

## **The role of different monocyte subsets and macrophages in asthma pathogenesis**

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### **ABSTRACT**

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Monocytes are comprised of three phenotypically and functionally distinct subsets: classical CD14<sup>++</sup>CD16<sup>-</sup>, intermediate CD14<sup>++</sup>CD16<sup>+</sup> and non-classical CD14<sup>+</sup>CD16<sup>++</sup> cells that can play differential roles in regulation of both systemic and local inflammatory processes. In addition, these monocyte subsets represent differential developmental stages with CD16-positive monocytes being the most mature cells that can be considered direct precursors of tissue macrophages.

Monocytes and, most significantly, monocyte-derived macrophages constitute an important component of both normal and asthmatic airways. Here we summarize the current knowledge on the roles of monocytes and macrophages in asthma pathogenesis. In addition, we discuss here the usefulness of standard and potential monocyte-directed anti-asthmatic therapeutic approaches.

**Key words:** monocytes, asthma, monocyte subsets, macrophages, glucocorticoids

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## **INTRODUCTION**

Asthma affects approximately 300 million people worldwide and thereby it constitutes one of the most serious global health problems. Asthma is a chronic inflammatory disease of large and small airways that is closely related to complex interactions among numerous inflammatory cells and soluble mediators. To date, the majority of studies have underscored the important roles of eosinophils, neutrophils, mast cells and T cells in the pathogenesis of asthma [1-4].

However, despite the fact that monocytes and especially monocyte-derived macrophages constitute the largest component of human airways, the role of these cells in regulation of asthmatic airway inflammation has not been fully elucidated. Here we summarize the most current knowledge on the monocyte-related alterations of immune system that contribute to asthma development and progression. Moreover, despite more than half-a-century history of the use of glucocorticoids (GC) in the asthma treatment, these drugs remain a mainstay therapy of this disorder. Therefore, here we discuss the effects of GC therapy on monocytes and macrophages and the novel approaches that could allow for enhancement of beneficial GC effects.

### **Main monocyte subsets**

Prior to currently acknowledged division of monocyte population into classical CD14<sup>++</sup>CD16<sup>-</sup>, non-classical CD14<sup>+</sup>CD16<sup>++</sup> and intermediate CD14<sup>++</sup>CD16<sup>+</sup> cells, monocytes have been divided to CD16-negative (referred to as CD14<sup>+</sup>CD16<sup>-</sup> or CD14<sup>high</sup>CD16<sup>low</sup>) and CD16-positive (referred to as CD14<sup>low</sup>CD16<sup>+</sup> or CD14<sup>low</sup>CD16<sup>high</sup>) monocytes [5-7].

Introduction of additional markers allowed for further characterization of these subsets indicating that CD14<sup>low</sup>CD16<sup>+</sup> monocytes expressed higher levels of CD43, CD115, CXCL16, CXCR1 and C3AR1 expression whereas CD14<sup>high</sup>CD16<sup>-</sup> monocytes exhibited higher levels of CD1d, CD93 and CD114.

Importantly, analysis of genes associated with proliferation, differentiation and cell cycle indicated that CD16-positive monocytes represented later stage of differentiation (assessed by substantial expression of broad spectrum of markers characteristic for dendritic cells and macrophages: SIGLEC10, CD43, RARA and CD97, CD115, C3AR1 respectively) in contrast to CD16-negative monocytes demonstrating high levels of genes coding myeloid (CD1d, CD14, CD93, MNDA) and granulocyte markers (CD114, FPR1) [6,8].

Transcriptomic and proteomic studies confirmed functional differences between CD16-positive and CD16-negative monocytes. Mature monocytes expressing CD16 showed higher levels of

genes and corresponding proteins associated with phagocytic activity mediated through Fcγ receptor: actin-related protein 2/3 complex (ARPC5), CD16, hemopoietic cell kinase (HCK), tyrosine-protein kinase Lyn (LYN), hemoxygenase 1 (HMOX), villin 2 (VIL2).

In contrast CD16-negative monocytes expressed higher levels of myelo-peroxidase (MPO), cathepsin G (CTSG), protein S100-A9 (S100A9), eosinophil cationic protein (RNASE3) and lysozyme (LYZ) which indicated their greater capability to kill microbes as compared to CD16-positive monocytes [7].

Similarly, other studies demonstrated that CD16-negative and CD16-positive monocytes significantly differed in immune status, trafficking properties, metabolism and signaling pathways [6].

