Letter to the Editor

p38α MAP kinase promotes asthmatic inflammation through modulation of alternatively activated macrophages

Dear Editor,

Asthma is characterized by reversible airflow obstruction, bronchial hyper-reactivity, and chronic airway remodeling (Al-Muhsen et al., 2011). Pulmonary macrophages have been implicated in asthmatic inflammation (Lee et al., 2015; Qian et al., 2015). The p38 mitogen-activated protein kinase (MAPK) plays an essential role in inflammation, but its role in asthma has not been determined (Kim et al., 2008). Here, our data show that macrophage-specific p38α MAPK-deficient mice displayed attenuated asthmatic inflammation in response to three allergens (dust mite, ragweed, and *Aspergillus*; DRA). Furthermore, we found that the protective effect was strongly associated with a reduction in the alternatively activated macrophage (AAM) polarization *in vivo* and *in vitro*. Taken together, our data indicate that p38α MAPK in macrophages contributes to AAM polarization and could be a therapeutic target for asthma.

To determine whether p38α MAPK modulates asthma via macrophage polarization, we generated macrophage-specific p38α MAPK-deficient mice (p38α MAPK^Lyz2-KO) (Supplementary Figure S1). Then, in DRA-induced asthma model (Figure 1A), the pulmonary eosinophil infiltration was significantly attenuated in p38α MAPK^Lyz2-KO mice (Figure 1B and C).

We also found a reduction in the number of total cells and eosinophils in bronchoalveolar lavage fluid (BALF) of the p38α MAPK^Lyz2-KO mice (Figure 1D). In response to DRA challenge, total IgE in serum of wild-type (WT) mice was 1398 ± 221.3 ng/ml, while that in p38α MAPK^Lyz2-KO mice was extremely reduced to 861.2 ± 236.1 ng/ml (Figure 1E). Furthermore, compared with WT mice that displayed an impressive mucous gland and goblet cell hyperplasia by Periodic acid-Schiff (PAS) staining, p38α MAPK^Lyz2-KO mice displayed reduced goblet cell hyperplasia (Figure 1F). These data indicate that p38α MAPK is critically involved in modulation of asthmatic inflammation, which is markedly reduced in p38α MAPK^Lyz2-KO mice.

Pulmonary macrophages are generally linked to immune response in asthma, which are polarized into two subpopulations: classically activated macrophages (CAMs) and AAMs. In asthma, macrophage phenotype is characterized by low expression of MHCII, CD86, and iNOS2 but high levels of AAM markers, such as Arg1 (Arginase 1), Ym-1 (Chitinase-like protein 3), Fizz-1/RELM-α (found in inflammatory zone 1), and CCL17 (Girodet et al., 2016). Our previous results revealed that alveolar macrophages from mild asthmatic patients express high levels of autotaxin and macrophages from DRA-induced mouse asthma model exhibit AAM polarization (Park et al., 2013; Ackerman et al., 2016). To further determine the effect of macrophage polarization on asthmatic inflammation, we conducted the adoptive transfer experiment

(Supplementary Figure S2A) (Qian et al., 2015). The p38α MAPK^Lyz2-KO mice receiving p38α MAPK^+/+ macrophages significantly increased the eosinophil infiltration (CD11c^− SiglecF^+) by up to 88% of all BALF cells. In contrast, mice receiving p38α MAPK^−/− macrophages markedly attenuated the percentage of eosinophils by up to 59% (Supplementary Figure S2B). Furthermore, our results showed that adoptive transfer of p38α MAPK^+/+ macrophages significantly increased the total cell number in BALF, the amount of IgE in serum, and PAS-positive goblet cells compared with adoptive transfer of p38α MAPK^−/− macrophages (Supplementary Figure S2C–E). Thus, these data indicated that p38α MAPK can promote the allergic inflammation, at least in part, through modulating macrophage polarization.

Next, to determine whether p38α MAPK modulates AAM polarization *in vivo*, we measured the expression of AAM markers in the lung tissue of DRA-induced mice. As shown in Supplementary Figure S3A, DRA significantly induced Arg1, Ym-1, and Fizz-1 expression, and the expression of these markers was significantly attenuated in p38α MAPK^Lyz2-KO mice. Furthermore, as illustrated in Supplementary Figure S3B, mice receiving IL-4-induced p38α MAPK^+/+ macrophages showed a higher expression of Arg1, Ym-1 and Fizz-1 than mice receiving p38α MAPK^−/− macrophages.

