A Critical Role of SHP-1 in Regulation of Type 2 Inflammation in the Lung

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Asthma is a chronic inflammatory disorder of the airways. Type 2 T helper (Th) cell–dominated inflammation in the lung is a hallmark of asthma. Src homology 2 domain–containing protein tyrosine phosphatase (SHP)-1 is a negative regulator in the signaling pathways of many growth factor and cytokine receptors. However, a direct role of SHP-1 in the IL-4/IL-13 signaling pathway has not been established. In this study, we sought to define the function of SHP-1 in the lung by characterizing the pulmonary inflammation of viable motheaten (*mev*) mice, and to investigate the molecular mechanisms involved. Pulmonary histology, physiology, and cytokine expression of *mev* mice were analyzed to define the nature of the inflammation, and the gene-deletion approach was used to identify critical molecules involved. In *mev* mice, we observed spontaneous Th2-like inflammatory responses in the lung, including eosinophilia, mucus metaplasia, airway epithelial hypertrophy, pulmonary fibrosis, and increased airway resistance and airway hyperresponsiveness. The pulmonary phenotype was accompanied by up-regulation of Th2 cytokines and chemokines. Selective deletion of IL-13 or signal transducer and activator of transcription 6, key genes in the Th2 signaling pathway, significantly reduced, but did not completely eliminate, the inflammation in the lung. These findings suggest that SHP-1 plays a critical role in regulating the IL-4/IL-13 signaling pathway and in maintaining lung homeostasis.

Keywords: Src homology 2 domain–containing protein tyrosine phosphatase-1; protein tyrosine phosphatase; motheaten mouse; type 2 T helper cell inflammation; lung

Asthma affects more than 20 million people in the United States alone. Chronic type 2 T helper (Th) cell–dominated inflammation in the airways is a key feature of asthma. The Th2 cytokines, IL-4 and IL-13, play a central role in mediating allergic inflammatory responses. IL-4 and IL-13 use IL-4 receptor (IL-4R) α for downstream signaling, including activation of signal transducer and activator of transcription (STAT)-6 and transcription of target genes. Although activation of STAT6 and other key components in response to IL-4 and IL-13 has been well documented, the molecular mechanisms responsible for termination of IL-4 and IL-13 signaling are less clear. Some of the molecules involved in the negative regulation of IL-4Rα signaling may include suppressor of cytokine signaling, B-cell lymphoma (BCL)-6, SH2 domain-containing inositol 5-phosphatase (SHIP)-1, and Src homology 2 domain–containing protein tyrosine phosphatase (SHP)-1 (1, 2).

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CLINICAL RELEVANCE

This study revealed a critical role of the phosphatase Src homology 2 domain–containing protein tyrosine phosphatase-1 in maintaining lung homeostasis and in regulating type 2 T helper cell inflammation in the lung. More attention should be given to the process of negative regulation in understanding and controlling pulmonary inflammation.

SHP-1 as a negative regulator, has been shown to associate with a variety of cytokine and growth factor receptors and immunoreceptors, including IL-3R, B-cell receptor (BCR), T-cell receptor (TCR), killer-cell immunoglobulin-like receptor (KIR), and Erythropoietin receptor (EPOR). Once phosphorylated and activated, SHP-1 binds to and dephosphorylates its target molecules and terminates the signaling. In the absence or decreased expression of SHP-1, cytokine/growth factor signaling will go unchecked, which may lead to abnormal or pathological responses (3, 4).

SHP-1 has been shown to regulate IL-4 and IL-13 signaling in cultured cells by binding to IL-4Rα and dephosphorylating the downstream target, STAT6, therefore decreasing the transcription of target genes (5). It was also shown *in vitro* that SHP-1 is a key enzyme for early dephosphorylation of STAT6 after activation by IL-4, and this process largely depends on the binding of functional SHP-1 to the immunoreceptor tyrosine–based inhibitory motif of IL-4Rα (6, 7), which may function as a docking site for phosphatases, including SHP-1. So far, there has been no report that links SHP-1 deficiency directly with allergic disorders in humans. Support for SHP-1 having a role in regulating allergic responses was suggested in a study of an asthma model using heterozygous motheaten (*me*) mice, which have reduced SHP-1 phosphatase activity. When immunized and challenged with ovalbumin, these mice mounted an enhanced Th2 response and airway hyperresponsiveness (AHR) as compared with wild-type (WT) mice (8). More recently, we reported that SHP-1 deficiency in mice increases cellular and tissue susceptibility to oxidant stress that may lead to enhanced inflammatory responses to aeroallergen stimulation (9).

The *me* mice and viable *me* (*mev*) mice are two natural mutant strains deficient in SHP-1 (10–12). These mice develop spontaneous inflammatory disorders in multiple organs (10, 13, 14). Different terms have been used to describe the lung pathology of these mice, including pneumonitis, unusual pneumonia, and interstitial lung disease (10, 11, 15, 16). However, the exact nature of the inflammation in the lung is still not clear. Furthermore, the molecular mechanisms by which SHP-1 deficiency causes abnormalities in the lung are not defined.