Last decade brought a number of studies suggesting that CD16-positive monocytes can be further delineated into two separate subsets [5, 8, 9]. Based on the differential levels of CD14 (LPS receptor) and CD16 (FcRγIII receptor) expression, a subset of intermediate CD14<sup>++</sup>CD16<sup>+</sup> monocytes has been distinguished. In healthy individuals, intermediate monocytes represent approximately 5-10% of monocytes. Since that moment current division of monocytes on classical CD14<sup>++</sup>CD16<sup>-</sup>, intermediate CD14<sup>+</sup>CD16<sup>++</sup> and non-classical CD14<sup>+</sup>CD16<sup>++</sup> monocytes and such nomenclature has been applied [5,10,11].

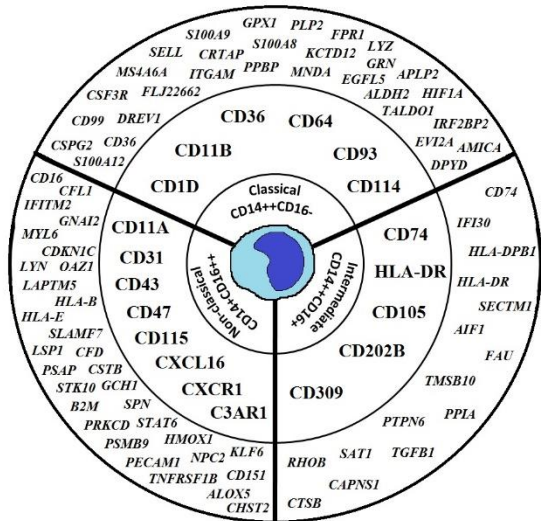
Several genes including genes coding proteins associated with response to microbes and phagocytosis (S100A8, S100A10, LYZ, CD14, CD93) and processing and presentation of antigens linked to MHC class II molecules (CD74, IFI30, HLA-DR paralogues) were found to be highly expressed in intermediate monocytes as compared to non-classical monocytes.

Furthermore, intermediate monocytes exhibited highest expression of VEGFR2 indicating pro-angiogenic properties of that subset. Notably, it has been showed that CD14<sup>++</sup>CD16<sup>+</sup> monocytes are the main producers of reactive oxygen species (ROS) on the basis of preferential higher expression of ROS-associated genes (CYBA, NCF2, TSPO) (Fig. 1).

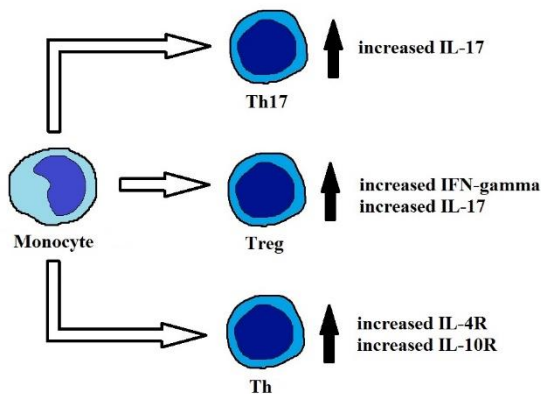
In addition, intermediate monocytes were found to be the subset most preferentially involved in regulation of T-cell-related immune responses [5, 8]. In allergic rhinitis patients, the monocyte expression of IL-4R and IL-10R was positively correlated with the expression of these receptors on CD4<sup>+</sup> T cells indicating that both cell types present similar patterns of responses to pro- and anti-inflammatory mediators [12].

Moreover, CD14<sup>+</sup>CD16<sup>++</sup> monocytes appeared to be most potent stimulators of IL-17 production by T cells [13, 14]. Similarly, activated monocytes induced production of pro-inflammatory

cytokines such as IL-17 and IFN-gamma by regulatory T cells [15] (Fig. 2).



