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Macrophage Polarization and Allergic Asthma

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Abstract

Allergic asthma is associated with airway inflammation and airway hyper-responsiveness. Macrophage polarization has been shown to have a profound impact on asthma pathogenesis. Upon exposure to local micro-environments, recruited macrophages can be polarized into either classically activated (or M1) or alternatively activated (or M2) phenotypes. Macrophage polarization has been heavily associated with development of asthma. The process of regulation of macrophage polarization involves an intricate interplay between various cytokines, chemokines, transcriptional factors, and immune-regulatory cells. Different signals from the microenvironment are controlled by different receptors on the macrophages to initiate various macrophage polarization pathways. Most importantly, there is an increased attention on the epigenetic changes (e.g., microRNAs, DNA methylation and histone modification) that impact macrophage functional responses and M1/M2 polarization through modulating cellular signaling and signature gene expression. Thus, modulation of macrophage phenotypes through molecular intervention by targeting some of those potential macrophage regulators may have therapeutic potential in the treatment of allergic asthma and other allergic diseases. In this review, we will discuss the origin of macrophages, characterization of macrophages, macrophage polarization in asthma, and the underlying mechanisms regarding allergen-induced macrophage polarization with emphasis on the regulation of epigenetics, which will provide new insights into the therapeutic strategy for asthma.

INTRODUCTION

Allergic disease prevalence has increased significantly in recent decades[1]. The majority of school age children and adults with asthma have concomitant allergic sensitization, which has been associated with asthma inception and severity[2]. In the United States, children living

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in inner-city households are exposed to higher levels of certain allergens (e.g., cockroach, house dust mite, and mouse) than those living in suburban homes[3–5]. The indoor environmental allergens are of particular interest in the study of determinants of allergic diseases because of constant exposure during early childhood and the potential for intervention[6]. However, the causal relationship between individual allergen exposure and the development of these conditions is currently not well-established. This may be due primarily to the complexity of interactions between various environmental factors, the timing and dose of exposure, and innate immune cells.

Macrophages are the most abundant immune cells in the lung (approximately 70% of the immune cells)[7] and play an important role in environmental allergen-induced airway inflammation in asthma[8,9]. Although it is still unclear as to the origin of lung macrophages, recent studies suggest that these macrophages developed from either differentiation of blood monocytes or the proliferation of resident macrophages[10]. Furthermore, macrophages are an extremely heterogeneous population, displaying a combination of inflammatory and anti-inflammatory functions[11]. The two extremes in the spectrum of macrophage function are represented by the classically activated (or M1) and the alternatively activated (or M2) phenotypes[12]. M1 macrophages, induced by IFN-γ and lipopolysaccharide (LPS), upregulate the expression of genes involved with the clearance of pathogens and drive inflammation in response to intracellular pathogens. In contrast, M2 macrophages, induced by interleukin (IL)-4 and IL-13, up-regulate the expression of genes involved with wound healing, clearance of dead and dying cells and tissues, and are involved in anti-inflammatory responses[13–15]. These two macrophage states mirror the Th1-Th2 polarization of T cells[10,14,16,17]. Increased M2 macrophage polarization and activation was observed in asthma, which has been suggested to play an important role in allergic asthma[18,19]. Therefore, a better understanding of the molecular mechanisms regulating macrophage polarization is essential to understand the causal relationship between allergen exposure and development of allergic diseases like asthma.

Numerous studies have summarized the regulation of macrophage polarization by various cytokines, chemokines and transcriptional factors[20–25]. In this review, we will mainly discuss the advanced concepts of epigenetic changes involved with the macrophage polarization, including miRNAs, DNA methylation and histone modification, which may regulate macrophage polarization through modulating cellular signaling and signature gene expression. These studies on the modulation of macrophage polarization are critical to better understand the macrophage-mediated clearance of pathogens and innate immunity, and in developing treatment for asthma.

ORIGIN OF MACROPHAGES

Innate immunity comprises a number of components including the skin, enzymes, phagocytes and even the micro-biome[26]. Among the phagocytes, macrophages are the major effector cells of the innate immunity.

History of macrophages

Macrophage was first described by Elie Metchnikoff in 1893. In his experiments, 'phagocytes' attacking and engulfing the microbes were observed in starfish challenged by a rose thorn[27]. He classified these phagocytes into macrophages ("the big eaters") and microphages ("the small eaters", now better known as neutrophils). This led to development of theory of phagocytosis[27]. The next landmark came in 1924 when Aschoff defined macrophages as a part of the reticulo-endothelial system (RES)[28]. RES was defined as a network of phagocytic cells (reticulum) in various organs which was present in the vicinity of vascular endothelium (endothelial). It was postulated that macrophages originated from the RES and resided within the parent tissue. This finding was challenged in 1968 when Van Furth *et al* formulated the mononuclear phagocyte system, according to which all macrophages were derived from terminal differentiation of circulating monocytes[29]. The blood monocyte derivation of macrophages was confirmed by others in different parts of the world at that time[30–32]. This theory held true until recently when the dual origin of tissue macrophages was identified: macrophages developed from differentiation of circulating monocytes and are derivatives of primitive macrophages which were seen in embryonal yolk sac and fetal liver[33]. Seeding of tissues with these primitive macrophages occurred before birth in the embryonal period where they proliferated to form the resident tissue macrophage population.

