

Correspondence

Reply

To the Editor:

Recently, we reported that deletion of the *Atg5* gene, specifically in CD11c⁺ dendritic cells, leads to impairment of the autophagy pathway, causing unprovoked spontaneous airway hyperreactivity and severe neutrophilic lung inflammation in mice.¹ Our data also demonstrated that adoptive transfer of *Atg5*-deficient, but not wild-type, dendritic cells augments lung inflammation and increases neutrophils in lungs. Most importantly, we observed that neutrophilic asthma in *Atg5*-deficient mice is steroid resistant and IL-17A dependent. Since our report, few other studies have highlighted the importance of autophagy among CD11c⁺ cells, and reported the induction of proinflammatory IL-17-producing T cells as a result of chronic inflammation.^{2,3}

Mabalarajan⁴ suggests that β -actin may not be a suitable house keeping protein for Western blot assays, and that a difference in overall protein density may be responsible for our observations. First, we followed a protocol from “Guidelines for the use and interpretation of assays for monitoring autophagy,” which was developed by a consortium of experts in the field, to properly analyze the autophagy pathway and to avoid misinterpretations. According to the guidelines, increased LC3I/LC3II ratio in combination with increased amount of P62 is indicative of impairment of autophagy.⁵ We carefully normalized the density of the bands to actin loading control, and, more importantly, we also reported the LC3I/LC3II ratio. Second, our conclusions were not based on solely one experiment. We confirmed our results by confocal microscopy, adoptive transfer of CD11c⁺ cells, *in vitro* functional assays, and finally by the generation of 2 different bone marrow chimeras (Fig 2). The results of both chimera models supported the notion that bone marrow-derived immune cells rather than nonhematopoietic cells (including alveolar and bronchial epithelial cells) contribute to the severity of airway hyperreactivity and neutrophilia induced by deficiency in *Atg5*.

Unlike other *Atg* family members, *Atg5* has been previously characterized as a central autophagy protein, and deficiency in *Atg5* was shown to be embryonically lethal in murine models. Therefore, we have successfully generated 2 models: inducible *Atg5* knockout mice under *rosa*, and noninducible depletion of *Atg5* in tissue-specific cells such as CD11c⁺ cells or alveolar epithelial cells. As indicated in the article, constitutive depletion of *Atg5* among the CD11c⁺ population resulted in unprovoked severe neutrophilic asthma in mice, to the degree that the host

cannot be further challenged by allergens. Surprisingly, Mabalarajan⁴ failed to notice that CD11c-specific *Atg5*-deficient mice clearly show a high number of neutrophils in their airways at the steady state (Fig 5, H).

The observation that impaired autophagy is involved in the pathogenesis of asthma is supported by several lines of research showing the association of *Atg5* polymorphisms with asthma in human studies and the fact that T_H2 cytokines and viral infection inhibit autophagy significantly.^{6,7} We agree that more studies in the field of *Atg* alternative pathways are needed; nevertheless, our report indicates that *Atg*-dependent autophagy plays a protective role among the CD11c⁺ population and inducers of this pathway might represent novel therapeutic targets for the treatment of asthma.

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