**Fig. 1.** Protein and molecular markers (inner and outer circle respectively) specifically upregulated within distinct monocyte subsets at different maturation stages



**Fig. 2.** Role of monocytes in modulation of selected immune responses mediated by T cells

### The role of monocytes in asthma pathogenesis

Recent reports indicated that monocytes that were previously underestimated as regulators of asthmatic inflammation, appear to play an important role in pathogenesis of this disorder [16-19]. Monocytes of asthmatic patients were shown to produce eminent amounts of reactive nitrogen species, exhibited higher activities of nitric oxide synthase (NOS) and total free radicals (TFRA), the feature that was closely related to asthma severity [17]. Monocytes were also found to produce soluble CD86 (sCD86), a pro-inflammatory mediator that is highly elevated in sera of asthmatic patients undergoing exacerbations. Concentrations of sCD86 showed negative correlation with airway responsiveness and arterial carbon dioxide tension [18]. Rizzo et al demonstrated that monocytes of

asthmatic patients presented defective immunosuppressive activities related to decreased secretion of inhibiting factors such as soluble HLA-G protein [19].

It became widely acknowledged that different monocyte subsets can play differential roles in the regulation of numerous inflammatory and infectious diseases including Sjögren's disease, type 1 diabetes, cardiovascular disease, acute and chronic liver disease, HIV infection and many others [10,20-25]. Previously we demonstrated enhanced frequencies of intermediate CD14<sup>++</sup>CD16<sup>+</sup> monocytes in severe asthmatic patients, the phenomena indicating that this subset might be responsible for maintenance of chronic inflammatory state in the course of the disease [16, 26]. This finding however does not exclude the contribution of other monocyte subsets to the pathogenesis of asthmatic airway inflammation. One has to keep in mind that non-classical CD14<sup>+</sup>CD16<sup>++</sup> monocytes possess "patrolling" properties, they are the first to infiltrate the inflamed tissue and as the most mature cells differentiate into new macrophages and dendritic cells of which roles are described in the following parts of the current review [9, 22].

Indeed, experimental studies revealed that different monocyte subsets present differential capabilities of differentiating into macrophages. Mouse Gr1<sup>low</sup>CX3CR1<sup>high</sup> monocytes unlike Gr1<sup>high</sup>CX3CR1<sup>int</sup> (equivalent of non-classical and classical monocytes in humans, respectively) were shown to preferentially give rise to lung macrophages. In addition, both abovementioned monocyte subsets have the potential to differentiate into dendritic cells in both physiological and inflammatory milieu [27]. Interestingly, analysis of microRNA (miRNA) expression within monocyte subsets resulted in discovery of high miR-155 and low miR-124 levels in non-classical CD14<sup>+</sup>CD16<sup>++</sup> monocytes indicating their M1 macrophage-oriented phenotype. On the contrary, intermediate CD14<sup>++</sup>CD16<sup>+</sup> monocytes exhibited M2 macrophage-like properties displaying increased miR-124 and lack of miR-155 expression. Interestingly, classical CD16-negative monocytes demonstrated combination of M1- and M2-like properties with high levels of miR-155 and miR-124 and additional eminent expression of miR-424 that was defined as a marker of immature monocytes [28-31].

### Contribution of monocytes to maintenance of macrophage and dendritic cell pool in the lungs

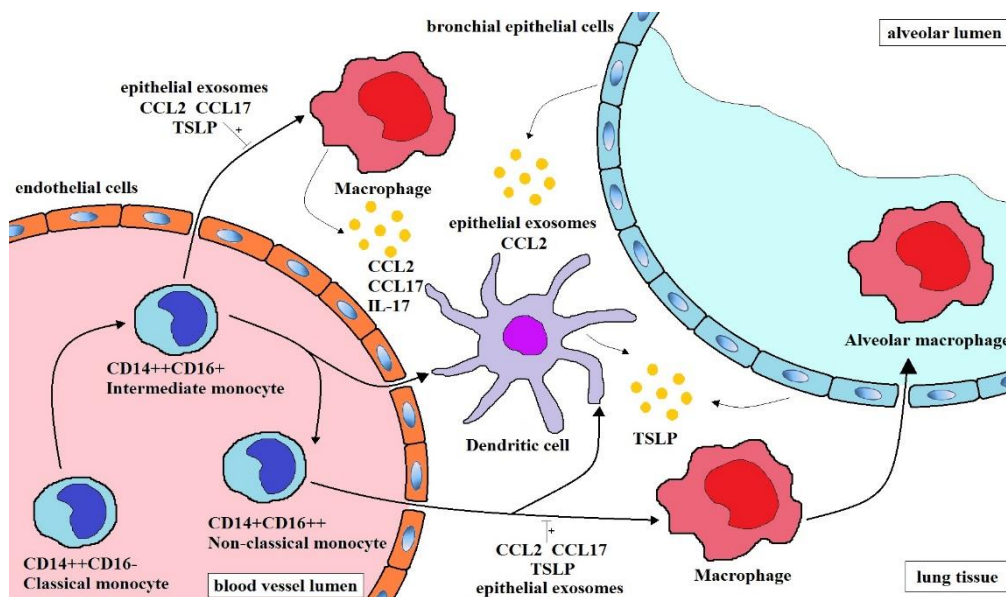
Detection of increased numbers of monocytes found in BAL fluid in murine model of asthma induced by ovalbumin (OVA) clearly confirmed involvement of monocytes in pathogenesis of this disorder [32]. In OVA-

