Receptor-associated Signals
Responses by Orchestrating Multiple T Cell
Receptor-associated Signals

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Background: The MAP kinase p38α is a critical regulator of innate and adaptive immunity.

Results: p38α-deficient T helper cells produce more IL-4 in response to antigen exposure and preferentially differentiate into T helper type 2 (Th2) cells.

Conclusion: p38α negatively regulates Th2 responses in vitro and in vivo through modulating multiple TCR-associated signals.

Significance: Interfering with p38α pathway may present new therapeutic strategy for Th2 immunity-mediated diseases.

Mitogen-activated protein kinase p38α is a critical regulator of certain inflammatory diseases. However, its role in T helper type 2 (Th2) responses and allergic inflammation remains unknown. Here we show an increase in the production of interleukin-4 (IL-4) in p38α−/− CD4+ T cells in response to antigen stimulation. p38α-deficient naïve CD4+ T cells preferentially differentiate into Th2 cells through increased endogenous production of IL-4. Consistent with those results, we also observed decreased expression of p38α during T helper cell differentiation. Furthermore, deficiency of p38α alters the balance in the expression of NFATc1 and NFATc2 under steady-state conditions and enhances the expression and nuclear translocation of NFATc1 in CD4+ T cells upon antigen stimulation. Knockdown of NFATc1 significantly inhibits Th2 differentiation in p38α−/− T cells but not in p38α+/− T cells. p38α deficiency also inhibits the activation of Akt but enhances the activation of ERK in response to T cell receptor engagement without impacting IL-2/Stat5 signaling. In a model of ovalbumin-induced acute allergic airway inflammation, mice with induced deletion of p38α show elevated serum ovalbumin-specific IgE level, increased infiltration of eosinophils, and higher concentrations of Th2 cytokines including IL-4 and IL-5 in the bronchoalveolar lavage fluid relative to control mice. Taken together, p38α regulates multiple T cell receptor-associated signals and negatively influences Th2 differentiation and allergic inflammation.

Interfering with p38α pathway may present new therapeutic strategy for Th2 immunity-mediated diseases.

The abbreviations used are: TCR, T cell receptor; OVA, ovalbumin; BAL, bronchoalveolar lavage; Th1, T helper type 1; NFAT, nuclear factor of activated T cell; BAL, bronchoalveolar lavage.
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Our studies demonstrate that disruption of p38α in CD4+ T cells promotes IL-4 production in response to antigen stimulation. p38α-deficient CD4+ T cells preferentially differentiate into Th2 phenotype through increased endogenous production of IL-4. During T helper cell activation, mRNA and protein levels of p38α are decreased. p38α deficiency alters the balance in the expression of NFATc1 and NFATc2 and increases protein level and nuclear translocation of NFATc1 in CD4+ T cells upon TCR stimulation. Down-regulation of NFATc1 reverses enhanced Th2 differentiation due to absence of p38α. Deletion of p38α also impairs TCR-induced activation of Akt and enhances the activation of ERK. In ovalbumin-induced acute allergic airway inflammation, p38α−/− mice show increased infiltration of eosinophils and a higher concentration of Th2 cytokines IL-4 and IL-5 in bronchoalveolar lavage fluid relative to control mice. We conclude that p38α is a negative regulator of Th2 responses in vitro and in vivo by affecting multiple TCR-associated signals.

**EXPERIMENTAL PROCEDURES**

**Mice**—Mice carrying the floxed p38α allele (p38αf/f) have been previously described (14, 15). Mx-Cre mice were purchased from The Jackson Laboratory (Bar Harbor, ME). p38αf/f mice were crossed to Mx-Cre mice. Heterozygous Mx-Cre+/p38αf/f (p38α+/−) mice were crossed to p38αf/f mice to obtain Mx-Cre−/p38αf/f (p38α−/−) mice. To activate the interferon-γ-inducible Mx-Cre recombine, 6–8-week-old mice were injected intraperitoneally 3 times with polyIC. All experiments were performed at least 8 weeks after polyIC treatment. All studies were performed in a C57Bl/6 genetic background. All mice were maintained in specific-pathogen-free conditions, and experiments were approved by the Indiana University Laboratory Animal Research Center.