Lung macrophages

Lung macrophages include a heterogeneous population of mononuclear phagocytes which are classified into two main categories: alveolar macrophages and interstitial macrophages[34–36]. Alveolar macrophages consist of airway macrophages and macrophages which truly reside in the alveoli; both these populations of cells are thought to arise from similar progenitor cells[7]. Alveolar macrophages reside on the epithelial surface of the lung, and in contrast to other resident macrophages, they are in direct contact with the environment (e.g., allergens, particulate matter, and commensal bacteria)[10]. Embryonically derived fetal monocytes appear to colonize the lung shortly after birth and differentiate into alveolar macrophages[37]. Alveolar macrophages normally live independently of blood monocyte input. However, if alveolar macrophages are damaged or depleted, monocytes recruited from the circulation become the dominant source of new macrophages[38,39] and contribute to re-populate the alveolar macrophage niche, where both tissue-resident alveolar macrophages (TR-AMs) and monocyte-derived alveolar macrophages (Mo-AMs) contribute to repopulation (Figure 1)[40]. Interestingly, using a novel lineage tracing system in mice to identify TR-AMs and Mo-AMs during the development of fibrosis and over the subsequent life span of the animal, Misharin *et al* showed that Mo-AMs drive lung fibrosis and persist in the lung over the life span[41]. These results suggest heterogeneity in alveolar macrophage function during fibrosis with important implications for the design of targeted therapy. In contrast, interstitial macrophages are the macrophages which reside in the interstitium and are less well studied when compared to alveolar macrophages. Recent studies have challenged the current paradigm that interstitial macrophages (IMs) are one uniform cell population[42]. Three subpopulations were identified, including IM1 ($MHCII^{lo}CD206^{high}$), IM2 ($MHCII^{+}CD206^{high}$), and IM3 ($MHCII^{high}CD206^{lo}CCR2^{+}$). Limited studies suggest

that the major function of interstitial macrophages is to maintain immune homeostasis in the respiratory tract and induce immune tolerance to benign antigens[43].

Functionally, alveolar macrophages are the major effector cells of immune responses and take part in pro and anti-inflammatory functions. In allergic asthma, after allergen exposure, there is rapid recruitment of monocytes and increase in Mo-AMs that promote acute inflammatory responses, while TR-AMs proliferate locally and suppress allergic inflammation (Figure 1)[9,44,45]. It was suggested that these Mo-AMs fight the perceived danger signals from allergen challenge by promoting an inflammatory responses and subsequent expansion of suppressive TR-AMs to restore homeostasis. While it is still as yet unclear about the polarization of either TR-AMs or Mo-AMs and their influence on asthma phenotypes, the newly established lineage tracing system will provide a unique opportunity to trace macrophage polarization and determine their function in asthma in the future.

MACROPHAGE POLARIZATION IN ASTHMA

Macrophage polarization is defined as a dynamic process by which a macrophage expresses different functional phenotypes in response to different micro-environmental signals[25]. Upon exposure to pathogens, the microenvironment in the tissue is altered which leads to the polarization of macrophages. As summarized in Figure 2, we hypothesize that allergen exposure (i.e. cockroach allergen) activates lung epithelial cells and innate immune cells to produce a variety of cytokines, which not only recruit blood monocytes to lung tissues but also direct primary macrophage polarization into different subtypes with different roles in asthma. These macrophage subtypes differ in their stimulators, cell surface markers, and cytokine/chemokine secretions.

Classically (M1) activated macrophages and asthma

LPS, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are potent stimulators for the polarization of macrophages into the M1 phenotype[25,46]. Recent studies suggest that oxidized low density lipoprotein (ox-LDL)[47], high-mobility group box 1 (HMGB1) protein[48], and caveolin-1 (Cav-1) are also involved in M1 macrophage polarization[49]. Phenotypically, M1 macrophages highly express CD80, CD86, MHCII, TLR4, and iNOS, and produce high levels of pro-inflammatory Th1 cytokines (e.g., IL-6, IL-12, IL-1β, and TNF-α) and chemokines (e.g., CCL2, CCL5), which play a predominant role in the clearance of intracellular pathogens and recruitment and activation of T and B cells. Macrophages are increased in allergic and non-allergic asthma. In light of macrophage polarization, attempts were made to identify a predominant phenotype in either allergic or non-allergic asthma. A very interesting study was performed by Robbe *et al.* to identify the M1 or M2 cells in two distinct mouse models: house dust mite (HDM) induced allergic mouse model and farm dust extract (FDE) induced non-allergic mouse model[50]. Intriguingly, the FDE model showed M1 polarization with increased expression of Th1 and Th17 cells, whereas the HDM model showed M2 polarization with a predominant Th2 cell response. This raises the possibility that M1 cells are the major effector macrophages in non-allergic asthma whereas M2 cells predominate in allergic asthma. Furthermore, M1

macrophages have been linked with the pathophysiology of severe asthma, particularly for those with a poor response to systemic corticosteroids[51].

Alternatively (M2) activated macrophages and asthma

In contrast to M1 macrophages that are activated by IFN-γ and LPS, M2 macrophages are induced by IL-4, IL-13 or IL-10. Further studies demonstrated that M2-polarized macrophages can be further divided into three subpopulations, M2a, M2b, and M2c, according to specific stimulators (Figure 2)[17,52]. The M2a subtype is induced by IL-4, IL-13, fungal and helminthic infections. M2b is elicited by IL-1 receptor ligands, immune complexes and LPS, whereas M2c is stimulated by IL- 10, TGFβ1 and glucocorticoids. Similarly, there is no unique surface marker for the identification of M2 macrophages. Phenotypically, they are characterized by high expression of MRC1, CD163, Arg-1 and low expression of iNOS, MHCII and CD86[53,54]. Recent studies found that both histamine receptor H1 (HRH1) and E-cadherin, highly expressed on M2 macrophages both *in vitro* and in bronchoalveolar lavage fluid (BALF) of asthmatic patients, may be additional markers for M2 macrophages[19]. M2 macrophages, in general, also express Ym1/2 (chitinase like protein) and FIZZ1 (resistin like molecule alpha)[55,56]. Characteristically, M2a cells secrete high levels of IL-13 and chemokines, including CCL-17, CCL-18, CCL-22 and CCL-24, which activate Th2 cells and promote eosinophil infiltration into the lungs[57,58]. M2c cells have greater expression of IL-10, an anti-inflammatory cytokine, but lower expression of NF-κβ and co-stimulator molecules including CD40, CD86, and HLA-DR[59].