challenged mice, the enlargement of monocyte pool was confirmed even in the bone marrow. Similar enlargement was found in case of eosinophils but not T cells, B cells and hematopoietic stem cells [33].

In animal asthma model, circulating monocytes were shown to infiltrate lung tissues and give rise to monocyte-derived pulmonary dendritic cells and macrophages [27]. Notably, capability of monocytes to transmigrate through endothelium remains predominantly a feature of CD16-positive monocytes and seems to be related to increased expression of several chemokine receptors (CXCR1, CCR2, CCR5, CCR7) and adhesion molecules (CD31, CD54, CD105) as well as and higher soluble CD146 (sCD146) binding activity [34,35].

In some contrast, however, recently published study suggested that in mouse model of asthma alveolar macrophages at early stages of allergic lung inflammation were not dependent on circulating monocytes and their maintenance was associated with local proliferation.

However, the same authors did not exclude the possibility that monocytes could contribute to maintaining the pool of alveolar macrophages in the course of asthma [36]. In contrast, another report demonstrated that bronchial allergen challenge attracted monocytes to have differentiated into alveolar macrophages in site of inflammation. This differentiation occurred in response to chemotactic protein CCL2/MCP-1 produced by stimulated bronchial epithelial cells and rhinovirus-infected macrophages [37,38]. In addition, Th2-oriented cytokine such as human TSLP (thymic stromal lymphopietin) that can be released by bronchial epithelial cells and is over-expressed in asthmatic patients was shown to augment the expression of CD80 on circulating monocytes. This suggests that pro-allergic mediators can further activate monocytes and monocyte-related immune responses in order to enhance the intensity of asthmatic inflammation [39,40] (Fig. 3.).



**Fig. 3.** Migration pathways and main functions of monocytes and lung macrophages. Graphic presentation of monocyte development in blood and their further trafficking into lung tissue

### The role of macrophages and dendritic cells in regulation of immune responses airways of asthmatic patients

Monocytes constitute the exclusive reservoir of macrophage precursors. On the other hand, macrophages were demonstrated to constitute a major population of cells detected in bronchoalveolar lavages (BAL). Further expansion of lung-associated macrophages was demonstrated in allergen challenged asthmatic mice [41]. In addition, alveolar macrophages in mouse asthma model were shown to promote Th2-oriented immune

responses fueling allergic airway inflammation and subsequent lung remodeling [37].

Notably, recent studies showed that asthma was associated with increased numbers of CD68+ macrophages that could be detected in bronchial tissue, especially in the course of rhinovirus-induced complications. Interestingly, bronchial macrophage-related parameters assessed in these studies were not correlated with lung function parameters [42]. On the other hand, in rhinovirus-infected asthmatic subjects, macrophage accumulation was linked with increased production of CCL2 by these cells

suggesting a positive feedback loop that could contribute to self-enhancing airway inflammation [38]. Previously, we demonstrated that bronchial macrophages in asthmatic patients were capable of responding to both pro- and anti-inflammatory signals [43].

Macrophages can also activate and recruit other inflammatory cells involved in asthmatic inflammation. Recruitment of T cells can be supported by macrophages-secreted CCL17 [44].

Moreover, alveolar macrophages in asthmatic patients produced high levels of IL-17 thereby indicating that macrophages but not only Th17 cells can constitute a major source of IL-17 in the course of asthma [45]. Importantly from clinical point of view, higher incidence of bacterial infections in severe asthmatics was linked to impaired phagocytic activity of monocyte-derived alveolar macrophages [46]. In some contrast, a study conducted in mild asthmatic patients showed that both phagocytosis index and percentage of phagocytic macrophages were higher than in healthy subjects [47].

