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Alveolar Macrophages in Allergic Asthma: the Forgotten Cell Awakes

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Abstract

Purpose of Review—The role of alveolar macrophages in innate immune responses has long been appreciated. Here, we review recent studies evaluating the participation of these cells in allergic inflammation.

Recent Findings—Immediately after allergen exposure, monocytes are rapidly recruited from the bloodstream and serve to promote acute inflammation. By contrast, resident alveolar macrophages play a predominantly suppressive role in an effort to restore homeostasis. As inflammation becomes established after repeated exposures, alveolar macrophages can polarize across a continuum of activation phenotypes, losing their suppressive functions and gaining pathogenic functions.

Summary—Future research should focus on the diverse roles of monocytes/macrophages during various types and phases of allergic inflammation. These properties could lead us to new therapeutic opportunities.

Keywords

Asthma; Macrophage; Phenotypes; Eosinophilic; Neutrophilic; Inflammation

Introduction

Asthma, rhinitis, food allergy, and eczema represent common allergic diseases that have increased in prevalence over the last several decades and, today, are responsible for enormous morbidity and economic cost worldwide [1]. These diseases involve allergen-triggered responses, resulting in a prototypic eosinophil-predominant program termed type 2 inflammation [2]. Recognition of the central importance of type 2 inflammation has appropriately fostered attention on the responsible cells and cytokines [3]. As a consequence of such an intense focus, other cells and mechanisms have been relatively overlooked. One

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Compliance with Ethical Standards

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such “forgotten cell” is the macrophage [4]. Macrophages are best known for their central role in innate immune responses, but recent studies suggest that these cells may also be crucial participants in allergic diseases. In this review, we highlight emerging data on the role of macrophages and the potential of macrophage-directed therapy in allergic inflammation. Although we will focus our attention on asthma, it is likely that these findings are applicable to other allergic diseases as well.

Pathogenesis of Asthma

Asthma is a syndrome in which the airways are chronically inflamed, leading them to contract more readily than is normal in response to a wide variety of stimuli, a condition termed airway hyperresponsiveness. Typical triggers of bronchoconstriction include not only allergens, but also irritant chemicals, cold air, and exercise. Increased mucus secretion and thickening of the airway walls also contribute to narrowing of the airway lumen, leading to symptoms of shortness of breath, wheeze, cough, and chest tightness [5].

We will provide a brief overview of current understanding of the pathogenesis of typical allergen-induced asthma. Inhaled allergens are detected by pattern recognition receptors (PRRs) on the surface of airway epithelial cells, activating these cells to secrete interleukin (IL)-33, IL-25, granulocyte-macrophage-colony stimulating factor (GM-CSF), and thymic stromal lymphopoietin. These epithelial-derived mediators promote type 2 inflammation through their indirect actions on dendritic cells (DCs) and their direct actions on type 2 innate lymphoid cells (ILC2s). DCs are stimulated to take up antigen and migrate to draining lymph nodes, where they present antigen to naive T cells, stimulating their differentiation into T helper (Th) 2 cells. Both Th2 cells as well as ILC2s elaborate characteristic type 2 cytokines including IL-4, IL-5, and IL-13 [5, 6]. Collectively, these type 2 cytokines are responsible for IgE production (which in turn triggers mast cell release of bronchoconstrictor substances), eosinophil accumulation and activation, and airway abnormalities including mucus metaplasia, bronchial hyperresponsiveness, and airway wall thickening due to smooth muscle cell hyperplasia and subepithelial fibrosis [5]. Classic type 2 or allergic asthma tends to respond to treatment with corticosteroids as well as a growing menu of targeted biologics (anti-IgE, anti-IL-5, and anti-IL-4/IL-13) [3]. However, we now appreciate that a substantial subset of asthma is predominantly nonallergic or “low type 2” in nature. This phenotype may be characterized by infiltration of neutrophils and is often more severe and difficult to treat, being relatively resistant to corticosteroids [7].

What about Macrophages?

No anatomic site is so continuously challenged by such a variety of foreign substances—including allergens, microbial pathogens, chemicals, and particulates—as are the airways. In the face of this constant assault, maintenance of physiologic homeostasis (i.e., gas exchange) requires an exquisite capacity to appropriately calibrate inflammatory responses in the airways and, where necessary, to restrain them. Ideal properties of a cell whose role is to orchestrate and calibrate such responses would include its normal residence at the mucosal surface, a high degree of functional plasticity, and the ability to communicate with neighboring cells in a paracrine manner. Although historically viewed mainly as professional

phagocytes and largely ignored in the context of allergic inflammation of the airways, lung macrophages fulfill these criteria and are prime candidates to serve as “conductors” capable of either promoting or suppressing inflammatory responses in the airways [4].