Given that both IL-4 and IL-13 are major inducers of M2 macrophage polarization, M2 cells are suggested to be the major macrophages in allergic asthma. Furthermore, IL-33 has been recently suggested to be a potent inducer of M2 macrophages[60–62]. In particular, IL-33 released from airway epithelial cells after antigen challenge can modulate M2 macrophage polarization through ST2[60]. Intriguingly, ST2 also contains binding sites for the other cytokine IL-4, IL-5, and IL-13 and the chemokine CCL-17, CCL-18 and CCL-24[61,62]. In addition, eosinophils, innate lymphoid type 2 (ILC2), $CD4^+CD25^+$ regulatory (Treg) cells and mesenchymal stem cells (MSCs) were recently reported to be major regulatory cells in driving the polarization of M2 macrophages[63–67]. Recently, Girodet and colleagues provided evidence to support a direct contribution of M2 macrophages in allergic airway responses and the pathogenesis of asthma[19]. Particularly, they found that M2 macrophages were significantly increased with higher expression of both MRC1 and MHC-II in BALFs from patients with asthma when compared with healthy control subjects (>2.9 fold). These data point to a potentially important role for M2 macrophages in asthma, and pharmacologic interventions that target M2 development and function might be a promising approach in asthma therapy and synergistic with current asthma therapies.

Although the current M1/M2 paradigm with its polarized extremes is oversimplification, this *in vitro* construction provides a useful guide for studying microphage biology *in vivo*. Moreover, both M1 and M2 macrophages do exist *in vivo*. However, there are obvious limitations, e.g., macrophages placed into culture may no longer resemble these which exist *in vivo*, existence of an intermediate stage of macrophages that bridges the M1/M2 extremes. Recently, several other methods were suggested to delineate macrophage

phenotypes. For example, naming macrophages according to the stimuli they encounter [e.g., M(IL-4), M(LPS)], according to what they do in their natural habitats (e.g., pruning macrophages, thermos-regulating macrophages), or according to their function which similar to Th1, Th2 or Th17. However, all of these descriptive systems have their limitations. In addition, we wish to emphasize that polarized M1/M2 macrophages act as antigen presenting cells that efficiently activate Th1, Th2, Th17, or Treg cells[23]. M1 macrophages cause activation of Th1 cells via production of TNFα and IL-12 mediated through CD86 and MHCII markers, leading to activation of non-allergic inflammation. Similarly, M2a cells activate Th2 cells via IL-4 and IL-13 production mediated by CCL17 and MRC1, leading to development of allergic asthma. M2b cells activate Treg cells via IL-10 and TGFβ production mediated by CCL24 and MRC1, leading to development of allergic tolerance and deceased inflammation.

REGULATION OF MACROPHAGE POLARIZATION

The process of regulation of macrophage polarization is a complex interplay between various cytokines, chemokines, and signaling molecules (Figure 3). Studies have revealed that different signals from the microenvironment are controlled by different receptors on the macrophages to initiate various macrophage polarization pathways.

Polarization of macrophages to M1 phenotype

The LPS/TLR4 pathway has been considered as one of the major pathways in M1 macrophage polarization[68]. For example, The LPS/TLR4 pathway activates NF-κB, interferon regulatory factor 3 (IRF3) and promotes the secretion of pro-inflammatory cytokines (e.g., IL-6, TNFα, and iNOS), inducing M1 polarization[69]. This was supported by studies showing that the LPS/TLR4 pathway induced M1 polarization by activating STAT1 in a MyD88 independent fashion[70]. Additionally, IRF5 is also critical for M1 polarization and the induction of IL-12, IL-23 and TNF[71]. A role for Bruton's tyrosine kinase (Btk) was implicated in macrophage polarization in response to LPS stimulation[72]. Specifically, absence of Btk was shown to skew macrophages towards the M2 phenotype. Several other molecules are also involved, including P2Y(2)R, G-protein coupled receptor, which plays a role in inducing the production of nitric oxide (NO) via iNOS[73], SOCS3, which activates NF-κB/PI-3 kinase pathways to produce NO[74], and Activin A, which down-regulates IL-10[75].

Polarization of macrophages to M2 phenotype

IL-4 and IL-13 skew macrophages toward the M2 activation state via STAT6[76]. Macrophage M2 phenotype is also promoted by several transcription factors, including IRF4, peroxisome proliferator activated receptor gamma (PPARγ)[77–79], and Krueppel-like factor 4 (KLF-4)[80]. It has been suggested that STAT6, PPARγ, KLF-4, and IRF4 may coordinate the M2 polarization of macrophages. MRC1 has been suggested to mediate allergic sensitization and asthma to allergens, including cockroach allergens[81,82]. We found that MRC1 on macrophages tends to polarize macrophages towards M2 phenotype[83].