**Antibodies, Cytokines, and Reagents**—Anti-CD3, anti-CD28, anti-IFN-γ, PE-anti-IL17A, PE-anti-ROTY, FITC-anti-CD4, annexin V, and 7-Aminoactinomycin D antibodies were purchased from BD Biosciences. PECy7-anti-IL-4 was purchased from eBioscience (San Diego, CA). Akt, phospho-Akt, ERK, phospho-ERK, Stat5, phospho-Stat5, 1xβar, and p38α antibodies were purchased from Cell Signaling Technology (Beverley, MA). Anti-actin antibody was purchased from Miltenyi Biotec (Auburn, CA). For Th0 differentiation, purified naïve T cells in complete RPMI 1640 medium with IL-2 (5 units/ml) were stimulated with plate-bound anti-CD3 (2 μg/ml) and soluble anti-CD28 (1 μg/ml) antibodies. Cells were expanded on day 3. For nuclear translocation assay, the supernatants were immunoblotted with the indicated antibodies.

**Isolation of Cells and T Helper Cell Differentiation**—Naive CD4+CD25−CD62L+CD44+ T cells (1 × 10^6 cells/ml) were purified from spleen via magnetic isolation (Miltenyi Biotec, Auburn, CA). For Th0 differentiation, purified naïve T cells in complete RPMI 1640 medium with IL-2 (25 units/ml) were stimulated with plate-bound anti-CD3 (2 μg/ml) and soluble anti-CD28 (1 μg/ml) antibodies. Cells were expanded on day 3. For nuclear translocation assay, the supernatants were immunoblotted with the indicated antibodies.

**Preparation of Nuclear Extracts**—CD4+ T cells were either left unstimulated or stimulated with anti-CD3 and anti-CD28 antibodies. Nuclear extracts were prepared by first incubating the cells with a buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 2 mM MgCl2, 0.1 mM EDTA, 1 mM DTT, and protease inhibitors for 15 min, then 0.4% Nonidet P-40 was added, and cells were vortexed. Cells were then spun to pellet at 6000 rpm for 5 min in a benchtop centrifuge. The nuclear pellets were resuspended in an extraction buffer containing 20 mM HEPES (pH 7.9), 1.5 mM MgCl2, 0.2 mM EDTA, 0.63 mM NaCl, 25% glycerol, 0.5 mM DTT, and protease inhibitors, then vigorously vortexed for 30 min in the cold room. The lysed nuclei were centrifuged at maximum speed for 5 min. For nuclear translocation assay, the supernatants were immunoblotted with the indicated antibodies.

**Retroviral Transduction and T Cell Differentiation**—The pMKO.1 GFP retroviral vector (Addgene plasmid 10676, Cambridge, MA) was kindly provided by Dr. William Hahn. Mouse
NFATc1 shRNA sequences were obtained from Origene and were cloned into pMKO.1 GFP vector. Recombinant retrovirus was generated as described (16). For retroviral transduction, purified naïve CD4⁺ T cells were activated by plate-bound anti-CD3 and soluble anti-CD28 and were “spininfected” with fresh retroviral supernatant containing Polybrene. After viral transduction, GFP-positive cells were sorted and cultured under Th0 condition for further analysis.