During the last two decades, we have learned a great deal about the origins, plasticity, and functions of macrophages in tissue homeostasis and in innate and acquired immunity [8, 9]. Depending on their localization, we can broadly define two well-studied populations of lung macrophages: (1) alveolar macrophages (AMs) located on the alveolar epithelial surface and (2) interstitial macrophages (IMs) located within alveolar walls [10]. A third population that is especially relevant in the context of asthma is airway macrophages found on the epithelial surface of the airways. Unfortunately, these cells are exceedingly poorly characterized. Available information—though extremely dated [11]—suggests that they are phenotypically and functionally very similar to AMs and may represent AMs that have migrated up the tracheobronchial tree. In this review, we will classify them together with, and refer to them as, AMs.

It was long thought that resident tissue macrophages, including those of the lung, originate from blood monocytes, which are themselves produced in the bone marrow [12, 13]. However, within the last 5 years, this dogma has been turned on its head, and we now recognize that under normal circumstances, most tissue macrophages, including AMs, instead arise from embryonic precursors before birth and are maintained throughout life by local proliferation [14–18, 19•, 20, 21••]. Under certain experimental conditions, it has been shown that AMs can be replaced by IMs [22–25]. It is still unclear whether IMs and airway macrophages are likewise of embryonic origin, derived from monocytes, or both.

Macrophages can polarize into many different phenotypes, depending on signals present in their environment, which may be hard to define in vivo. This high degree of plasticity is now appreciated as a defining characteristic of macrophages, including AMs [26]. This property also complicates attempts to simplistically categorize their role in asthma. Previously, macrophages were classified as M1 and M2 in an attempt to mirror the Th1/Th2 dichotomy of T cells [27]. M1 or “classically activated” macrophages have high microbicidal and tumoricidal activity, as exemplified by production of nitric oxide and pro-inflammatory cytokines (such as IL-12 and tumor necrosis factor alpha (TNF- α)), while M2 or “alternatively activated” macrophages have antiparasitic and tissue remodeling activity, as exemplified by production of arginase-1 and transforming growth factor-beta [28, 29]. It has become clear, however, that this dichotomous classification underrepresents the functional and physiological diversity of macrophages. For example, it was observed that macrophages classified in the M2 category actually comprise a collection of many functionally different subsets [30], some of which more closely resemble M1 than M2 macrophages [31]. This stimulated efforts to divide M2 macrophages into an ever-expanding number of subsets. It also became clear that macrophage phenotypes generated in vitro by stimulation with specific cytokines (e.g., interferon (IFN) γ for M1 and IL-4/IL-13 for M2) inadequately model those found in vivo, where phenotypes appear as a continuum rather than discrete entities [32, 33]. This classification dilemma hindered consensus and progress in macrophage biology for years. A recent proposal to instead operationally describe macrophages, according to the markers they express and/or the signals that induce them, has

been a welcome step forward, avoiding the complexity of contrasting classifications and different definitions of activation [34].

Origins of Macrophages in Asthma

Does the embryonic origin and local maintenance of AMs during homeostatic conditions also apply to these cells during allergic inflammation? Indeed, studies have shown that maintenance of the AM pool during type 2 inflammation is largely dependent on local proliferation [35, 36••]. However, it has also been demonstrated that immediately after allergen exposure, macrophages can arise from recruited monocytes [37•]. When transgenic macrophage Fas-induced apoptosis mice were utilized to conditionally deplete blood monocytes, the appearance of new AMs soon after allergen challenge was significantly abrogated. In parallel, this depletion of blood monocytes in allergen-challenged mice resulted in less eosinophilic inflammation [37•]. This study did not, however, exclude the possibility that these new populations of AMs could also arise from proliferating, resident AMs under the influence of mediators elaborated by pathogenic, recruited monocytes. A similar attenuation of allergic inflammation was observed in a different study when circulating monocytes were depleted by intravenous injections of clodronate [36••]. By contrast, when clodronate was instead administered via the intratracheal route to deplete resident AMs in this allergic model, eosinophilic inflammation was enhanced [36••]. Together, these studies suggest that resident AMs primarily serve to maintain lung homeostasis by suppressing inflammation, while immigrating monocytes primarily promote allergic inflammation [36••, 37•, 38–40]. The picture that emerges is one of rapid recruitment of monocytes to fight the perceived dangers of the allergen by mounting an inflammatory response and subsequent expansion of suppressive AMs in an attempt to restore homeostasis (Fig. 1a).