EPIGENETIC REGULATION OF MACROPHAGE POLARIZATION

It is becoming increasingly clear that epigenetic mechanisms have a significant role in regulating macrophage activation and polarization in response to local environmental signal[84,85]. Studies have focused on the role of epigenetic changes in macrophage polarization that regulates immune responses and inflammatory gene expression in allergy and asthma. Epigenetic changes can occur in response to environmental stimuli, which may impact disease states[86]. Three main epigenetic mechanisms have been identified, including miRNAs, DNA methylation and histone modification, which may contribute to the altered cellular signaling and signature gene expression during M1 and M2 polarization.

miRNAs

miRNAs are a class of small and non-coding RNAs that regulate gene-expression programs by reducing the translation and stability of target mRNAs, thus regulating gene expression and cell function[87–89]. miRNAs have emerged as regulators of phagocyte activation[90,91], Th2 polarization[88,89], and pathogenic airway inflammation[89,92–94]. While miRNA-mediated macrophage polarization is a highly conserved process[95], recent studies suggest that miRNAs may have the ability to control the balance of M1 and M2 macrophage polarization and skew immune responses[96–98] through regulating different transcriptional factors[99,100]. Here, we will specifically review several of the most intensively studied immune system-related functional miRNAs that have been shown to play important roles in macrophage polarization and in the development of allergic diseases and asthma. (Table 1).

miR-155—Extensive studies have linked miR-155 to the development of allergic asthma[101–106]. Very recent studies suggested that miR-155 is a critical regulator of type 2 innate lymphoid cells (ILC2)[104] and a novel target in allergic asthma[107]. Interestingly, studies have shown that miR-155 enhanced COX-2 expression in asthmatic human airway smooth muscle cells[108]. Most interestingly, miR-155 has been shown to stimulate M1 macrophage polarization and inhibit M2 macrophage polarization[109]. For example, miR-155 inhibits STAT6 by targeting IL-13Rα1[97] and suppresses TGFβ/Smad signaling pathway by inhibiting Smad2[110]. Studies also showed that miR-155 enhances M1 macrophage polarization by the repression of negative regulators of pro-inflammatory responses including SOCS1[111], SHIP1[112], and BCL6[113]. miR-155 promoted M1 macrophage polarization through the expression of BCL6, a negative transcription factor of the pro-inflammatory NF-kB pathway. Akt kinases differentially contribute to macrophage polarization, with Akt1 ablation giving rise to an M1 and Akt2 ablation resulting in an M2 phenotype. miR-155 was found to be essential in Akt isoform-dependent M1/M2 polarization of macrophages by targeting C/EBPβ[114]. However, it was also noted that miR-155 may act as either “pro-inflammatory” or “anti-inflammatory” miRNA by controlling the balance of M1 and M2 macrophages in some diseases[84,115].

miR-146—miR-146a has also been linked to asthma and allergy[116,117]. miR-146 has two isoforms, miR-146a and miR-146b. miR-146a is highly expressed in M2 macrophages and plays a pivotal role in macrophage polarization. It was revealed that miR-146a modulated macrophage polarization by targeting Notch1 and PPARγ, which are highly expressed in M2

macrophages[118]. Furthermore, miR-146a acts as a negative regulator of classical NF-kB, IRAK, and TRAF6, which promotes M2 macrophage polarization and suppresses pro-inflammatory cytokine production[119–121]. In contrast to miR-146a, miR-146b is highly expressed in M1 macrophages and modulates the shaping of macrophage phenotypes. Mechanistically, miR-146b suppresses the M1 macrophage signature genes by targeting IRF5[122]. Furthermore, miR-146b modulates the TLR4 signaling pathway by direct targeting of multiple elements, including TLR4, MyD88, IRAK-1, and TRAF6[123]. Most importantly, these studies suggest that miR-146b may be a molecular effector of IL-10 anti-inflammatory activity.

Let-7c—Let-7c has been reported to promote M2 phenotype through targeting p21-activated kinase 1 (PAK1) that is up-regulated in M1 polarized macrophages[124]. Banerjee *et al* found that let – 7c was highly expressed in M2 macrophages, and over-expression of let-7c promoted M2 macrophage but suppressed M1 polarization via targeting C/EBPδ[125]. Let-7c levels are also greater in alveolar macrophages from fibrotic lungs as compared with those from normal lungs.

miR-21—miR-21 mediates homeostatic M2 macrophage polarization via CSF-1R signaling. Especially, the CSF-1R signaling suppresses the M1 inflammatory phenotype and promotes the M2 polarization by targeting pro-inflammatory molecules Erk1/2 and NF-κβ[126]. Furthermore, miR-21 has been shown to inhibit the pro-inflammatory tumor suppressor, PTEN and programmed cell death protein-4 (PDCD4) which favor pro-inflammatory response and enable M2 polarization of macrophages[91]. The regulation of LPS-TLR4 signaling by miR-21 increases IL-10 production which inhibits the transcriptional elongation of the TNFα gene[127]. The increased IL-10 promotes the activation of JAKs, STAT3 phosphorylation and subsequently favors M2 macrophage polarization. Interestingly, miR-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression[96].

miR-511-3p—miR-511-3p, the active strand of miR-511, is an intronic miRNA encoded by both mouse and human *MRC1* genes. miR-511-3p is transcriptionally co-regulated with *Mrc1* in macrophages and regulates the activation of macrophages[128,129]. Recent studies suggest that miR-511-3p controls macrophage-mediated microbial responses and enhances intestinal inflammation[130]. Studies from our research group have demonstrated that MRC1 tends to polarize macrophages towards M2 phenotypes[83]. In agreement with these observations, miR-511-3p over-expression using miR-511-3p mimic suppressed M1 with reduced expression of *IL-1β, IL-6*, and i*NOS* and promoted M2 macrophage polarization with enhanced expression of *Fizz1, Ym1*, and *Arg1*. Mechanistically, our studies suggest that the miR-511-3p-modulated macrophage polarization may be through an interaction with PTGDS (prostaglandin D2 synthase). Other potential targets of miR-511-3p include ROCK2, LTBP1, CCL2 as direct targets, and TLR4 and C/EBPα as indirect targets (Figure 4). These findings imply that miR-511-3p has an effect on macrophage polarization that may control allergen-induced inflammatory responses.