To understand the nature of the *me* lung pathology, we examined *mev* mice, performed comprehensive analyses of the histopathology and pulmonary physiology of these mice, and investigated the cellular and molecular mechanisms that are

involved. Our studies demonstrate that type 2 inflammation is a prominent feature of the *mev* lung pathology, and Th2 cytokines, especially IL-13, are up-regulated in the lung. Experiments using cytokine and STAT6 gene knockout (KO) mice revealed that the Th2 signaling pathway is largely responsible for the pulmonary phenotype resulting from the SHP-1 deficiency.

MATERIALS AND METHODS

Animals

The *mev* (*Ptpn6*^{mev}) mice and IL-4–null mice on C57BL/6 genetic background were from the Jackson Laboratory (Bar Harbor, Maine). Heterozygous mice (*Ptpn6*^{mev}/+) were interbred to generate WT, heterozygous, and homozygous mice (*Ptpn6*^{mev}/*Ptpn6*^{mev}, hereafter abbreviated as *mev*). The genotype of these mice was determined using PCR primers and protocols described by the Jackson Laboratory. STAT6-null mice on BALB/c genetic background were purchased from the Jackson Laboratory, and were backcrossed to C57BL/6 background for more than 10 generations. IL-13–null mice were generated as described previously (17), and backcrossed to C57BL/6 background for more than 10 generations. Crossbreeding between *mev* mice and IL-4, IL-13, or STAT6-null mice was performed to generate *mev* mice on respective gene KO background. Mice were used for experiments at 7 to 9 weeks of age, unless indicated otherwise. All mice were housed in cages with microfilters in the specific pathogen-free environment. All procedures performed on mice were in accordance with the National Institutes of Health guidelines for humane treatment of animals and were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University.

Lung and Bronchoalveolar Lavage Samples

Lung tissue and bronchoalveolar lavage (BAL) samples were obtained as previously described by our laboratories (18, 19). Briefly, mice were anesthetized; the trachea was isolated by blunt dissection; and a small-caliber tubing was inserted and secured in the airway. Three successive volumes of 0.75 ml of PBS with 0.1% BSA were instilled and gently aspirated and pooled. BAL samples were centrifuged, and supernatants were stored at −70°C until assayed. Cells in 100 μl aliquots were counted. A total of 100,000 viable BAL cells were centrifuged onto slides by a Cytospin 3 (Thermo Shandon Ltd, Runcorn, UK) and stained with Hema 3 System (Fisher Scientific Co., Newark, DE). The numbers and types of cells in the pellet were determined. The lung was perfused with cold PBS through the right ventricle with cut vena cava until the pulmonary vasculature was cleared of blood. The whole lung was either excised for RNA and protein analyses or inflated with fixatives for histology.

Histology Evaluation

Hematoxylin and eosin, periodic acid Schiff (PAS), Masson's trichrome, and Alcian blue (AB) stains were performed on lung sections after fixation with Streck solution (Streck Laboratories, St. La Vista, NE), as previously described (19). The same microscopic magnification was used for the sample slides from WT and *mev* mice under comparison.

Assessment of Pulmonary Physiology

To assess pulmonary physiology, invasive pulmonary function tests were performed. The baseline airway resistance and AHR to methacholine (MCh) challenge were assessed. Mice were anesthetized with intraperitoneal injection of 100 μl/30 g of a mixture of ketamine (15 mg/ml) and xylazine (15 mg/ml). A tracheotomy was performed using an 18-gauge intravenous adaptor, and a polyethylene cannula was inserted into the distal trachea. The mice were connected to a computer-controlled small animal ventilator (FlexiVent; SCIREQ, Montreal, PQ, Canada) and ventilated at a frequency of 150 breaths/minute and a volume of 6 ml/kg, with a positive end-expiratory pressure of 3 cm H_2O. Airway resistance was measured by using the forced oscillation technique (20). Dose–response curves to inhaled MCh (Sigma, St. Louis, MO) were determined. MCh was diluted in sterile PBS to 0, 1.56. 3.12, 6.25, and 12.5 mg/ml, and delivered through an in-line nebulizer. After each dose, data were collected at 1-minute intervals and then averaged. The values for airway resistance (cm H_2O/ml/s) were plotted as a function of MCh doses.

mRNA Analysis

Total cellular RNA from lung tissues was obtained using Trizol reagent (Invitrogen, Carlsbad, CA). The mRNA of specific genes was evaluated by RT-PCR using specific primers, as previously described (19). The mRNA of β-actin was used as an internal control.

Cytokines and Chemokines

The levels of cytokines and chemokines in the BAL were determined using commercially available ELISA kits per the manufacturer's instructions (R&D Systems, Minneapolis, MN; eBioscience, San Diego, CA).

Western Blot Analysis

Antibody to MUC5AC was purchased from Neomarker (Fremont, CA). Antibodies to STAT6 and phosphorylated STAT6 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Statistical Analysis

Most of the data were assessed by Student's *t* test and expressed as mean (± SEM). Differences between groups with *P* values of 0.05 or less were considered statistically significant.

