2 and S1PR3 analogs significantly enhance host antibacterial capabilities by inducing macrophage polarization toward the M1 phenotype, while S1PR1 analogs fail to inhibit infection due to their inability to effectively activate the M1 effector mechanism [14, 16]. Therefore, S1PR2/3 analogs offer a novel strategy for antibacterial therapy targeting host immunity. Future research should further clarify the M1 polarization phenotype induced by S1PR2/3 analogs and validate their efficacy and safety as host-directed therapy through in vivo experiments.

3. Regulatory Mechanisms of Macrophage Polarization by Mycobacterium Tuberculosis Effector Proteins

Mycobacterium tuberculosis regulates host macrophage polarization by secreting a variety of effector proteins, thereby driving the formation of granulomas. ESAT-6 exhibits a significant phase-dependent regulation of macrophage polarization and apoptosis, which may be related to its key role in the dynamic homeostasis of granulomas. In the early stage of infection (within 24 h), ESAT-6 activates the TLR4/MyD88/NF-κB pathway by upregulating the expression of TLR4, MyD88 and NF-κB proteins, inducing THP-1 cells to polarize to the M1 type, accompanied by an increase in M1 macrophage apoptosis [17]. However, with the prolongation of the treatment time (30-42 h), the activity of this signaling pathway is significantly reduced, the M1 polarization effect is inhibited, while the M2 polarization effect continues to be enhanced, forming an immunophenotype dominated by the M2 type [17]. Further mechanistic studies have shown that the regulation of M1/M2 polarization and apoptosis by ESAT-6 depends on the dynamic activation and inactivation of the TLR4/MyD88/NF-κB pathway [17]. This time-dependent regulatory pattern may reveal that early M1 polarization briefly activates the inflammatory response to recruit immune cells to form granulomatous structures; subsequently, M2 polarization dominance and M1 cell apoptosis inhibit excessive inflammation and maintain an anti-inflammatory microenvironment, which ultimately facilitates Mycobacterium tuberculosis's immune escape and chronic persistent infection.

The secretory antigen complex Ag85B induces macrophage M1 polarization via the TLR4/TRAF6/NF-κB signaling pathway, contributing to bronchial epithelial cell damage

and an imbalance between TH17 and Treg cells[18]. Adhesion factor HBHA may inhibit macrophage autophagy and induce M2 polarization through the TLR4-dependent PI3K-AKT-mTOR signaling pathway, weakening antibacterial immune responses [19]. Latency-associated protein HSP16.3 activates the downstream AKT/ERK/p38-MAPK signaling cascade by binding to macrophage surface chemokine receptors CCRL2 and CX3CR1, inducing macrophages to convert to an anti-inflammatory M2 phenotype, thereby facilitating bacterial immune escape. Rv1987 (antigen MPT64) induces macrophage M2 polarization by activating the PI3K/Akt1/mTOR signaling pathway. Mycobacterium tuberculosis PPE36 (Rv2108) inhibits M1 polarization by suppressing the ERK signaling pathway, reducing the secretion of pro-inflammatory and chemokine factors, suppressing cytokine storms, and alleviating inflammatory damage to immune organs, thus providing a microenvironmental support for bacterial immune escape. In summary, host factors and bacterial factors regulate macrophage polarization through multiple signaling pathways, and their regulatory effects and biological outcomes are summarized in Table 1.

Table 1. Regulation of Macrophage Polarization and Biological Outcomes by Host Factors and Bacterial Factors

Influencing factors	Effect on polarization	Biological outcome
ESAT-6	Supports M2 polarization and M1 to M2 reprogramming	Decreased expression of pro-inflammatory factors
Ag85B	Promotes macrophage polarization toward the M1 phenotype by engaging the TLR4/TRAF6/NF-κB axis.	Damage to bronchial epithelial cells and dysregulation of the TH17/Treg cell equilibrium
HBHA	TLR4 induces M2 polarization and drives M1 macrophages to transform into the M2 phenotype.	Decreased expression of pro-inflammatory factors
Rv1987 protein	Inducing M2 polarization of macrophages by activating the PI3K/Akt1/mTOR signaling pathway	The bactericidal ability is significantly reduced, and the survival rate of bacteria within macrophages increases.
PPE36(Rv2108)	Suppressing M1 polarization by inhibiting the ERK signaling pathway	Decreased expression of pro-inflammatory factors
HSP16.3	M2 polarization induced by CCRL2/CX3CR1	Increased expression of anti-inflammatory factors
BHLHE40	Supports M1 polarization	Increased expression of pro-inflammatory factors
ATF2	Supports M1 polarization and M2 to M1 reprogramming	Increased expression of pro-inflammatory factors
LincRNA-Cox2	Promote M1 polarization	Increased expression of pro-inflammatory factors
LncRNA XIST	Supporting M2 polarization by activating the NF-κB pathway	The bactericidal ability is significantly reduced, and the survival rate of bacteria within macrophages increases.
circTRAPPC6B	Targeting miR-892c-3p enhances the expression of IL-6 and IL-1β and reprograms mycobacterium-induced macrophages from M2 to M1.	Increased expression of pro-inflammatory factors
circRNA-000352	Upregulation of CTLA4, along with downregulation of miR-224-5p, miR-324-5p, and miR-488-5p, facilitated M2 polarization.	Decreased expression of pro-inflammatory factors
CYP2C19	PPARγ is activated by the production of 11,12-EET and 14,15-EET, which in turn promotes M2 polarization.	Increased expression of anti-inflammatory factors
S1PR2-3 analogues	Promote M1 polarization	Decreased expression of anti-inflammatory factors

4. Conclusion

Macrophage M1/M2 polarization balance is subject to complex regulation by both host and pathogen factors, and is crucial in determining the fate of tuberculous granulomas. Current research indicates that transcription factors BHLHE40 and ATF2, as well as non-coding

RNAs such as LincRNA-Cox2 and XIST, participate in polarization by regulating pathways such as IRF/STAT and NF-κB; while bacterial effector proteins such as ESAT-6 and Ag85B dynamically regulate host immunity in a time-dependent manner.

However, current research still has the following limitations: First, most evidence comes from in vitro models, and the intercellular dialogue and in vivo dynamic evolution of polarization regulation in the complex microenvironment of granulomas remain unclear; second, the cross-regulatory pathways between non-coding RNAs and metabolites need further analysis; third, the spatiotemporal expression patterns of bacterial effector proteins and their synergistic mechanisms with host factors still need to be explored. Future research should focus on multi-factor synergistic regulatory networks and the translational application of host-guided therapies.

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