DNA methylation

DNA methylation is one of the major epigenetic regulatory systems and is generally linked with transcriptional silencing. DNA methylation occurs via DNA methyltransferases (DNMT) which adds methyl groups to 5′CpG dinycleotides leading to condensation of chromatin and prevention of transcription[131]. DNA methylation has been associated with asthma since altered DNA methylation status may cause differential gene expression of cytokines (e.g., IL-4, IFN-γ)[132] and transcription factor (e.g., FOXP3)[133]. Early studies in human cohorts have demonstrated an association of DNA methylation in a few candidate genes with asthma phenotypes[134,135]. A study in African American inner-city children identified 81 differentially methylated regions (DMRs) in PBMCs associated with allergic asthma[136]. Several immune genes were hypomethylated in those asthmatic patients, including IL13, RUNX3, and TIGIT. However, these studies did not provide evidence for the role of DNA methylation in the control of gene expression and macrophage polarization[137]. Intriguingly, recent studies suggest a significant association for DNA methylation and differential expression of M1/M2 genes[138]. DNMT1, 3a and b are differentially expressed in M1 and M2 macrophages and play a critical role in gene silencing[139]. Moreover, DNMT3b knockdown promoted macrophage polarization to M2 phenotype and suppressed macrophage inflammation, whereas overexpressing DNMT3b did the opposite[138]. Mechanistic studies suggest that DNMT3b regulates macrophage polarization through binding to the methylation region of PPARγ1, a key transcriptional factor that regulates macrophage polarization. Furthermore, treatment with DNMT inhibitor 5-Aza 2 deoxycytidine (Aza) promotes an anti-inflammatory M2 macrophage phenotype and attenuation of acute lung injury[140]. These findings indicate that DNMT3b is crucial in regulating macrophage polarization through epigenetic mechanisms. So far, although there is no study on DNMT3b in asthma, we postulate that DNMT3b may be one of the major genes that are involved in regulating macrophage polarization and lung inflammation in asthma. In addition, several other asthma-associated genes were reported to be involved in controlling macrophage polarization by the regulation of promoter methylation (e.g., galectin-3[141], BMP-2[142]). Thus, it would be of interest to identify these genes, which may provide a novel strategy for the prevention and therapy of allergic asthma.

Histone modification

Histone modification refers to the process in which histones may undergo divergent epigenetic changes, including methylation, acetylation, phosphorylation, ubiquitylation and SUMOylation, which could function as epigenetic markers of chromatin state linked with either transcriptional activation or repression[84,85]. Distinct histone-modifying enzymes have been identified that control macrophage activation and polarization to M1 or M2 phenotypes (e.g., methyltransferases, demethylases, acetyltransferases and deacetylases)[139,117]. Several M2 marker genes, e.g., Ym1 and arginase 1, are epigenetically regulated by reciprocal changes in H3K27 methylation. Under M2 condition, the expression of Jmjd3, a H3K27 demethylase, is increased, leading to a decreased methylation of H3K27 in the promoter of M2 marker genes[143]. Aryl hydrocarbon receptor (AhR)-mediated polarization has been associated with the development and resolution of several diseases such as bacterial and parasitic infections[144]. Most intriguingly, a recent study by Liao *et al.* suggests an existence of a new regulatory epigenetic synergism between AhR and IL-4 in macrophage

polarization[145]. Jmjd3 expression was increased in M2 macrophages, which could be inhibited by IL-4 neutralizing antibody[145]. Importantly, GSKJ4, a selective and potent Jmjd3 inhibitor, blocked the binding of AhR to the promoter of CCL1, leading to a decreased expression of CCL1. Jmjd3 was also shown to be crucial for M2 macrophage polarization and host responses against helminth infection[142]. Jmjd3-deficient mice exhibit decreased expression of M2-associated genes[143] and the inhibition of jmjd3 showed impaired pro-inflammatory macrophage response[146], showing the importance of Jmjd3 in macrophage polarization and activation. Interestingly, histone methylations in several pro-inflammatory genes are also associated with macrophage polarization (e.g., TNF α[147148], IL-6[147]). Thus, targeting histone methylations of genes required for M1 or M2 polarization could be beneficial as a therapy for multiple inflammatory diseases. Indeed, emodin, a Chinese herb-derived compound with the potential to inhibit inflammation, is uniquely able to suppress the excessive response of macrophages to both M1 and M2 stimuli by inhibiting the removal of H3K27 trimethylation and the addition of H3K27 acetylation, respectively[149]. Furthermore, ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine metabolism, is a regulator of macrophage function. Odc-deficient macrophages had increased H3K4 monomethylation and H3K9 acetylation, accompanied by decreased H3K9 di/trimethylation in primary macrophages[150].

In contrast, histone acetylation is the most studied histone modification where acetylation of lysine residues is achieved by histone acetyltransferases (HATs), but deacetylation occurs by histone deacetylases (HDACs). It appears that HATs are linked to transcriptional activity whereas HDACs are associated with transcriptional repression[85]. Thus, imbalance of HATs/HDACs may cause differential gene expression leading to different diseases like asthma. HDAC activity has been associated with steroid resistance and severity of asthma[151,152]. Moreover, HDAC3 is required for the activation of hundreds of, mainly STAT-1-dependent, inflammatory genes in M1 macrophages[153]. Macrophages lacking HDAC3 show an M2-line phenotype in the absence of external stimuli and are hyper-responsive to IL-4, suggesting that HDAC3 may promote M1 and inhibit M2 polarization[154]. Throughout the macrophage genome, HDAC3 deacetylates histone tails at regulatory regions, leading to repression of many IL-4-regulated genes, which are characteristics of M2 macrophages. Recent studies suggest that TGFβ transcriptionally downregulates TIMAP (TGFβ-inhibited membrane-associated protein) through HDAC3-associated Smad signaling, which is associated with TGFβ-induced macrophage polarization, suggesting that the strategies targeting the HDAC3/TIMAP axis might improve TGFβ-associated pathological processes in various diseases[155]. Thus, pharmacological blockade of HDAC3 functions could be of benefit in the treatment of inflammatory diseases. Additionally, the commensal microbe-derived butyrate, a novel activator of STAT6-mediated transcription, drives M2 macrophage polarization through H3K9 acetylation[156].