One has to keep in mind that monocytes can give rise not only to macrophages but also to dendritic cells. Mouse model experiments showed that monocyte-derived dendritic cells can prime naïve CD4<sup>+</sup> T cells and thereby they can play a critical role in maintenance of normal immune responses [27]. Plantinga et al also showed that monocyte-derived DCs, unlike conventional DCs that are essential for physiological priming of Th2 cells, are primarily responsible for development of asthmatic airway inflammation through chemokines release [48]. In another study inflammatory dendritic cells (referred to as iDCs) were confirmed to originate from blood monocytes which migrated into lungs in CCR2-dependent manner. These iDCs were suggested to be have independently accounted for development of airway hyper-responsiveness [49,50].

#### **Currently available therapies regulating monocyte-dependent immune responses**

Given the predominant involvement of CD16-positive monocytes in the pathogenesis of asthma, it is tempting to hypothesize that targeting these particular cell subsets could be of potential clinical benefit. Indeed, GC being indispensable drugs for asthma treatment were already demonstrated by us and others to preferentially deplete CD16-positive monocytes but not CD16-negative classical monocytes [16,51]. Mechanism of GC-mediated depletion of intermediate and non-classical monocytes was associated with enhancement of apoptosis of these cells. In part, this was caused by significantly higher expression of GC receptor (GCR) in CD16-positive monocytes than in CD16-negative monocytes [51].

In addition, GC affected monocyte function by diminishing levels of monocyte-related nitric oxide and nitric oxide synthase activities in asthmatic patients [17]. Quite surprisingly, GC therapy of asthmatic patients resulted in significant decrease of IL-10 receptor expression on both CD14<sup>++</sup>CD16<sup>+</sup> and CD14<sup>+</sup>CD16<sup>++</sup> monocytes probably representing the mechanism of counterbalancing of otherwise immunosuppressive actions of GC [52]. GC did not significantly affect phagocytic capacities of airway macrophages, however, they also failed to recover them [46,53].

Interestingly, recent studies indicated that monocyte phenotype and function can be affected by another potent immunomodulatory agent with high potential of being used in asthma treatment, namely vitamin D (vit. D). Vit. D represents an interesting pharmaceutical tool enabling for reduction of GC doses used in asthma treatment [54,55]. Significant role of vit. D used alone or as a supplement to GC therapy was demonstrated in context of inhibition of inflammatory factors (p38 MAP kinase) and simultaneous induction of anti-inflammatory pathways (MKP-1 kinase) [56-58]. Besides, recently we demonstrated that active form of vit. D3 preferentially decreased numbers of CD16-positive and TNF- $\alpha$  secreting monocytes in asthmatic patients [59].

Moreover, 1,25-dihydroxycholecalciferol was shown to significantly diminish production of TNF- $\alpha$ , IL-6, IL-8 and CXCL10 in LPS-stimulated monocytes [60,61]. It was hypothesized that immune-suppressive effects of vit. D3 administration exerted preferentially on CD16-positive monocytes could have been related to increased expression of vitamin D receptor (VDR) within CD16<sup>+</sup> monocytes [61].

Interestingly, monocytes collected from human individuals having supplemented with  $\gamma$ -tocopherol (form of vitamin E) that were stimulated *ex vivo* with LPS released significantly lower amounts of such pro-inflammatory cytokines as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, MCP-1 and MIP-1 as compared to individuals not supplemented with this agent [62].

Notably, zinc deficient asthmatic rats were found with increased numbers of monocytes, eosinophils and neutrophils in BAL fluid accompanied by elevated concentrations of MCP-1/CCL2 and eotaxin/CCL11. This may suggest that zinc supplementation could have a role in decreasing airway inflammation [63].

Interestingly, recent studies revealed beneficial effects of bone marrow-derived mononuclear cells (including both monocytes and lymphocytes) delivered to asthmatic mice on lung function, airway hyper responsiveness and fiber deposition [33].

## CONCLUSIONS

Accumulating body of evidence indicates that macrophages and such monocyte subsets as intermediate CD14<sup>++</sup>CD16<sup>+</sup> and non-classical CD14<sup>+</sup>CD16<sup>++</sup> cells can play a number of crucial roles in regulation of asthmatic airway inflammation. Despite the data reported by our and other groups demonstrating that currently used GC-based anti-asthmatic therapeutic regimens efficiently target CD16-positive monocytes, further research on novel monocyte-oriented agents is still warranted.

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## Conflicts of interest

The authors declare that there are no conflicts of interest.

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