**RESULTS**

**Effects of p38α Deletion on the Development, Proliferation, and Apoptosis of T Cells** —To address the genetic role of p38α in regulation of immune response, a newly established p38α conditional deletion system was utilized (14, 15). Previous studies have used Mx-Cre mice to efficiently delete the targeted gene to study its effect on CD4⁺ T helper cells (17, 18). By crossing mice bearing floxed p38α alleles with Mx-Cre bearing mice, p38α protein in splenocytes and purified CD4⁺CD25⁺CD62LhiCD44⁺ naïve T cells from p38α⁻/⁻ mice was completely deleted after three injections of polyIC (Fig. 1A). Based on previous reports and our observations, there was no significant phenotypic difference between wild type and p38α⁺/⁻ mice. Thus, in this study, we used p38α⁺/⁻ mice as the control mice. Flow cytometric analysis revealed that CD4⁺CD8⁺ ratios were similar in the thymus and spleen of p38α⁻/⁻ and p38α⁺/⁻ mice (Fig. 1B). These results suggest that inactivation of p38α does not perturb the development of CD4⁺ and CD8⁺ cells. We next investigated T cell function in the absence of p38α.
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FIGURE 2. Absence of p38α enhances IL-4 production when exposed to antigen. A, cultured splenocytes from control or OVA-sensitized p38α+/− and p38α−/− mice were re-stimulated with OVA (100 μg/ml) for 72 h. Concentration of IL-4, IL-5, and IFN-γ in the supernatants was measured by ELISA (n = 5 per group). *, p < 0.05. Data are the mean ± S.D. B, naive CD4+CD25−CD62L−CD44+ T cells from p38α+/− and p38α−/− mice were stimulated with immobilized anti-CD3 and anti-CD28. After 48 h, the concentration of IL-4 and IFN-γ in the supernatants was measured as panel A. *, p < 0.05. Data are the mean ± S.D.; n = 5.

the p38 pathway promotes cell cycle progression in multiple cell types (14, 19). To determine whether p38α affects T cell proliferation in response to antigen exposure, splenocytes from p38α+/− and p38α−/− mice were stimulated with αCD3 and αCD28 antibodies. [3H]Thymidine incorporation assay showed that p38α−/− T cells proliferated modestly but significantly more than p38α+/− T cells (Fig. 1C). Studies have shown that IL-4 prevents the apoptosis of CD4+ T cells in the absence of TCR activation (20). We, therefore, questioned whether deletion of p38α imposed more resistance in T cells to apoptotic stimuli. Under culture conditions lacking IL-4, apoptotic CD4+ T cells detected by annexin V and aminopterin were stained and counted. p38α−/− T cells detected by annexin V and aminopterin were stained and counted were not altered as a result of p38α deficiency (Fig. 1D). These results suggest that inactivation of p38α is dispensable for T cell development and apoptosis but modestly influences T cell proliferation.

p38α-deficient T Helper Cells Produce More Th2 Cytokines in Response to Antigen—CD4+ helper T cells are the essential regulator of adaptive immunity. After being activated, naïve CD4+ precursor cells differentiate into cytokine-secreting effector cells. Th1 produce interferon-γ, and Th2 cells make IL-4, IL-5, and IL-13 (21). Next, we examined the cytokine profile of CD4+ T cells from p38α−/− mice after TCR stimulation. Control and p38α−/− mice were immunized with OVA plus aluminum hydroxide, and total splenocytes were isolated and re-stimulated with OVA to determine the antigen-specific T cell response. As shown in Fig. 2A, enhanced production of IL-4 and IL-5 was detected by ELISA from p38α−/− splenocytes compared with control splenocytes. However, the production of IFN-γ, a key Th1 cytokine, was lower in p38α−/− splenocytes relative to control splenocytes. These results suggest that deletion of p38α causes a systemic increase in Th2 immunity. To further determine whether the enhanced Th2 cytokine production is due to an intrinsic T cell hyperresponsiveness to antigen stimulation, purified CD4+CD25−CD62L−CD44+ naïve T cells from control and p38α−/− mice were stimulated with plate-bound antibody to CD3 and anti-(α)CD28 for 48 h. The absence of p38α triggered greater production of IL-4. Meanwhile, the production of IFN-γ moderately decreased in p38α−/− T cells compared with control T cells (Fig. 2B). These results demonstrate that inactivation of p38α promotes the production of Th2 cytokines in T cells when exposed to antigen stimulation.

