Editorial: Searching for definitive evidence of the role of eosinophils in lung disease: Are we there yet?

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Eosinophils are innate immune cells belonging to the granulocytic compartment of circulating WBCs. Although they were identified more than a century ago in blood, sputum, and lung lavage samples, they remain a controversial cell type for their role in immunity, particularly in lung immune responses. Several studies using congenitally eosinophil-deficient strains of mice, PHIIL, AdBGATA, and iPHII, have demonstrated a prominent role for pulmonary eosinophils in regulating immune responses in allergic asthma as well as in pulmonary fibrosis, although some reports have cast these findings into doubt [1–3]. Possible reasons for the discrepancy in these reports may be that the three eosinophil-deficient strains of mice so far developed rely on the use of cytocidal reagents (diphtheria toxin) or gene targeting of key transcription factors (i.e., GATA-1). Potential confounding effects of these strategies could obscure our understanding of eosinophil effector functions in lung pathologies.

Recently, a novel, double-knockout mouse strain of two gene products that are almost exclusively expressed in eosinophils, MBP-1 and EPX, was shown to result in eosinophil deficiency with no apparent effects on other leukocyte lineages [4]. Single-gene knockouts of MBP-1 and EPX were shown to have no effects on AHR or pulmonary health [5]. Interestingly, unlike the single-gene knockout strains, eosinophil progenitors that lack genes expressing both of the key granule proteins MBP-1 and EPX exhibit negative feedback that blocks critical steps in the transcriptional development program in eosinophils, ultimately leading to a massive loss of mature eosinophils from the circulation and peripheral tissues. Thus, there is a tightly controlled feedback mechanism between granule formation and eosinophilopoiesis that regulates the development and maturation of eosinophils, potentially involving the transcription factor XBP-1 [6] as well as cystatin F [7]. The resulting eosinophil deficiency in the MBP-1−/−/EPX−/− strain, with its reduced risk of off-target effects, would, therefore, be ideal for studying models of eosinophilic diseases, without the need for cytocidal reagents or targeting of transcription factors; have broad tissue specificity; and hopefully, go some way toward resolving lingering doubts surrounding the role of eosinophils in lung disease.

In this issue, Ochkur et al. [8] report important findings in the Journal of Leukocyte Biology showing that the double-knockout of MBP-1 and EPX results in the loss of salient features of eosinophil-associated pulmonary diseases, measured as AHR, inflammation of lung tissues leading to mucus overproduction (measured as GM/MA in lung sections), and reduced IL-4 and IL-13 production in the airways, similar to what has been observed in PHIIL, AdBGATA, and iPHII strains (Fig. 1). First, the MBP-1−/−/EPX−/− double-knockout mice were subjected to an acute model of allergen sensitization and challenge using OVA, followed by methacholine challenge, which is an acute model of immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. J. Exp. Med. 192, 271–280.


Abbreviations: AHR = airway hyperresponsiveness; Ears = eosinophil-associated RNases; ECP = eosinophil cationic protein; EDN = eosinophil-derived neurotoxin; ES cell = embryonic stem cell, HE2 = human eotaxin-2 transgenic mice, iS = IL-5 transgenic mice, GM/MA = goblet cell metaplasia/epithelial cell mucin; MBP-1 = major basic protein-1, EPX = eosinophil peroxidase; PHIIL = phosphodiesterase 6A; dblGATA = double GATA; iPHII = immunoglobulin M antibodies; PHIL = phosphodiesterase 6A; dblGATA = double GATA; iPHII = immunoglobulin M antibodies; PHIL = phosphodiesterase 6A; dblGATA = double GATA; iPHII = immunoglobulin M antibodies.

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asthma that has been conventionally used to understand immune mechanisms of allergic airway inflammation. The MBP-1\(^{-/-}\)/EPX\(^{-/-}\) double-knockout mice showed almost complete depletion of blood and tissue eosinophils, as detected by Ab labeling of Ears, which are the mouse homologs of human ECP and EDN, with comparable numbers to PHIL and ΔdblGATA strains. Following OVA challenge, MBP-1\(^{-/-}\)/EPX\(^{-/-}\) mice showed substantially less lung tissue eosinophils compared with wild-type controls, and this trend was associated with reduced IL-4 and IL-13 levels detected in bronchoalveolar lavage. In addition, a decrease in GM/MA was observed, suggesting reduced inflammation that leads to excess mucus production.

Then, the MBP-1\(^{-/-}\)/EPX\(^{-/-}\) double-knockout mice were crossed with an I5\(/e2) strain that generates substantial numbers of eosinophils and promotes a potent, activating environment for lung eosinophils so that they undergo massive degranulation in the airways, leading to greatly increased AHR, GM/MA, and extensive airway remodeling [9]. Offspring from the double-knockout mice bred against the I5\(/e2) strain were demonstrated to have substantially reduced eosinophil numbers in bronchoalveolar lavage fluid, along with IL-4 and IL-13, compared with I5\(/e2) mice. In parallel with this, I5\(/e2)/MBP-1\(^{-/-}\)/EPX\(^{-/-}\) mice showed significantly decreased airway resistance in response to methacholine challenge when compared with I5\(/e2) mice. Notably, a more pronounced decrease in GM/MA was observed in I5\(/e2)/MBP-1\(^{-/-}\)/EPX\(^{-/-}\) mice compared with the acute OVA sensitization and challenge model.