CONCLUDING REMARKS

Significant progress has been made in identifying the origin, characteristics, and role of macrophages in physiology and pathophysiology over the last few decades. However, considering the fact that macrophages shows significant heterogeneity in function, a great deal of questions still remain unanswered. For example, although macrophages have been

defined as two separate polarization states, M1 and M2, with inflammatory and anti-inflammatory functions, these states have been largely defined by *in vitro* studies, and tissue macrophages are possibly activated along a continuum between M1 and M2 states *in vivo* with mixed stimuli. Moreover, no specific M1/M2 markers have been identified for *in vitro* or *in vivo* studies and between human and mouse macrophage polarization. Furthermore, macrophages have been widely recognized as extremely attractive therapeutic targets for inflammatory diseases, and most of the therapies are targeted at macrophage markers and their related signaling pathways. Thus, better understanding of macrophage diversity and definition of these cells is essential. Most importantly, considerable progress has been made in understanding the underlying mechanisms associated with the regulation of macrophage polarization, which involves an intricate network of various regulatory cytokines, chemokines, transcriptional factors, and immune-regulatory cells. Due to this intricate network of cellular and marker interplay, it opens up an array of therapeutic targets for the future to modulate macrophage polarization and immune responses in asthma. Furthermore, there is an increased attention on the epigenetic changes that impact macrophage functional responses and M1/M2 polarization. Attempts have been made to study epigenetic mechanisms and identify epigenetic markers that regulate macrophage polarization and activation in asthma. Taken together, modulation of macrophage polarization through targeting those identified potential macrophage regulators may have therapeutic potential in the treatment of allergic asthma and other allergic diseases.

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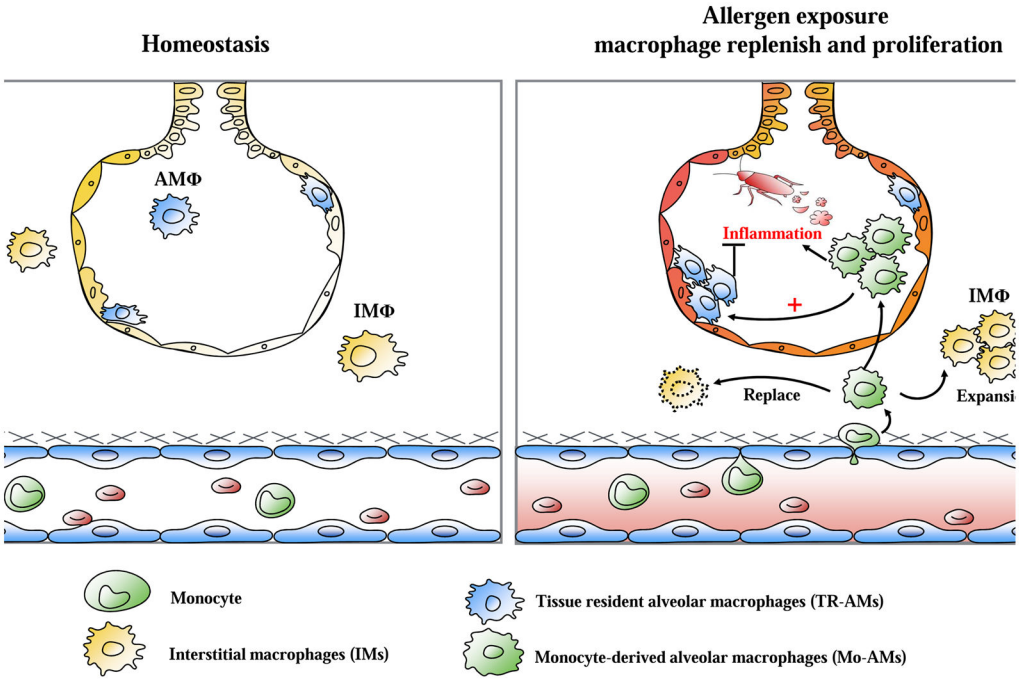

Figure 1.

Schematic diagram of repopulation strategies of macrophages. During homeostasis, majority of resident macrophages (i.e., alveolar macrophages) are derived from embryonic progenitors with small contribution from blood monocyte-derived macrophages. However, after allergen exposure inflammatory mediators produced by damaged epithelial cells or activated resident innate immune cells cause the influx of blood monocytes, which then differentiate and expand in the airway and re-populate the alveolar macrophage niche, where both tissue-resident alveolar macrophages (TR-AMs, suppress inflammation) and monocyte-derived alveolar macrophages (Mo-AMs, promote inflammation) contribute to repopulation.