Deletion of p38α Promotes Th2 Cell Commitment through Enhanced Endogenous Production of IL-4—Because p38α−/− CD4+ T cells showed enhanced production of IL-4 and IL-4 is required for the initiation of Th2 cell differentiation (21), we examined if p38α regulates the differentiation of Th2 cells. To assess whether deficiency of p38α in T cells interfered with their ability to differentiate into IL-4-producing Th2 cells, IFN-γ-producing Th1 cells, and IL-17-producing Th17 cells, naïve CD4+CD25−CD62L−CD44+ T cells were purified from control and p38α−/− spleens and cultured under neutral (Th0), Th1, Th2, or Th17 polarizing conditions. In the Th0 condition, the frequency of IL-4-producing cells increased almost 2-fold as measured by flow cytometry (Fig. 3A), and a more potent production of IL-4 and IL-5 was detected by ELISA in supernatants derived from p38α−/− cultures (Fig. 3B). In contrast, p38α deficiency resulted in fewer IFN-γ-producing cells, and reduced production of IFN-γ was seen by ELISA in supernatants from control T cells (Fig. 3, A and B). Both p38α+/− and p38α−/− CD4+ T cells barely differentiated into Th17 cells or produced IL-17A under Th0 conditions (data not shown).

In Th2 conditions, with excess exogenous IL-4, p38α−/− T cells developed into IL-4+ cells as efficiently as control T cells, and no significant difference in the secretion of IL-4 was observed between p38α−/− and control T cells (Fig. 3, C and D). Both p38α+/− and p38α−/− CD4+ T cells were extensively differentiated into Th2 cells or produced IL-17A under Th0 conditions (data not shown).

Noubade et al. (22) reported that p38 controls Th17 development in a T cell-intrinsic manner. However, Huang et al. (23) recently reported that p38α plays a selective role in dendritic cell-mediated Th17 differentiation but not in T cell-intrinsic Th17 differentiation. In our experiments we detected no significant difference in the differentiation of Th17 cells between p38α+/− and p38α−/− CD4+ T cells under Th17 skewing conditions (Fig. 3, C and D). These results suggest that p38α may not regulate Th17 differentiation directly. The differences in the conclusions among different research groups could be a result of differences in the specificity of the mouse models used.

We further wondered whether p38α modulates the expression of the master transcription factors involved in T helper cell differentiation, including T-bet (Th1), Gata-3 (Th2), and RORγt (Th17). Consistent with the differentiation data, deletion of p38α slightly increased the expression of Gata-3 and inhibited the expression of T-bet. At the same time, p38α defi-
ciency did not significantly alter RORγt expression (Fig. 3, E and F).

Next, we showed that the presence of anti-IL-4 abolished the ability of p38α−/− naïve T cells to preferentially commit to the Th2 phenotype and mildly increased Th1 differentiation in p38α−/− T cells (Fig. 3G). This observation is consistent with the concept of inhibitory action of IL-4 on Th1 development (24, 25). Together, these results demonstrate that in the absence of polarizing cytokine, TCR-induced IL-4 production by CD4+ T helper cells is enhanced due to the absence of p38α, and p38α-deficient naïve T cells preferentially differentiate into Th2 cells due to increased endogenous production of IL-4.

**p38α Expression Is Reduced during T Helper Cell Differentiation**—In T helper cells, short-term stimulation of TCR (less than an hour) can activate p38 through alternative mechanisms that are independent of MKK3/6 (7, 8). However, the expression of p38α during a time span required for T helper cell differentiation remains unknown. We collected protein samples from wild type naïve CD4+ T cells that were induced to differentiate under un-polarized conditions (Th0). Western
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**FIGURE 4.** p38α expression is compromised during T helper cell differentiation. **A**, naïve CD4$^+$ T cells were activated with immobilized anti-CD3 (2 μg/ml) and anti-CD28 (1 μg/ml) and cultured under Th0 conditions for the indicated times. Protein levels of p38α and NFATc1 were detected by immunoblotting. **B**, naïve CD4$^+$ T cells were pretreated with bafilomycin A1 (100 ng/ml) and MG132 (20 μM), respectively, then activated by anti-CD3 and anti-CD28. p38α levels were measured by immunoblotting. **C**, naïve CD4$^+$ T cells were cultured as panel A. Total RNA was extracted, and mRNA levels of p38α were detected by real-time PCR. p38α expression relative to β-actin is shown as the mean ± S.D. from triplicate wells. *, p < 0.05. Data are representative of three independent experiments.