“Expected” Macrophage Phenotype in Allergic Asthma

The dominant paradigm of macrophages in allergic asthma has focused on the role of alternatively activated AMs elicited by exposure to IL-4/IL-13. This macrophage phenotype positively correlated with severity of airway inflammation in many different studies. However, whether this phenotype is the cause or the consequence of allergic inflammation remains to be determined, as literature on this topic is contradictory. An adoptive transfer study of in vitro differentiated IL-4/IL-13-stimulated macrophages into the lungs of allergic mice showed that these macrophages actively contribute to the exacerbation of the disease and are not just bystanders [41]. These findings were later confirmed by a few other studies [42–44], which found enhanced allergic inflammatory responses in lung tissue as a result of alternative activation of macrophages. In contrast to the aforementioned studies, it was later demonstrated that macrophages induced by IL-4/IL-13 to express the murine M2 marker Ym1/chitinase 3-like 3 are not necessary for allergic inflammation and are only a consequence of elevated type 2 responses [45]. In this study, the contribution of Ym1+ macrophages to acute and chronic house dust mite (HDM)-induced allergic lung inflammation was investigated by using *LysM^{cre}* mice with abrogated IL-4R α signaling in macrophages. The investigators observed that airway hyperreactivity, type 2 responses, mucus hypersecretion, eosinophil infiltration, and collagen deposition were not significantly

abrogated despite decreased alternative activation of macrophages. Recent data, however, showed that these LysM^{Cre} mice exhibit deletion of IL-4R α signaling in mature tissue-resident macrophages, but not in immature macrophages arising from proliferation or from recruited monocyte precursors [46]. In addition, it was found that Ym1+ macrophages derived from monocytes or from tissue macrophages are phenotypically and functionally distinct [47]. Taken together, these findings suggest that Ym1+ macrophages newly derived from either proliferation or from recruited monocytes are pathogenic in allergic inflammation, whereas mature Ym1+ resident macrophages are not (Fig. 1b). These data also highlight a cautionary note about the interpretability of data from transgenic mice with specific Cre drivers to establish roles of different lung macrophage populations in allergic asthma, given that the origins of IMs and the relationship between IMs and AMs remain uncertain.

“Unexpected” Macrophage Phenotype in Allergic Asthma

Typical type 1 cytokines (IFN- γ , TNF α , and IL-12) commonly coexist with type 2 cytokines in asthma. Since AMs respond differently to these two groups of cytokines, it may be expected that different macrophage phenotypes could similarly coexist. Indeed, our recent investigation demonstrated the presence of a mix of macrophage phenotypes in both mouse models of allergic inflammation as well as in human asthmatics [48, 49•]. Such data have therefore motivated studies on the involvement of classically activated macrophages in asthma, with results suggesting diverse possible roles [50–52]. Functional studies have shown that IFN γ -stimulated macrophages prevent the onset of allergic inflammation in mice by suppressing DC maturation [53]. This would suggest that these macrophages might serve as a counter-regulatory mechanism to dampen inflammation. On the other hand, we observed by immunohistochemical analysis that the presence of higher numbers of macrophages expressing the IFN γ -activated transcription factor interferon regulatory factor 5 (IRF5) in bronchial biopsies of asthma patients was associated with more severe airflow obstruction (lower FEV₁/FVC) [49•]. Thus, classical activation of macrophages may not only help prevent allergic sensitization, but may also promote the development of a more severe asthma phenotype in the context of established disease. IL-12 production by IRF5+ macrophages may explain their dual role. It was shown that neutralization of IL-12 during the allergen sensitization phase aggravated development of allergic inflammation in mice, but neutralization of IL-12 during allergen challenges abolished eosinophilic inflammation and airway hyperresponsiveness [54].

Diverse Macrophage Phenotypes in Nonallergic Asthma

The presence of type 1 cytokines, along with other classic inflammatory cytokines such as IL-6, would be expected during host responses to viruses and to a variety of exogenous and endogenous ligands for PRRs [55]. We will briefly summarize what is known about macrophage responses to these stimuli and the possible role of macrophages in driving neutrophilic inflammation in these contexts.