RESULTS

Pulmonary Inflammation

To begin to define the nature of the inflammation seen in the lung of *mev* mice, we compared the lung histopathology of WT and *mev* mice. All the mice examined in this study were 7 to 9 weeks old. Lung sections from WT mice had no infiltration of inflammatory cells. In contrast, patchy cellular infiltration of the airways and lung parenchyma was readily seen in lung sections from *mev* mice, which ranged from mild infiltrates to total lobar consolidation (Figure 1A). Under higher magnification, clusters of eosinophils (arrows) and enlarged macrophages were readily seen in the airways and lung parenchyma of *mev* mice (Figure 1A). These macrophages had crystalline inclusions typical of murine Th2 inflammation, which we and others have previously shown to correspond to the chitinase-related protein, Ym1/2 (21, 22). Consistent with these findings, BAL total cell counts and differential analysis in *mev* mice showed significant increases in all cell types, including macrophages, eosinophils, lymphocytes, and neutrophils, as compared with WT mice (Figure 2B). These results indicate that the pulmonary inflammation seen in *mev* mice is a characteristic type 2 inflammatory response.

Mucous Metaplasia

To determine whether mucous metaplasia occurred in the airways, we analyzed lung sections from WT and *mev* mice using PAS and AB staining for neutral and acidic mucins, respectively. No PAS- or AB-positive staining was found in the small airways in the lung sections of WT mice, with occasional positive cells in the large airways (Figure 3A). In contrast, markedly increased PAS-positive and AB-positive goblet cells were readily seen in the airways of *mev* mice (Figure 3A). To further confirm this observation, we assessed the expression of mucin gene, MUC5AC, at mRNA and protein levels in the lung. RT-PCR results showed that low levels of MUC5AC mRNA were seen in the lung of WT mice, whereas increased MUC5AC mRNA was found in the lung of *mev* mice (Figure 3B). Western slot blot using anti-MUC5AC antibody showed markedly increased MUC5AC in the BAL fluid from *mev* mice as compared with that of WT mice (Figure 3C). These results indicate that SHP-1 deficiency resulted in goblet cell metaplasia, mucous hyperproduction, and up-regulation of MUC5AC gene expression in the airways.

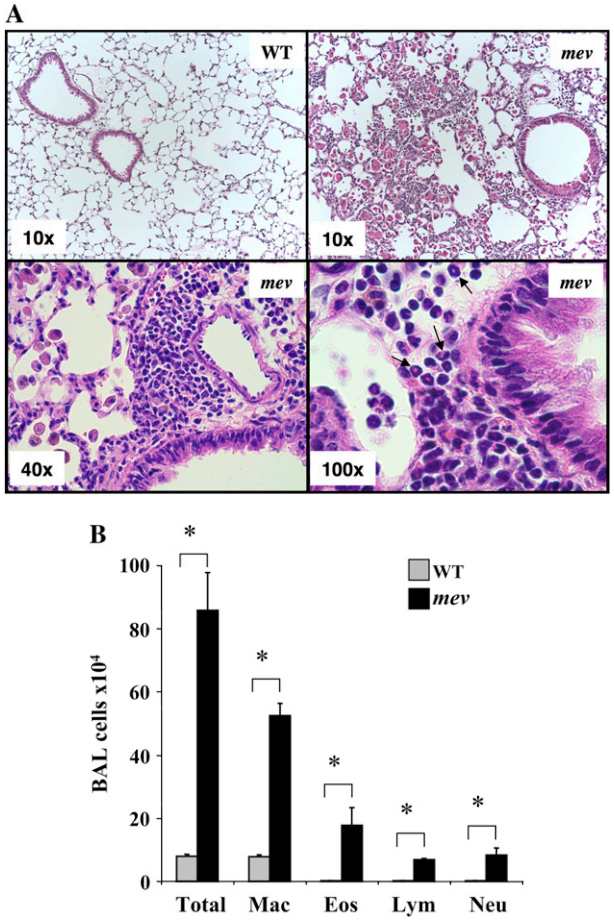

Figure 1. Comparison of lung histology and bronchoalveolar lavage (BAL) cellularity of viable motheaten (*mev*) mice and wild-type (WT) littermate controls. (*A*) Representative hematoxylin and eosin–stained lung sections from 9-week-old WT and *mev* mice. *Arrows* indicate eosinophils. (*B*) BAL samples were obtained from WT and *mev* mice ($n = 6$ each), and total cell counts and differentials were determined ($^*P < 0.05$). Eos, eosinophils; Lym, lymphocytes; Mac, macrophages; Neu neutrophils. Data represent mean and SEM values.

Fibrosis in the Lung

As part of the airway remodeling, there is increased airway wall thickness, which includes an increase in connective tissue. To assess fibrosis in the lung, Masson's trichrome staining was used to detect collagen deposition. As shown in Figure 4A, trichrome-positive–stained material can be seen around the airways of WT mice (Figure 4A). However, significantly increased collagen deposition was readily seen around the airways and in the parenchyma of *mev* mice (Figure 4A). To understand the molecular mechanisms of the fibrotic change, we compared the expression of transforming growth factor (TGF)-β1 in the airways of WT and *mev* mice by ELISA. As shown in Figure 4B, either total or activated TGF-β1 was barely detectable in the BAL from WT mice. In contrast, TGF-β1 protein was highly increased in the lung of *mev* mice, including spontaneously activated TGF-β1 (Figure 4B). These data indicate that SHP-1 deficiency resulted in pulmonary fibrosis.