Asthma is a Th2-mediated inflammatory response that is associated with increased Th2-type cytokine production.
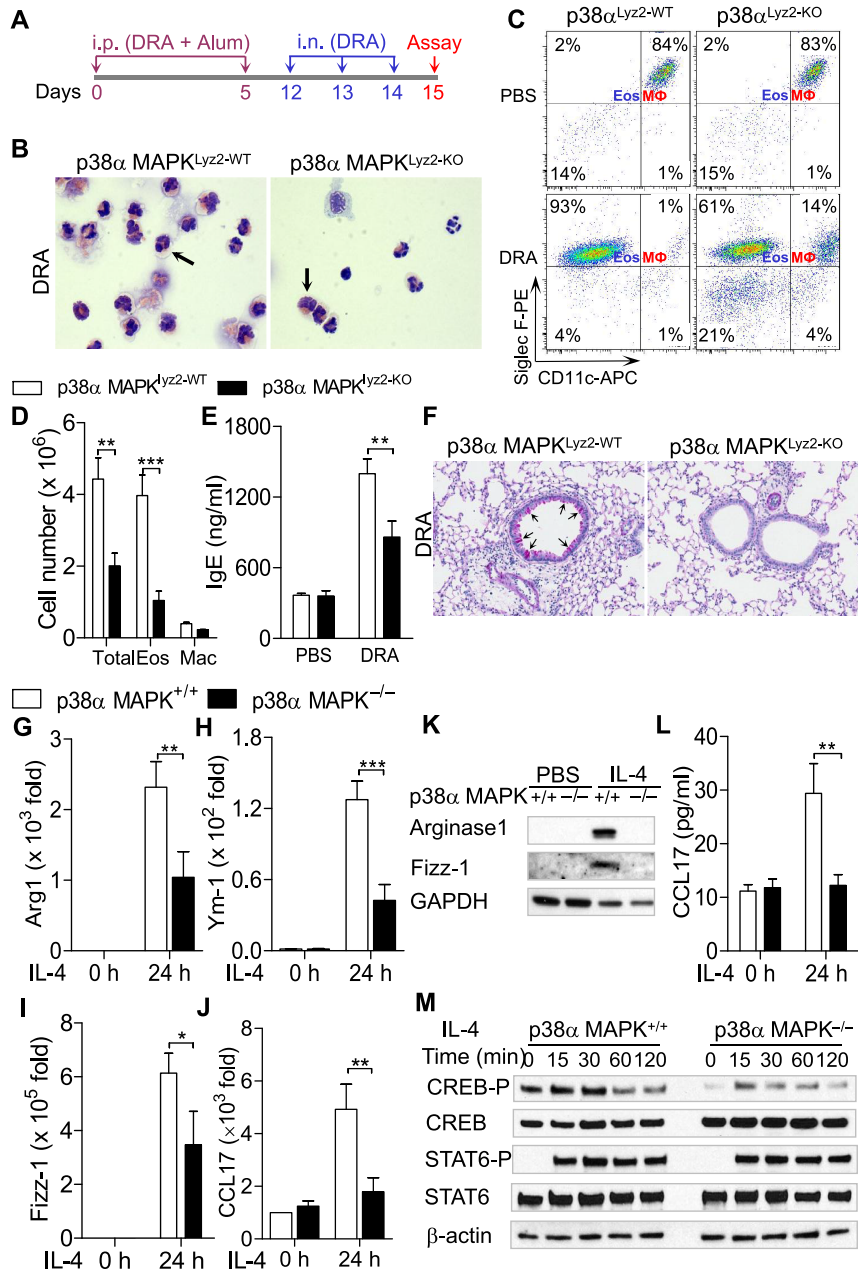
Figure 1 p38α MAP kinase promotes asthmatic inflammation through modulation of AAMs. (**A**) DRA-induced asthma model is presented in diagram. Mice were sensitized with 100 mg DRA + alum adjuvant (i.p., intraperitoneally) on Day 0 and Day 5 and then challenged with 60 mg DRA (i.n., intranasally) on Day 12, Day 13, and Day 14. On Day 15, asthmatic inflammation was detected. (**B**) BALF cells were stained with Hema-3 and black arrows point to eosinophils. (**C**) BALF cells were stained with Siglec F-PE and CD11c-APC and assessed by flow cytometry. Eosinophils (Eos, CD11c$^-$ Siglec F$^+$) and macrophages (MΦ, CD11c$^+$ Siglec F$^+$) were identified. (**D**) Total cells, eosinophils, and macrophages were determined based on the total number of cells and percentage of eosinophils and macrophages in BALF by cytospin. (**E**) Total IgE in serum was measured by ELISA. (**F**) The scanning of PAS-stained lung sections was performed by the Genie system and the black arrows point to PAS-positive goblet cells. (**G–J**) BMDMs isolated from p38α MAPK$^{Lyz2-WT}$ and p38α MAPK$^{Lyz2-KO}$ mice were challenged with IL-4 (5 ng/ml) for 24 h, and the mRNA expression levels of Arg1 (**G**), Ym-1 (**H**), Fizz-1 (**I**), and CCL17 (**J**) were measured by real-time PCR. The protein levels of Arginase 1 (**K**), Fizz-1 (**K**), and CCL17 (**L**) in p38α MAPK$^{+/+}$ and p38α MAPK$^{-/-}$ macrophages after treatment with IL-4 for 24 h were detected by immunoblot or ELISA. (**M**) The p38α MAPK$^{+/+}$ and p38α MAPK$^{-/-}$ BMDMs were stimulated with 5 ng/ml of IL-4 for 15, 30, 60, and 120 min, and phosphorylation of CREB and STAT6, as well as CREB, STAT6, and β-actin were detected with immunoblot. Results represent mean ± SEM (*n* = 6 for each group in **B–F**) and at least three independent experiments were performed in **G–M**. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. p38α MAPK$^{Lyz2-WT}$ vs. p38α MAPK$^{Lyz2-KO}$ mice received the same treatment.