Infections with common respiratory viruses such as rhinovirus are a major trigger of asthma exacerbations [56]. One mechanism contributing to virus-induced exacerbations is the direct

interaction of the virus with lung macrophages [57]. Mouse models support the role of macrophages in these exacerbations, but interestingly, the pathogenic macrophage phenotype involved varied with the underlying inflammatory context. Thus, in mice with a predominant type 2 inflammation, viral infection induced alternative activation of AMs and further increased eosinophil numbers. By contrast, mice with type 1 inflammation showed increased classical activation of AMs and neutrophilic inflammation upon virus infection [58].

The well-studied pathogen-associated molecule pattern, bacterial lipopolysaccharide or endotoxin, contaminates cigarette smoke as well as many types of organic dusts [59, 60]. This and other ligands for PRRs on macrophages lead to activation of the transcription factor nuclear factor-kappa-light-chain-enhancer of activated B cells (NF- κ B) [55]. In a recent study, we found that IRF5+ macrophages predominated in a mouse model of nonallergic inflammation induced by pulmonary challenge with farm dust extract (FDE). In vitro treatment of macrophages with FDE induced classical activation, implying that this polarization reflected a direct effect of endotoxins via PRRs present on macrophages [61]. Damage-associated molecular patterns such as high-mobility group box 1, generated endogenously by epithelial cells in response to allergens as well as environmental toxins, represent an alternative means for activating these receptors and NF- κ B [62•].

PRR-mediated responses, in turn, may lead to the production of neutrophil chemoattractants (such IL-8 and leukotriene B₄ [LTB₄]) by classically activated macrophages [63, 64]. Both higher levels of IL-8 and LTB₄ have been observed in airways of patients with severe, neutrophilic asthma [65, 66]. Indeed, macrophages producing increased levels of type 1 cytokines were also found in severe, corticosteroid resistant asthma, which is often associated with neutrophilic inflammation [67]. While it is tempting to speculate that classically activated macrophages are important effectors of “low type 2” or neutrophilic forms of asthma, this remains to be definitively investigated.

Macrophage Phenotypes in Homeostasis

An “asthma-suppressive” role for resident AMs has been noted above. Examining the properties of normal AMs during health indeed revealed a predominantly anti-inflammatory phenotype. More IL-10+ macrophages were found in healthy mice and humans than in models of airway inflammation and in biopsies of asthmatics [48,49•,61]. The role of IL-10 in the immune system has been studied intensively, and this cytokine was found to be important in the resolution of airway inflammation [68, 69]. These results focus attention on the loss of normal suppressive components of the AM phenotype in asthma, rather than merely on the acquisition of pathogenic features.

Emerging Therapies

Can knowledge of the biology and roles of macrophages be translated into novel and much-needed therapeutic approaches for the treatment of allergic diseases such as asthma? Several potential strategies can be envisioned.

If lung macrophages derived from recruited monocytes are indeed pathogenic [36••, 37•], then blocking their recruitment could ameliorate allergic inflammatory responses. Since

monocyte recruitment has been shown to depend on C-C chemokine receptor 2 [37•], its blockade could have potential utility. Indeed, this approach has been demonstrated to be efficacious in a primate model of allergic asthma, where it resulted in less airway hyperresponsiveness and lower numbers of eosinophils and AMs in bronchoalveolar lavage fluid [70].

As mentioned earlier, various biologic therapies targeting type 2 cytokine pathways have shown efficacy or promise in treating allergic asthma. Interrupting these pathways may be expected to similarly abrogate alternative activation of macrophages. However, as macrophage phenotypes are far more plastic than are those of T lymphocytes, an unintended consequence of such treatments could be reprogramming of macrophages towards classical activation [71]. Such a phenomenon was observed when ovalbumin-induced allergic airway inflammation was studied in an IL-4 receptor knockout mouse. When IL-4 receptor signaling was abrogated, macrophages switched exclusively to classical activation, which was associated with higher levels of type 1 cytokines and neutrophilic inflammation as compared to the patterns observed in wild-type allergic mice [58]. These results highlight the possibility that efforts to abrogate type 2 inflammation could, via a shift in macrophage polarization, merely divert the inflammatory response to an equally deleterious type 1-dominant form.