Airway Epithelial Hypertrophy

One of the pathological changes in the lung of patients with asthma is airway epithelial hypertrophy. Comparing the airways of *mev* mice with those of similarly sized WT mice, it was noticed that the airway epithelium in the lung of *mev* mice was thickened (Figures 1A, 2A, and 3A).

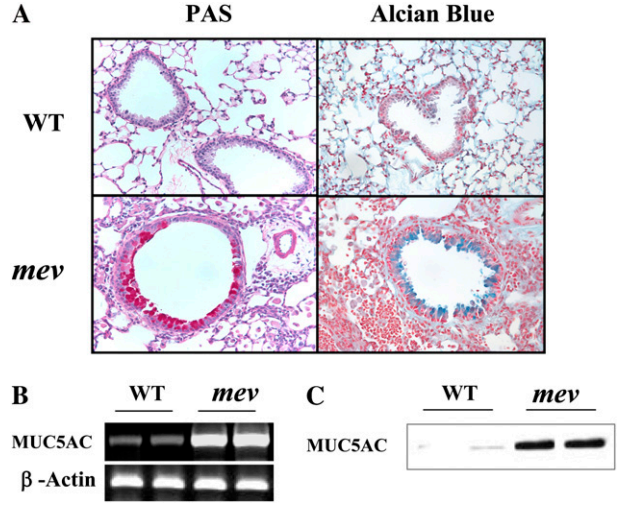

Figure 2. Mucous metaplasia and up-regulation of MUC5AC in the lung of *mev* mice. (*A*) Lung sections from WT and *mev* mice were stained with periodic acid Schiff for neutral mucin, and with Alcian blue (AB) for acidic mucin. (*B*) RT-PCR analysis of MUC5AC expression in the lungs of WT and *mev* mice. (*C*) Western slot blot of MUC5AC in BAL fluids from WT and *mev* mice.

Abnormal Pulmonary Physiology

Analysis of the lung mechanics using the forced oscillation technique demonstrated that, in the absence of any stimulation, *mev* mice already had significantly increased baseline airway

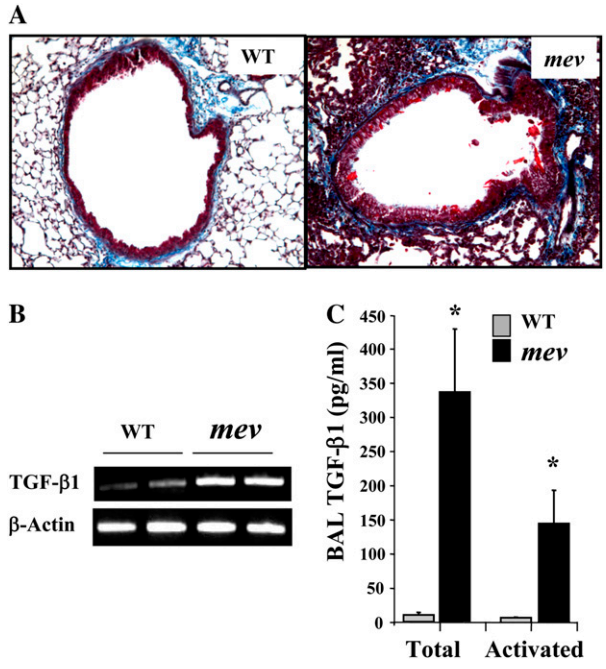

Figure 3. Pulmonary fibrosis. (*A*) Masson's trichrome staining of lung sections from WT and *mev* mice. (*B*) RT-PCR analysis of transforming growth factor (TGF)-β1 expression in the lungs of WT and *mev* mice. (*C*) Protein levels of total and active TGF-β1 in BAL fluids from WT and *mev* mice determined by ELISA ($n = 6$ for each group; $^*P < 0.05$). Data represent mean and SEM values.

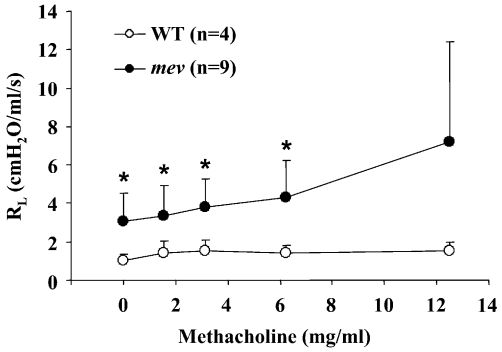

Figure 4. Airway resistance and airway hyperresponsiveness of WT and *mev* mice. Invasive pulmonary function tests were performed on anesthetized WT and *mev* mice using the FlexiVent System, as described in MATERIALS AND METHODS. Airway resistance in response to increasing concentrations of methacholine through inhalation was recorded and analyzed (*$P < 0.05$). Data represent mean and SEM values.

resistance compared with that of WT mice (Figure 4). When challenged, *mev* mice showed further increases in airway resistance in response to increasing concentrations of MCh, whereas WT mice only showed small increments (Figure 4). The variation of airway resistance within the *mev* group significantly increased when the concentration of MCh was 12.5 mg/ml, and some *mev* mice died at 25 mg/ml of MCh. Thus, no consistent data could be obtained at concentrations higher than 12.5 mg/ml.