Given that p38α MAPK modulates AAM polarization in DRA-induced asthma model, we next determined the role of p38α MAPK in AAM polarization *in vitro*. As shown in Supplementary Figure S4, IL-4 induced phosphorylation of p38 MAPK at 15 min and sustained for 4 h in bone marrow-derived macrophages (BMDMs), while p38 MAPK inhibitor SB203580 significantly attenuated IL-4-induced AAM markers in BMDMs. These results indicated that p38 MAPK signaling is required for AAM polarization.

To further investigate the function of p38α MAPK in AAM polarization, we challenged BMDMs from p38α MAPK[Lyz2-KO] mice with IL-4, in which p38α MAPK-deficient macrophages displayed a significant reduction in expression of Arg1, Ym-1, Fizz-1, and CCL17 (Figure 1G–J). In addition, protein levels of Arg1, Ym-1, and CCL17 were also attenuated in p38α MAPK-deficient BMDMs (Figure 1K and L). These data, collectively, suggested that p38α MAPK is required for IL-4-induced AAM polarization.

To determine how p38α MAPK modulates AAM polarization, we stimulated BMDMs with IL-4 for 15, 30, 60, and 120 min. IL-4 induced a similar STAT6 phosphorylation in both p38α MAPK^{+/+} and p38α MAPK^{−/−} macrophages, suggesting that p38α MAPK did not modulate AAM polarization via classic JAK/STAT6 signal. As a protein kinase, p38α MAPK further activates a series of transcription factors such as NF-κB, C/EBPβ, and CREB through phosphorylation of its downstream substrates (Zarubin and Han, 2005). Given that CREB is involved in AAM polarization (Luan et al., 2015; Sahu et al., 2017), we investigated whether the phosphorylation of CREB was modulated by p38α MAPK in BMDMs. The phosphorylation of transcription factor CREB was greatly reduced in p38α MAPK-deficient BMDMs upon IL-4 stimulation (Figure 1M). Because p38α MAPK/CREB is also required for CAM polarization in response to pattern-recognition receptors or type 1 cytokines (Kim et al., 2008), these data suggest that p38α MAPK/CREB signal pathway serves as an 'engine' to promote both CAM and AAM polarizations. In summary, our data indicate that p38α MAPK promotes asthma via enhancing AAM polarization and p38α MAPK is potentially a drug target for asthma.

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