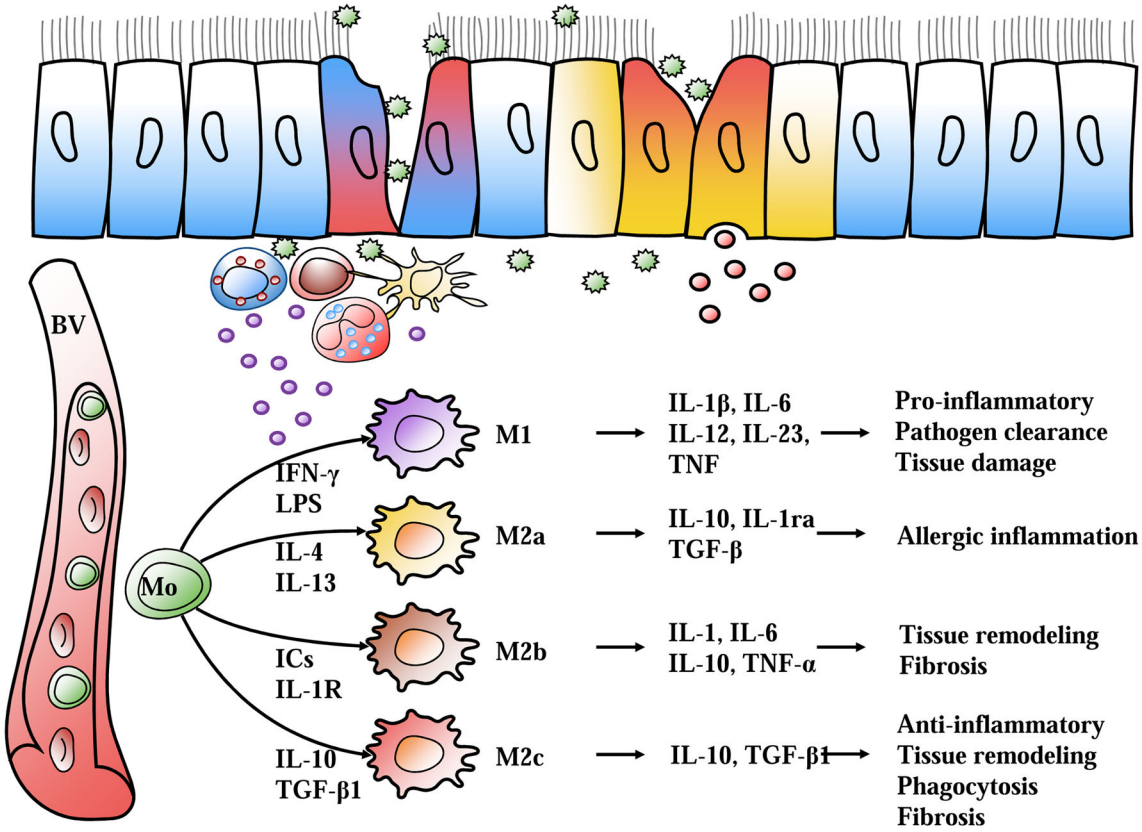

Figure 2.

Schematic diagram of macrophage subtypes. Allergen exposure (i.e. cockroach allergen) activates lung epithelial cells and innate immune cells to produce a variety of cytokines, which direct specific macrophage polarization subtype. M1 subtype is generally considered to be pro-inflammatory, M2a subtype is induced by IL-4 and IL-13, which are critical mediators of allergic inflammation. M2b and M2c subtypes predominately participate in tissue remodeling and fibrosis. BV (blood vessel), Mo (monocyte).

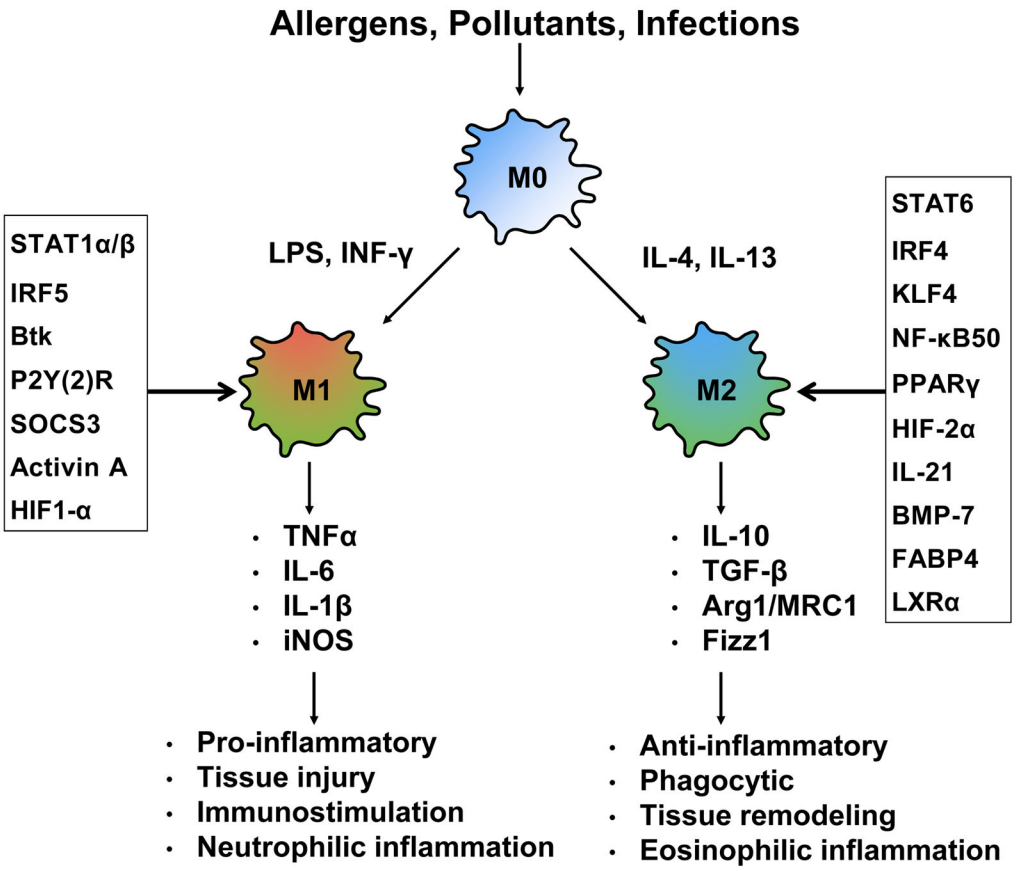

Figure 3.

Schematic diagram of macrophage activation with the most related signaling molecules involved in M1/M2 macrophage polarization. Lung primary macrophages are different into M1 and M2 phenotypes after exposed to allergens, pollutants, and infections, which are regulated by signaling molecules (box), respectively. M1 and M2 macrophages play distinct roles in inflammation and tissue remodeling by secreting different inflammatory mediators.