blotting results showed that compared with naïve CD4$^+$ T cells, p38α levels were significantly reduced during T cell differentiation (Fig. 4A), whereas the expression of NFATc1, a positive regulator of IL-4 production and Th2 differentiation (26), was increased (Fig. 4A). The inverse expression pattern between p38α and NFATc1 further suggests that repression of p38α pathway may be necessary for IL-4 secretion and Th2 development. We further examined the mechanism underlying down-regulation of p38α during T cell commitment. Treatment of cells with bafilomycin A1, a lysosome inhibitor, and MG132, a proteasome inhibitor, failed to prevent reduction of p38α expression (Fig. 4B). Thus, it is unlikely that decreased p38α expression is mediated via the degradation of p38α protein. In contrast, real-time PCR analysis revealed that mRNA levels of p38α were significantly reduced in differentiating CD4$^+$ T cells relative to naïve CD4$^+$ T cells (Fig. 4C), suggesting T helper cell activation negatively impacts the expression of p38α at the level of mRNA. These results further support that p38α is a negative regulator of Th2 development.

p38α is Required to Maintain Homeostasis of NFAT Transcription Factors in T Helper Cells and Down-regulation of NFATc1 Partially Reverses the Enhanced Th2 Development Due to p38α Deficiency—Next, we further investigated the mechanisms underlying negative regulation of p38α on IL-4 production and Th2 responses. Downstream of TCR, Ca$^{2+}$-dependent NFAT transcription factors play critical roles in regulating T cell functions. T cells express NFAT1/NFATc2, NFAT2/NFATc1, and NFAT4/NFATc3 (27). The balance among NFAT family members is required for proper generation of Th2 cells. NFATc1-deficient lymphocytes show decreased IL-4 production (26). However, NFAT1/NFATc2 knock-out mice show enhanced allergic immune response (28), and NFAT1/NFATc2-deficient T cells produce more IL-4 and preferentially differentiate into Th2 cells (29). T cells that lack both NFATc2 and NFATc3 differentiate into Th2 cells (30). We, therefore, assessed whether p38α modulates the expression of NFAT transcription factors in CD4$^+$ T helper cells. First, we detected basal protein levels of NFATc1 and NFATc2 in control and p38α$^{-/-}$ CD4$^+$ T cells. As shown in Fig. 5A, ratio of NFATc1: NFATc2 was higher in p38α$^{-/-}$ T cells relative to control T cells, whereas the protein level of JunB, another transcription factor involved in Th2 development (31), and Fyn, a tyrosine kinase downstream of TCR but upstream of PKC-θ/NF-κB involved in modulating Th2 cytokine secretion (32), was comparable between control and p38α$^{-/-}$ T cells. These results suggest that deletion of p38α skews the balance of NFATc1 and NFATc2 expression under steady-state conditions. Consistently, we found that inhibition of the p38 pathway by SB203580 in CD4$^+$ T cells also impairs the expression of NFATc2 but not NFATc1 (Fig. 5B). These results suggest that expression and activity of p38α plays an important role in maintaining basal NFATc2 expression compared with NFATc1 expression.

We next examined whether p38α regulates NFATc1 expression after T helper cell activation. As shown in Fig. 5C, TCR stimulation induced higher expression of NFATc1 in p38α$^{-/-}$ T cells compared with control T cells. TCR activation also increased the expression of NFATc2; however, its level was significantly lower in p38α$^{-/-}$ T cells relative to control T cells. Consistent with the results derived using whole cell lysates, p38α inactivation also resulted in increased nuclear NFATc1 (Fig. 5D). In contrast, nuclear localization of p65 RelA protein, a subunit of NF-κB complex, in p38α$^{-/-}$ T cells was comparable to control T cells after TCR stimulation. However, we observed the presence of nuclear P65 in p38α$^{-/-}$ T cells but not in control T cells under steady-state conditions, suggesting increased basal NF-κB activity in these cells due to p38α inactivation.

To further assess the role of NFATc1 in mediating the enhanced Th2 differentiation due to p38α deficiency, we knocked down NFATc1 expression in CD4$^+$ T cells by retroviral transduction of shRNAs as indicated (Fig. 5E). Down-regulation of NFATc1 did not significantly inhibit Th2 differentiation among p38α$^{+/−}$ T cells. However, knockdown of NFATc1 in p38α$^{-/-}$ T cells significantly reduced IL-4$^+$ population,
although it was still higher than the p38α−/− counterpart, indicating that in addition to NFATc1, other signaling molecules are likely involved (Fig. 5, F and G). Together, these data demonstrate that deletion of p38α alters the homeostasis of NFAT transcription factors, which may promote Th2 differentiation.