Expression of Cytokines and Activation of Th2 Signaling Pathway

To understand the underlying molecular mechanisms of the pulmonary inflammation in *mev* mice, we investigated the cytokine expression profiles in the lung. As determined by RT-PCR in the lung of *mev* mice, the mRNA levels of IL-4, IL-5, and IL-13 were increased, whereas that of IFN-γ was not (Figure 5A). Measurement using ELISA showed that both IL-4 and IL-13 were significantly increased in the BAL of *mev* mice (Figure 5B). IL-5 levels were increased in *mev* mice, but no differences were seen in IFN-γ levels between WT and *mev* mice (Figure 5B). To determine if the Th2 signaling pathway was activated, we examined the activation status of STAT6 in the lung tissue using antibodies against STAT6 and phosphorylated STAT6 by Western blot. As shown in Figure 5C, the total amount of STAT6 was comparable in the lung tissue of WT and *mev* mice. Phosphorylated STAT6 was barely seen in the lung of WT mice. In contrast, significantly increased levels of phosphorylated STAT6 were found in the lung tissue of *mev* mice, indicating activation of the Th2 signaling pathway in the lung of *mev* mice (Figure 5C). Furthermore, we examined the chemokine response in the lung. Th2 inflammation–related chemokines, such as eotaxin and monocyte chemotactic protein -1, -2, -3, and -5 (23), were upregulated in the lung of *mev* mice at the mRNA level, and some were significantly increased at the protein level in the BAL fluid of *mev* mice. However, the expression of IFN-γ–responsive gene, interferon-inducible protein (IP)-10, did not change (Figures 6A and 6B).

Role of Th2 Cytokines and Signaling Pathway

The pulmonary phenotype and cytokine expression profiles indicate that Th2 cytokines and the Th2 signaling pathway are involved in the lung inflammation of *mev* mice. To determine if specific molecules in the Th2 signaling pathway plays a role in

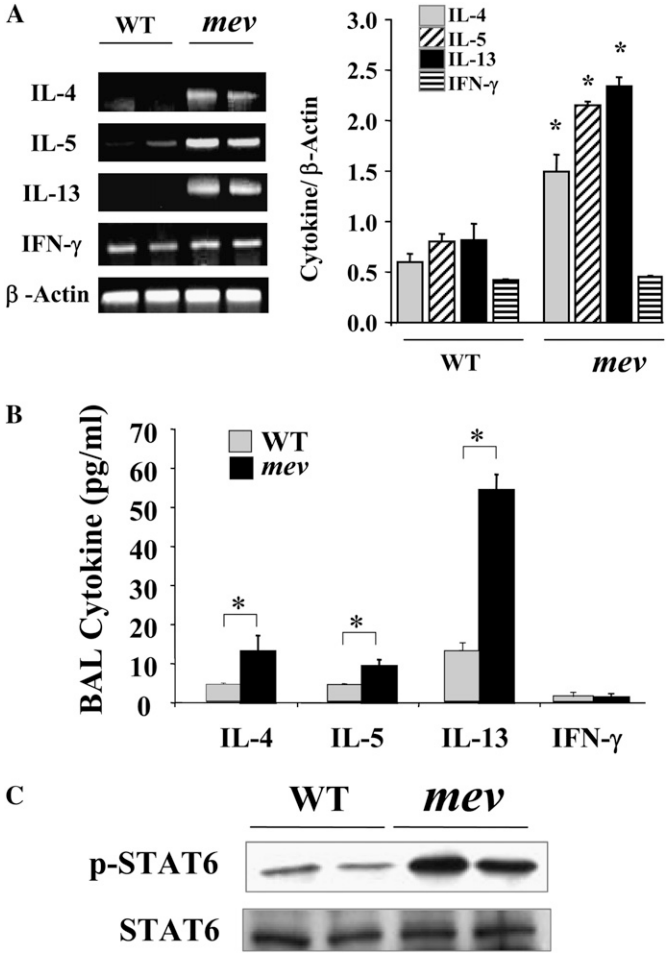

Figure 5. Cytokine expression and signal transducer and activator of transcription (STAT) 6 activation in the lung. (*A*) RT-PCR analysis of mRNA expression of T helper (Th) 2 and Th1 cytokines in the lung and densitometry of cytokine mRNA with β-actin mRNA as reference ($n = 6$ each group; *$P < 0.05$). (*B*) Protein levels of cytokines in the BAL fluids from WT and *mev* mice ($n = 6$ each) determined by ELISA (*$P < 0.05$). (*C*) Western blot analysis of total and phosphorylated STAT6 in the lungs of WT and *mev* mice. Data represent mean and SEM values.

the pulmonary inflammation of *mev* mice, we selectively deleted IL-4, IL-13, or STAT6 genes by crossbreeding with the respective KO mice, and examined the phenotypic changes in these mice.

Examination of the lung inflammation and mucous metaplasia demonstrated that ablation of IL-4 gene only slightly reduced the tissue inflammation and BAL cell counts (Figure 7). No significant difference was seen in mucus staining between the lungs of *mev* mice and *mev*/IL-4KO mice (Figures 7A and 7B). The percentage of cell types in the BAL was comparable in these mice. As a result of IL-4 gene deletion, the IL-4 cytokine levels in the BAL of *mev*/IL-4KO mice were similar to that of the WT mice, and significantly lower than that of the *mev* mice (Figure 7C). However, the BAL IL-13 levels in these mice did not decrease significantly (Figure 7C).