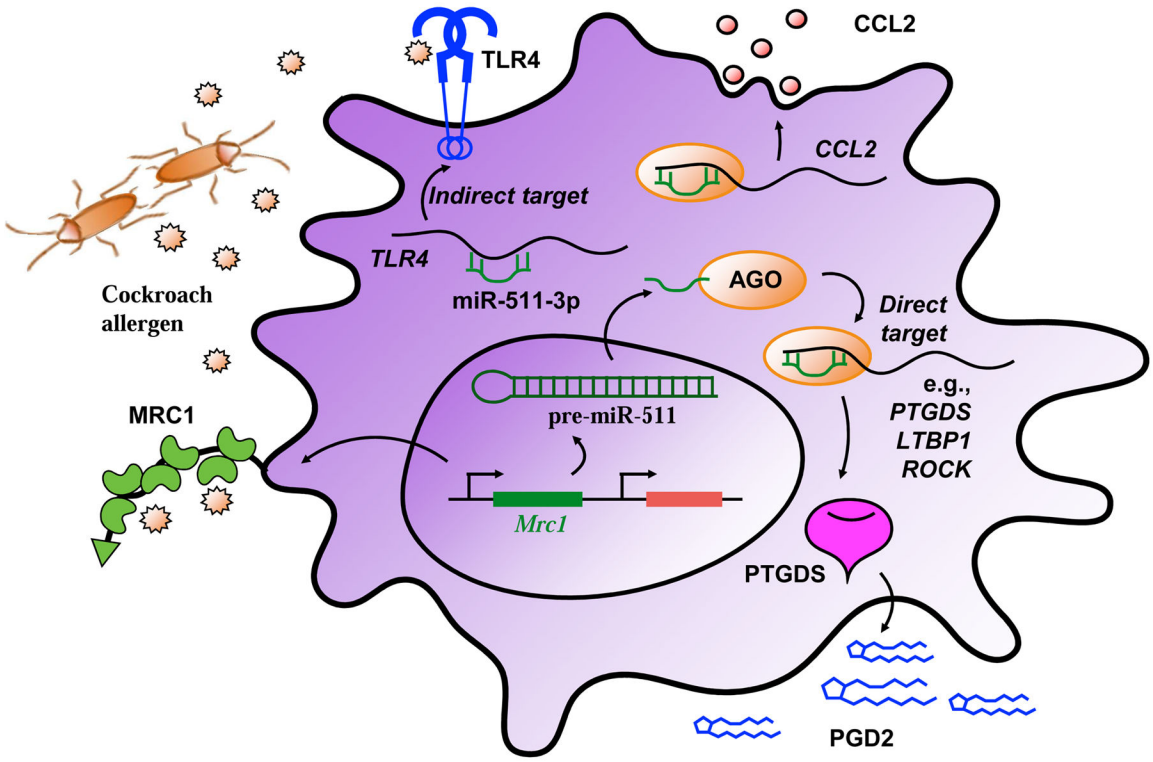

Figure 4.

Schematic diagram of miR-511-3p in macrophage polarization. miR-511-3p is an intronic miRNA encoded by *MRC1* gene. miR-511-3p is transcriptionally co-regulated with *Mrc1* in macrophages. miR-511-3p can regulate macrophage polarization into M1 or M2 by binding its direct (e.g., *PTGDS, LTBP1, ROCK, and CCL2*) or indirect (e.g, *TLR4*) target genes. AGO (argonaute), PTGDS (prostaglandin D2 synthase), PGD2 (prostaglandin D2), LTBP1 (latent-transforming growth factor beta-binding protein 1), ROCK (Rho-associated protein kinase), CCL2 (C-C motif chemokine ligand 2), TLR4 (Toll-like receptor 4).

Table 1

miRNA	Targets	Function	References
M1			
mi-155	SMAD2, C/EBP, IL13R 1, Pellino-1, SOCS1, BCL-6	Inhibits SMAD2: TGF-α,	(131)
		Akt dependent polarization via CEBP	(135)
		Down-regulates IL-13 receptor	(36)
		Increases expression of BCL6	(134)
miR-127	BCL6	Activates JNK pathway	(136)
miR-125b	IRF4	Enhances pro-inflammatory responses	(150)
miR-27b	PPAR-y	Increases LPS response	(151)
miR-223	pknox1	Pro-inflammatory activation of macrophages	(152)
miR-106a	IL-10	Decreases IL-10 leading to inhibition of M2 polarization	(153)
M2			
miR-146a	Notch 1, PPARγ, NF-κB, IRAK, TRAF6	Decreased inflammation	(38)
		Suppresses pro-inflammatory cytokine production	(138–140)
miR-146b	IRF5 TLR4, MyD88, IRAK-1, TRAF6	Suppresses M1 via IRF5	(141)
		Modulates TLR4 signaling pathway	(142)
Let 7c	p21-activated kinase 1(PAK1), CEBP	Inhibits PAK1 in M1 macrophages	(143)
		Promotes M2 and inhibits M1 via CEBP	(144)
		Upstream of IL-10	(146)
miR-21	SIRPb1, Erk 1/2, NF-κB, PTEN, PDCD4, TLR4, STAT3	Downstream of CSF-1R	(145)
		Prevents PGE2 mediated M2 polarization	
miR-511-3p	PTGDS, ROCK2, LTBP1, CCL2, TLR4, C/EBP	M2 activation	(147), (39) (148)
miR-124	C/EBP STAT3, TACE	Upregulation of M2 associated markers like Arg1, FIZZ1, TGF and downregulation of M1 markers	(154)
miR-125a	KLF13	Promotes M2 polarization	(155)
miR-125b	TNF-α; IRF4	Decreases inflammation	(156)