**Effects of the Absence of p38α on Downstream Pathways of TCR and IL-2—** TCR, IL-4, IL-2, and Notch signaling pathways play critical roles in IL-4 production and Th2 development (21, 33). Our observation that deletion of p38α has no significant influence on Th2 differentiation in the presence of exogenous IL-4 suggests that p38α may not affect IL-4/Stat6 signaling. We wondered whether inactivation of p38α alters downstream pathways from TCR and IL-2. Ca2+/calcinurin/NFATs, PI3K, and ERK can be activated by TCR and CD28 signaling (34–36). PKC-θ is also a critical downstream target of both TCR and CD28 and can activate NF-κB (37, 38). Studies have shown that PI-3 kinase/Akt, ERK, and NF-κB all mediate T cell differentiation. Activation of Akt promotes Th1 commitment (20). ERK is required for Th2 differentiation (39, 40). PKC-θ/NF-κB mediates Th2 development (20, 32). We consistently observed reduced basal phosphorylation of Akt in p38α−/− T cells versus control T cells (Fig. 6A), suggesting impaired PI3K/Akt signaling due to the absence of p38α in these cells. After stimulation with αCD3 and αCD28, the absence of p38α markedly impaired the phosphorylation of Akt (Fig. 6A). These biochemical findings are consistent with our results demonstrating that deletion

**FIGURE 5.** Altered NFAT homeostasis is required for enhanced Th2 differentiation due to p38α deficiency. A, naïve CD4+ T cells were collected from p38α+/+ and p38α−/− mice. Total cell lysates were prepared and analyzed by immunoblotting for NFATc1, NFATc2, JunB, Fyn, and p38α expression. B, CD4+ T cells were treated with SB203580 (SB; 10 μM), and protein levels of NFATc1 and NFATc2 were detected by immunoblotting. C, naïve CD4+ T cells from p38α+/+ and p38α−/− mice were stimulated with anti-CD3 and anti-CD28 for 48 h. Total protein was prepared, and expression of NFATc1 and NFATc2 was measured by immunoblotting. D, CD4+ T cells from p38α+/+ and p38α−/− mice were treated as in panel C. Nuclear protein was extracted, and protein levels of NFATc1 and P65 RelA were detected by immunoblotting. E, CD4+ T cells were infected with control or NFATc1 shRNA retrovirus. Protein levels of sorted GFP+ cells were measured by immunoblotting. F, CD4+ T cells from p38α+/+ and p38α−/− mice were “spininfected” with control or NFATc1 shRNA retrovirus as panel E. Sorted GFP+ cells were further cultured under Th0 conditions, and percentages of IL-4+ cells were analyzed by flow cytometry. G, shown is a quantitative analysis of the average percentage of IL-4+ cells (n = 3–4). *, p < 0.05. NS, not significant. Data are the mean ± S.D. Signals in A–E were quantified by densitometric scanning.
FIGURE 6. p38α modulates TCR and IL-2 signaling. A, naïve CD4⁺ T cells from p38α⁺/− and p38α⁻/⁻ mice were stimulated with anti-CD3 and anti-CD28 for indicated times. Total and phosphorylated Akt and Foxo3a were detected by immunoblotting. B, CD4⁺ T cells from p38α⁺/− and p38α⁻/⁻ mice were treated as in panel A, and total and phosphorylated ERK was detected by immunoblotting. C, after exposure to antigen, the protein level of IκBα was measured in p38α⁺/− and p38α⁻/⁻ T cells by immunoblotting. D, in vitro-expanded CD4⁺ T cells from p38α⁺/− and p38α⁻/⁻ mice were stimulated with IL-2, and total and phosphorylated Stat5 was detected by immunoblotting. Data are representative of two or three independent experiments. All signals were quantified by densitometric scanning.
of p38 inhibits the production of IFN-γ and Th1 differentiation. As a downstream target of Akt, phosphorylation of Foxo3a was also reduced in p38α−/− T cells relative to control T cells (Fig. 6A), suggesting that the absence of p38α induces higher Foxo3a activity in activated T helper cells. In contrast to Akt, inactivation of p38α significantly enhanced TCR-induced activation of ERK (Fig. 6B). In addition, we also observed reduced basal levels of IκBα protein in p38α−/− T cells (Fig. 6C), suggesting a higher basal IKKα/β activity in p38α-deficient T cells. However, after antigen stimulation the degradation of IκBα in control T cells was comparable to p38α−/− T cells, indicating that p38α is dispensable for the PKC-θ/NF-κB pathway in T helper cells (Fig. 6C), which is consistent with our p65 nuclear translocation findings observed in Fig. 5D. IL-2/Stat5 is required for optimal IL-4 production during T cell activation (21). To explore whether p38α modulates IL-2/Stat5 activation, T cells were subjected to IL-2 stimulation, and the level of tyrosine phosphorylation of Stat5 was assessed. Stat5 phosphorylation was similar in p38α−/− T cells and control T cells (Fig. 6D). These results suggest that the absence of p38α affects downstream output of TCR but not IL-2 signaling.