Deletion of IL-13 gene in *mev* mice dramatically decreased the inflammatory infiltration in the lung, although some residual inflammation was still present (Figures 7A and 7B). In contrast to *mev*/IL-4KO mice, *mev*/IL-13KO mice showed significantly reduced mucous metaplasia, with very few AB-positive cells in the airways of these mice (Figure 7A). The numbers of all cell

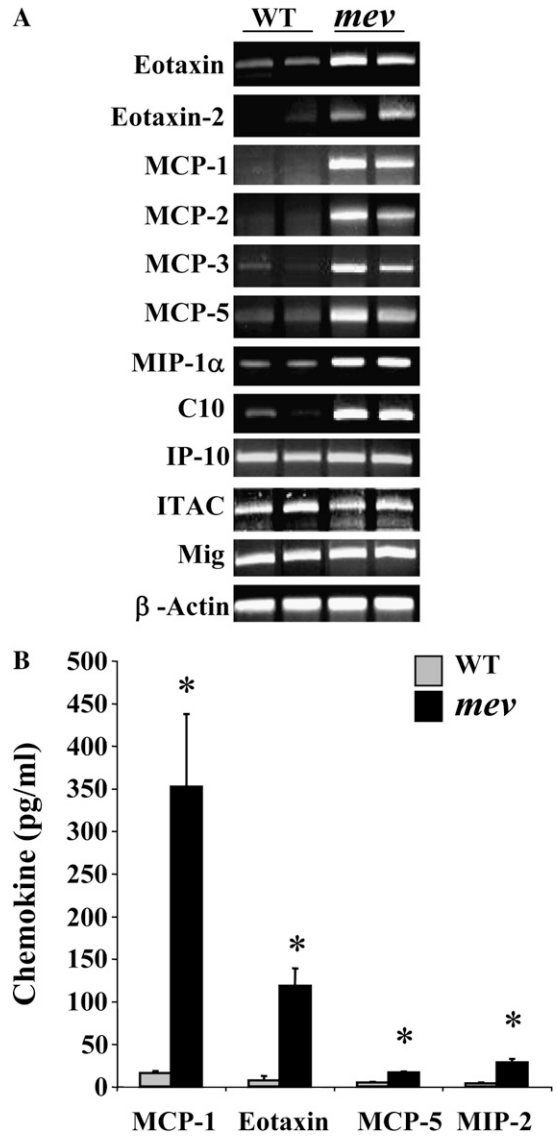

Figure 6. Chemokine expression and production in the lung. (*A*) Total lung RNA was obtained from WT and *mev* mice, and gene-specific primers were used in the RT-PCR analysis of chemokine expression, with β-actin as an internal control. (*B*) Protein levels of selected chemokines in BAL from WT and *mev* mice ($n = 6$ each group) measured by ELISA (*$P < 0.05$). Data represent mean and SEM values.

types were significantly decreased, but the percentages of the cell types did not change (Figure 7B). Disruption of IL-13 gene significantly reduced not only the levels of IL-13, but also that of IL-4 in the BAL fluid of *mev* mice (Figure 7C).

Deletion of the STAT6 gene in *mev* mice also significantly dereased the lung tissue inflammatory cell infiltration and BAL cellularity (Figures 7A and 7B), whereas no difference was seen in the percentage of inflammatory cells. AB-positive cells were hardly seen in the airways of *mev*/STAT6KO mice (Figure 7A). Lack of STAT6 gene resulted in significant reduction of both IL-4 and IL-13 in the BAL fluids of *mev* mice (Figure 7C).

The levels of IL-5 in the BAL fluid of these mice were close to or below the detection limit of the ELISA kit. Thus, no reliable data could be obtained.

Furthermore, we measured the levels of eotaxin in the BAL of different genotypes of mice (Figure 7D). Deletion of the IL-4 gene had little effect on the BAL eotaxin levels. Disruption of the IL-13 gene caused an obvious drop in eotaxin, but the difference between *mev* mice and *mev*/IL-13KO mice did not reach statistical significance. Finally, deletion of STAT6 gene caused a significant reduction in the eotaxin levels in the BAL (Figure 7D).

DISCUSSION

In several studies, SHP-1 has been implicated as a negative regulator of the Th2 signaling pathway. A glutamine-to-arginine substitution mutation (R576) in the cytoplasmic domain of the human IL-4Rα chain has been associated with atopy (24). Because the mutation is right next to a tyrosine residue, it was postulated that the R576 mutation may affect the binding of SHP-1. Indeed, phosphopeptides derived from the allele with the R576 mutation showed decreased binding of SHP-1. This also correlated with increased IL-4 signaling in peripheral blood mononuclear cells isolated from homozygous patients (24). In cultured cells, SHP-1 can dephosphorylate STAT6 after binding to the immunoreceptor tyrosine–based inhibitory domain of activated IL-4Rα (5–7). Reduced SHP-1 enzyme activity in the heterozygous *me* mice has been shown to lead to exaggerated responses to allergen stimulation in the lung (8). However, a functional role of SHP-1 in regulating Th2 activation pathway, and in controlling allergic inflammatory response in the lung, has not been established, particularly in the absence of obvious allergen stimulation.