If resident AMs are indeed protective against allergic inflammation, then supporting or even boosting their maturation and numbers could be beneficial. Indeed, maturation of resident AMs has been reported to be deficient in the context of allergic inflammation, and this was attributable to a relative deficiency of GM-CSF; intrapulmonary administration of GM-CSF was found to promote AM maturation and enhance their ability to protect against viral exacerbations of asthma in a mouse model [72•]. Such an approach might have the additional benefit of enhancing proliferation of AMs [73]. Although GM-CSF is generally viewed as promoting type 2 inflammation, it is possible that in the appropriate context, its beneficial effects would predominate. Alternatively, treatment with other mitogens such as M-CSF may be considered.

A more direct approach to exploiting the homeostatic potential of normal resident AMs is to adoptively transfer them into the lungs. Alternatively, as non-pulmonary macrophages and hematopoietic macrophage precursors have been shown to acquire AM phenotypic features upon exposure to the environment of the lung [74•, 75, 76], transfer of other normal macrophages may suffice. This approach has been employed in murine models of allergic asthma, with conflicting results [77, 78]. A creative variation on this theme is exemplified by the recent report in which macrophages were initially treated ex vivo with prostaglandin E₂ (PGE₂) (which exerts a variety of anti-inflammatory or suppressive actions on macrophages, including strongly boosting their IL-10 production) prior to their adoptive transfer into the lungs during HDM challenge. This significantly attenuated allergic lung inflammation, whereas transfer of naïve macrophages failed to do so [78].

A large body of research has documented the ability of another type of cell-based therapy—namely administration of mesenchymal stem cells—to ameliorate tissue injury. In a number of organs, including the lung [79], this ability is shared by extracellular vesicles derived

from these same cells [80]. As extracellular vesicles possess numerous potential advantages over live cells as therapeutic vehicles, it is likewise attractive to consider potential anti-inflammatory actions of those derived from AMs. We have recently reported that AMs can dampen inflammatory responses in lung epithelial cells via the transcellular delivery of suppressor of cytokine signaling (SOCS) proteins in extracellular vesicles [81••]. Interestingly, AM capacity to package SOCS proteins within secreted vesicles was potentiated by treatment with PGE₂ or IL-10. The utility of these AM-derived vesicles in allergic inflammation remains to be established.

Conclusion

AMs serve an important and well-established role in innate immune responses. Despite the fact that AMs are the most prominent immune cells normally residing on the respiratory mucosal surface and are capable of either promoting or suppressing inflammatory responses in the airways, the role of AMs in allergic asthma has been understudied until recently. Many gaps in knowledge remain to be filled, but precedent suggests that macrophages of different origins or phenotypes have the potential to be protective and/or harmful in different stages of allergic airway disease. The enhanced capacity for phagocytosis and antigen presentation of IRF5+ macrophages may be useful in the initial response immediately after allergen exposure [82, 83], while the enhanced tissue reparative functions of Ym1+ macrophages may be useful at later time points. Under homeostatic conditions, completion of these tasks should favor resolution of inflammation. In asthma, however, either or both of these macrophage phenotypes remain persistently activated, thereby contributing to chronic inflammation and further damage of the airways (Fig. 1b). Further studying the effect of recruited, pro-inflammatory monocytes, and the anti-inflammatory ability of resident AMs may give us valuable information on how monocytes/macrophages control inflammation in allergic asthma. These findings could yield new therapeutic opportunities that may also be applicable to other allergic diseases.

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Fig. 1.

a Immediately after allergen exposure, there is rapid recruitment of monocytes that predominantly serve to promote acute inflammatory responses. By contrast, resident AMs, which are largely self-maintaining, mainly act to suppress the acute inflammation in an attempt to restore homeostasis. It is as yet unclear whether AMs can arise directly from recruited monocytes or from IMs as intermediate progenitors. These newly recruited monocyte-derived cells appear to oppose the suppressive actions of resident AMs. Pathogenic cells may possibly also arise from proliferating resident AMs under the influence of mediators secreted by recruited monocytes. **b** Inflammation becomes chronic after repeated exposures to allergen with the increased recruitment of immune cells and consequent elevated levels of cytokines such as IL-4, IL-13, and IFN γ . In response to these signals, AMs can polarize across a continuum of activation phenotypes, losing their suppressive functions and gaining pathogenic functions. IL-4/IL-13-induced AMs promote type 2/eosinophilic inflammation, and IFN γ -induced AMs are associated with type I/neutrophilic inflammation. It is as yet unclear whether these activated phenotypes can arise from recruited monocytes directly or from IMs as an intermediate progenitor