An interesting comparison in eosinophil degranulation responses was made between the two models of allergic airway inflammation and the I5\(/e2) mouse strain. In the former model, OVA sensitization and challenge usually exhibits minimal eosinophil degranulation, whereas extensive eosinophil degranulation is evident in the I5\(/e2) strain. In comparing these two mouse models, degranulation does not appear to contribute to AHR, whereby the loss of AHR was equivalent in the allergen-challenged (which lack degranulation) and I5\(/e2) mice (with extensive degranulation). On the other hand, eosinophil degranulation appears to contribute more to the development of pulmonary fibrosis (measured as GM/MA) because a more-pronounced decrease in GM/MA was observed in I5\(/e2)/MBP-1\(^{-/-}\)/EPX\(^{-/-}\) mice compared with OVA-challenged MBP-1\(^{-/-}\)/EPX\(^{-/-}\) mice. The association of eosinophils with development of pulmonary fibrosis was originally reported in ΔdblGATA mice on a BALB/c background, in which the lack of eosinophils did not affect lung inflammation, but, instead, reduced long-term remodeling [10]. These findings indicate that eosinophil degranulation may be a greater contributor to airway remodeling than AHR, although a direct comparison was not made between these models.

A strength of these findings is that two distinct disease models were used to test the effect of MBP-1 and EPX double-gene deletion in airway physiology and responses, and both models support a critical role for eosinophils in lung disease, specifically in relation to AHR and mucus production. One would infer that this would not be necessary, given the previous findings in earlier mouse models; however, there have been concerns that eosinophil-deficient strains of mice do not show consistent results regarding the role of eosinophils in allergic asthma and other lung diseases [1-3]. Moreover, strain-specific differences (C57Bl/6 vs. BALB/c) in these eosinophil-deficient mouse models contribute to the controversy surrounding the role of eosinophils in allergic lung inflammation [11]. Another important consideration is that the OVA model of allergic inflammation is being supplanted by the house dust mite extract model, which is thought to better reflect human disease, and this may also reveal differences in the role that eosinophils may have in allergic lung inflammation.

Although findings from the MBP-1\(^{-/-}\)/EPX\(^{-/-}\) strain are perhaps not surprising given that they corroborate earlier reports of eosinophil-deficient mice showing reduced AHR and diminished mucus production, this study represents a major advancement because of the reduced risk of off-target effects in the MBP-1\(^{-/-}\)/EPX\(^{-/-}\) mice, at least in comparison with PHIL, ΔdblGATA, and iPHIL mouse models. Moreover, this report provides strong confirmation of the central role that eosinophils have in lung disease and AHR, in addition to the three previous mouse models. These observations from the novel MBP-1\(^{-/-}\)/EPX\(^{-/-}\) mouse strain will go some way toward resolving differences reported in other studies.

In light of these findings, we must always remain mindful of the possibility of spontaneous off-target mutations that may arise in ES cells or later, during breeding because of gene knockouts, particularly when more than one gene is ablated because these may still result in confounding effects [12]. However, the MBP-1\(^{-/-}\)/EPX\(^{-/-}\) double-knockout strain used in the present study was back-crossed on the C57Bl/6 background for at least 20 generations, which should reduce the incidence of unwanted, spontaneous mutations or other off-target effects in this strain.

In conclusion, this report provides further definitive evidence that eosinophils have an essential role in allergic airway inflammation and pulmonary pathologies related to tissue eosinophilia in the airways leading to tissue remodeling and fibrosis. Findings from this study should play down fears that the observed eosinophil-dependent airway
remodeling and immune modulation in eosinophil-deficient strains of mice is an artifact of diphtheria toxin treatment or off-target effects resulting from gene deletion of the GATA-1 transcription factor. The results presented in this report will serve to extend previous studies, and they continue to support a prominent role for the enigmatic eosinophil in allergic airway inflammation. Indeed, clinical findings using the anti-IL-5 Ab treatment to deplete eosinophils in asthmatic subjects suggest that targeting eosinophils is beneficial for the treatment and management of allergic disease [13]. Perhaps the moral of these mouse models is that they reveal distinct aspects of allergic lung inflammation in an outbred human population. Future studies using the MBP-1<sup>−/−</sup>/EPX<sup>−/−</sup> mouse strain are anticipated, which will allow us gain a greater understanding of the specific function of eosinophils in immunity, which remains elusive, in spite of extensive experimental analysis using animal models.

REFERENCES


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