**FIGURE 7.** p38α−/− mice display enhanced allergic airway inflammation. A, total BAL fluid cells from OVA-sensitized/challenged p38α+/− and p38α−/− mice and number of eosinophils in BAL fluid were counted. B, lung histopathology of OVA-sensitized mice after the final challenge is shown. Lung sections stained with hematoxylin and eosin show increased perivascular and peribronchial infiltration of leukocytes in p38α−/− mice. Scale bar, 50 μM. C, lung histopathology demonstrates perivascular and peribronchial infiltration of eosinophils by Giemsa staining. Scale bar, 12.5 μM. D, concentration of IL-4 and IL-5 in BAL fluid from OVA-sensitized/challenged p38α+/− and p38α−/− mice was detected by ELISA. E, serum OVA-specific IgE concentration in OVA-sensitized/challenged p38α+/− and p38α−/− mice was measured by ELISA. The number of mice per group in panels A–E was between four and eight. *, p < 0.05, data in A and D are mean ± S.D.; horizontal bars in E denote the mean.

Disruption of p38α Enhances the Susceptibility to Acute Allergic Airway Inflammation—To further assess the effects of p38α deletion on Th2 responses in vivo, OVA-induced airway allergic inflammation, which is mediated by Th2 immunity, was studied. We immunized mice intraperitoneally with OVA and the adjuvant aluminum hydroxide. After the final OVA challenge, we found that in p38α−/− mice, the total number of inflammatory cells in the bronchoalveolar lavage (BAL) fluid were almost 2.0-fold higher than control counterparts, and the
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number of eosinophils in the BAL fluid was similarly increased (Fig. 7A). Lung histology showed higher numbers of inflammatory cells around blood vessels and airways of p38α^{−/−} mice relative to controls (Fig. 7B). Giemsa staining showed less eosinophils around airways and the blood vessels of control mice compared with p38α^{−/−} mice (Fig. 7C). Th2 cytokines IL-4 and IL-5 in the BAL fluid from p38α^{−/−} mice were higher compared with control mice based on ELISA analysis (Fig. 7D).

Consistent with this result, deletion of p38α significantly elevated the OVA-specific IgE level in the serum (Fig. 7E). Thus, inactivation of p38α imposes hyper-responsiveness of Th2 immunity during acute allergic airway inflammation.

**DISCUSSION**

p38α is a master regulator of critical inflammatory mediators, including TNF-α, IL-1, and COX2, and activation of p38 is believed to play an essential role in the pathogenesis of diseases such as rheumatoid arthritis and Crohn disease. However, its role in other types of inflammatory responses such as allergic inflammation is unclear. Here, we provide evidence to demonstrate that p38α negatively regulates Th2 immunity. p38α deficiency elevates TCR-induced IL-4 expression and promotes T helper cell differentiation into Th2 phenotype. During T helper cell activation, mRNA and protein levels of p38α are decreased. p38α^{−/−} mice display more severe allergic airway inflammation relative to control mice. The absence of p38α alters the homeostasis of NFAT transcription factors and affects pathways downstream from TCR. Interfering with p38α may present a potential therapeutic strategy for Th2 immunity-mediated diseases.