Both *me* and *mev* mice are deficient in SHP-1 and have autoimmune diseases and immunodeficiency. These mice develop spontaneous inflammation in multiple organs, including the lung (10, 13–15, 25). However, the nature of the inflammation in the lung has not been defined, and the underlying mechanisms have not been explored. In this study, we investigated the pulmonary phenotype of *mev* mice, the molecular mechanisms, and the signaling pathways that are involved.

Analyses of the lungs of *mev* mice showed presence of large numbers of cells in the airways and in the lung parenchyma, including abundant eosinophils and lymphocytes. Lung histology showed prominent mucous metaplasia, epithelial hypertrophy, and subepithelial and parenchymal fibrosis. Pulmonary physiology assessment showed significantly increased baseline airway resistance and AHR in response to stimulation. Cytokine analyses revealed that Th2 cytokines IL-4 and IL-13 were highly up-regulated in the lung of *mev* mice, whereas the levels of IFN-γ and IP-10 did not change. Consistent with these results, IL-4/IL-13 downstream target genes, including chemokines, eotaxin and monocyte chemotactic protein-1, mucin, and TGF-β, were also up-regulated. Furthermore, studies using selective gene KO demonstrated that Th2 cytokine IL-13 and Th2 signaling molecule STAT6 were critical in mediating pulmonary inflammation and mucous metaplasia in *mev* mice, whereas IL-4 had some part in lung inflammation, but no effect on mucous production. Deletion of IL-13 gene significantly reduced IL-4 cytokine expression in the lung of *mev* mice, whereas deletion of IL-4 had no reciprocal effect on IL-13 expression. These studies reveal that *mev* mice develop a typical type 2 immune response in the lung, and the inflammation is largely dependent on Th2 cytokines, particularly IL-13, and on the Th2 signaling molecule, STAT6, suggesting that SHP-1 plays a critical role as a negative regulator in the Th2 signaling pathway and in maintaining lung homeostasis.

The observation that pulmonary inflammation in *mev* mice develops spontaneously in the absence of overt allergen stimulation suggests that innate immunity is involved. This is supported by findings in studies in which backcrossing *me* mice to strains deficient in myeloid progenitors showed that the *me* phenotype, including the lung pathology, was partially dependent on the

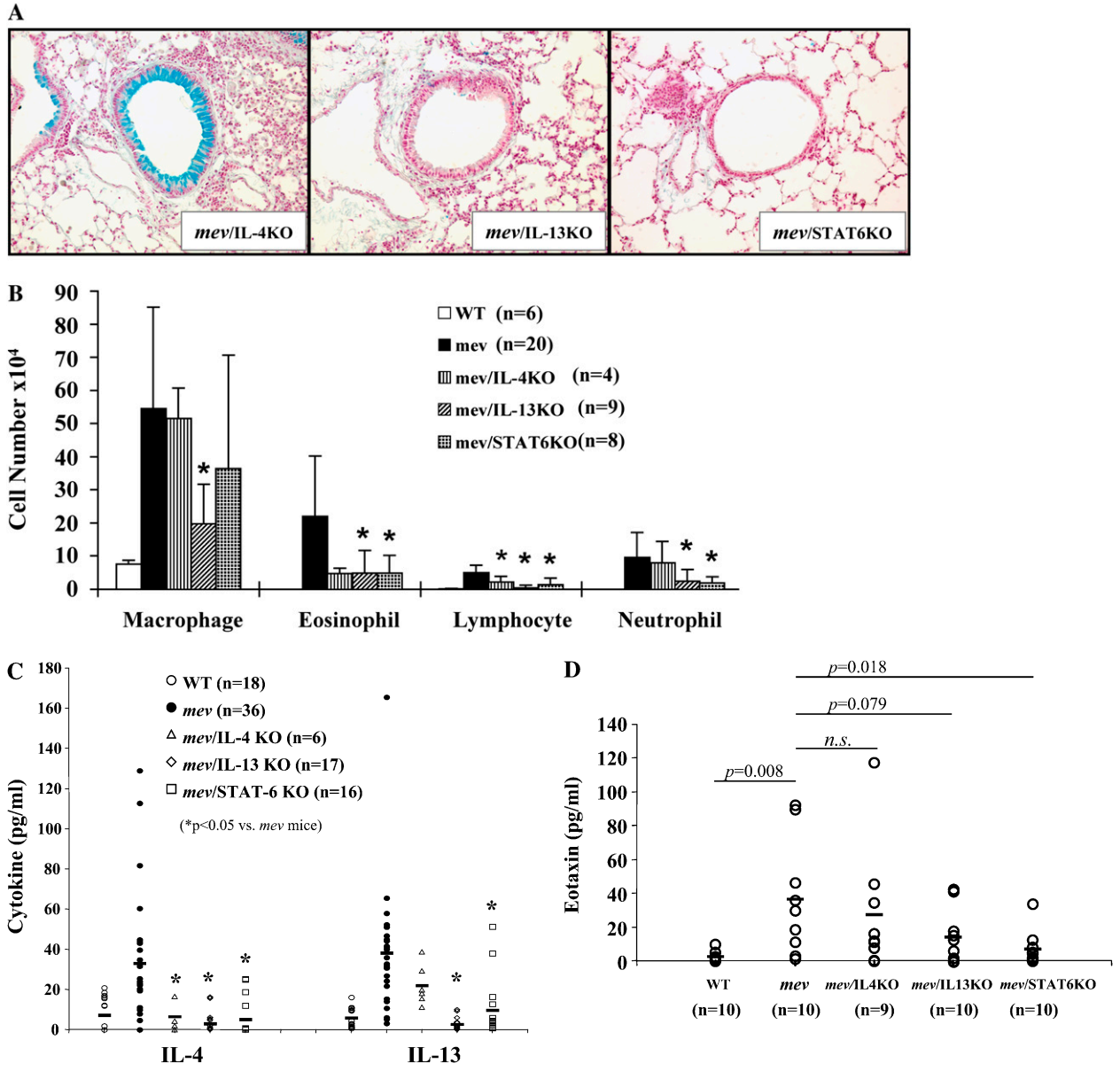

Figure 7. Effects of cytokine gene disruption on pulmonary inflammation and mucous metaplasia in *mev* mice. (*A*) AB staining of lung sections from *mev*, *mev*/IL-4 knockout (KO), *mev*/IL-13KO, and *mev*/STAT6KO mice. Representatives of multiple samples are shown. (*B*) Cellularity analysis of BAL samples from WT, *mev*, *mev*/IL-4KO, *mev*/IL-13KO, and *mev*/STAT6KO mice (*$P < 0.05$). Data represent mean and SEM values. (*C* and *D*) Effects of gene disruption on IL-4, IL-13, and eotaxin expression; IL-4 and IL-13 (*C*) and eotaxin (*D*) levels in the BAL fluids of WT, *mev*, *mev*/IL-4KO, *mev*/IL-13KO, and *mev*/STAT6KO mice were determined by ELISA.

myeloid cell population (26, 27). On the other hand, when *mev* mice were backcrossed to recombinant activating gene (RAG)-$1^{-/-}$ mice, which lack mature T and B cells, the pathology in these mice did not change, indicating that T and B cells, critical in adaptive immunity, are not required for the development of the *me* phenotype (28). We found that SHP-1 plays a role in host response to oxidant stress, and that deficiency in SHP-1 causes increased inflammation to aeroallergen, possibly through activation of innate immune cells (9). Together, these studies suggest that innate immunity may be critical for initiating the immune response seen in these mice, and that myeloid cells, particularly macrophages, monocytes, and mast cells, are acting as important initiating and effector cell types in the pathogenesis of pulmonary inflammation of these mice. Among these cell types, mast cells and basophils are capable of producing Th2 cytokines and other proinflammatory cytokines. The role of these cell types in generating the immune response in the lung of *mev* mice is under investigation.

It is interesting to note that disruption of IL-4 gene had no significant effect on the levels of IL-13 cytokine in the lung of *mev* mice, whereas disruption of IL-13 gene had significant inhibitory effects on the IL-4 levels in the lung (Figure 7C). Together with the effects of cytokine gene deletion on inflammation and mucous metaplasia, these observations indicate that IL-13 plays a major role in the development of the *mev* pulmonary phenotype, where IL-4 has a minor or no role. This is probably further evidence to support the view that the *mev* pulmonary phenotype is not mediated by adaptive immunity, as IL-4 is known to be critical in T cell

differentiation in adaptive immune responses, whereas IL-13 is more of an effector cytokine that can be produced by T cells and by cell types of innate immunity.

Further down the signaling pathway, STAT6 is a major signaling molecule, transducing the signals of IL-4 and IL-13 to the downstream target genes (29). However, other mechanisms are also known to be involved in this pathway. Signaling molecules from the insulin receptor substrate (IRS) family can interact with IL-4Rα and activate the phosphatidylinositol 3-kinase (PI3K) pathway (1). Some recent studies have shown that activation of the PI3K pathway is a critical part of immune responses to allergen stimulation in mouse models of asthma (30, 31). It has been demonstrated that SHP-1 regulates the IRS–PI3K pathway downstream of the insulin receptor (32). However, it is not known whether the IRS–PI3K pathway downstream of IL-4Rα plays a role in the *mev* lung phenotype due to loss of SHP-1. Our findings suggest that STAT6 is largely responsible for the Th2 inflammatory phenotype in the *mev* lung, but other mechanisms are also involved, and should be investigated further.

Our understanding of Th2 inflammatory disorders has advanced rapidly in recent years. However, the vast majority of our research effort is focused on the up-regulation of Th2 cytokines and the activation of Th2 pathways. Little is known about the negative regulation of Th2 inflammation. Our findings establish a direct functional relationship between tyrosine phosphatase SHP-1 and pulmonary Th2 inflammation. This may add a new facet to our understanding of the critical role of SHP-1 in controlling Th2 immune response, and to our understanding of allergic and asthmatic disorders from the perspective of negative regulation. Aberrant expression of SHP-1, or a functional deficiency in SHP-1, in humans may have unrecognized effects on some of the allergic and asthmatic disorders. On the other hand, enhanced SHP-1 function may offer an alternative mechanism for controlling Th2 inflammation.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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