Based on our results, we propose a model for p38α in the regulation of NFAT transcription factors, IL-4 production, and Th2 differentiation in response to T cell activation. In control T helper cells, the presence of p38α maintains NFAT (NFATc1 and NFATc2) homeostasis. Upon TCR stimulation, down-regulation of p38α shifts the balance toward NFATc1 from NFATc2, and increased expression of NFATc1 promotes IL-4 production and Th2 differentiation. However, in p38α^{−/−} T helper cells, the absence of p38α not only alters NFAT homeostasis under steady-state conditions, but once activated, these cells respond more rapidly, as they were already “primed.” After TCR stimulation, the absence of p38α inhibits Akt activation and enhances ERK activation, which may result in decreased Th1 and increased Th2 differentiation (20, 39) but may not affect IL-4/Stat6 and IL-2/Stat5 pathways. These data suggest that p38α utilizes multiple TCR-associated signals to influence Th2 commitment.

Prior studies involving TCR-mediated p38 activation have primarily focused on its phosphorylation by upstream kinases such as MKK3/6 as well as alternative pathways (7, 8). Here, we provide evidence to suggest that during T helper cell differentiation, expression of p38α is compromised. This may represent a novel mechanism to modulate p38α MAP kinase signaling. In T helper cells, this form of regulation is not restricted to the p38 pathway. Previous studies have shown that during T cell activation, TCR also induces JNK expression (41). Although it is known that p38α mediates stress response and regulates apoptosis in many cell types including in CD8 T cells (42), in CD4 T cells the PKC-θ/NF-κB pathway is a more important regulator of apoptosis. It is curious that T helper cells restrain prolonged activation of p38 during T cell activation. Although it is not clear how exactly this is achieved; temporospatial regulation of p38α could explain the diverse functions p38α is able to mediate in distinct cell types. We propose that during T cell differentiation, T helper cells utilize at least two mechanisms to regulate the p38α pathway. If antigen exposure is temporary and short-lived, TCR activates p38α through phosphorylation. In contrast, if antigen persists, prolonged process of differentiation inhibits the expression of p38α. Thus, the duration of antigen presence may regulate T helper cell functions through differential regulation of the p38α pathway, and p38α may play an important role in balancing Th1 and Th2 response.

Recently, studies utilizing mice deficient in the expression of MK2 have shown increased OVA-induced airway inflammation (43). In a skin inflammation model, conditional knock-of p38α in myeloid cells also enhances inflammatory response (44). Hyper-inflammatory phenotype is also observed in mice lacking both MSK1 and MSK2 (45). Thus, the divergence of pro-inflammatory and anti-inflammatory signaling in the p38 pathway highlights the importance to selectively target the inflammatory processes.

Because Mx-cre can disrupt p38α in hematopoietic cells, other immune cells in addition to T cells could contribute to the in vivo phenotype of allergic airway inflammation. Of note, we did not observe the phenotypic difference of B cell development between control and p38α^{−/−} mice. And a previous report has shown that B cells are not required for OVA-induced allergic airway inflammation (46). There is evidence that mast cells do not play an essential role in OVA-induced acute airway allergic inflammation (47). The amount of blood eosinophils in p38α^{−/−} mice is comparable to control mice.3 Most of our in vitro experiments are conducted in purified CD4^{+} T cells, and the results are consistent with the altered Th2 response in vivo. Thus, we believe that enhanced allergic airway inflammation in p38α^{−/−} mice is due to altered T cell function due to p38α deficiency in T cells.

Th2 immunity regulates both anti-parasitic and allergic responses; inhibition of p38 may enhance expulsion of intestinal parasites. However, in the case of allergic inflammation, inhibition of p38 may accelerate disease pathogenesis, and therefore, inhibition of p38 should be considered cautiously. Taken together, it will be helpful to further identify downstream regulators of p38 during modulation of Th2 immunity. These regulators may represent attractive targets for therapeutic intervention in asthma and other allergic inflammatory diseases.

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