Inhibition of immunoglobulin E attenuates pulmonary hypertension

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Pulmonary hypertension (PH) is a severe cardiopulmonary disease characterized by pulmonary vascular remodeling. Immunoglobulin E (IgE) is known to participate in aortic vascular remodeling, but whether IgE mediates pulmonary vascular disease is unknown. In the present study, we found serum IgE elevation in pulmonary arterial hypertension (PAH) patients, hypoxia-induced PH mice and monocrotaline-induced PH rats. Neutralizing IgE with an anti-IgE antibody was effective in preventing PH development in mice and rat models. The IgE receptor FcεRIα was also upregulated in PH lung tissues and FcεRIα deficiency prevented the development of PH. Single-cell RNA-sequencing revealed that FcεRIα was mostly expressed in mast cells (MCs) and MC-specific FcεRIα knockout protected against PH in mice. IgE-activated MCs produced interleukin (IL)-6 and IL-13, which subsequently promoted vascular muscularization. Clinically approved IgE antibody omalizumab alleviated the progression of established PH in rats. Using genetic and pharmacological approaches, we have demonstrated that blocking IgE-FcεRIα signaling may hold potential for PAH treatment.
sustained serum IgE elevation was observed in hypoxia-induced PH mice (Fig. 1b) and MCT-induced PH rats (Fig. 1c) when compared with control animals.

Adaptive immune response is observed in PH lung 16, so we hypothesized that the elevated IgE is produced from lung tissues. To characterize the local immune response in lung tissues and the cells involved, single-cell RNA-sequencing (scRNA-seq) was performed in lung tissues from mice exposed to hypoxia at different timepoints (Extended Data Fig. 1). The proportion of B cells (identified by their characteristic enrichment of Cd19, Fig. 1d,e) was increased under hypoxia (Fig. 1f). Differentially expressed genes (DEGs, Fig. 1g) and their enriched gene ontology (GO) terms between consecutive pairs of timepoints revealed ‘B cell activation’ and ‘B cell differentiation’ at the early stages of PH (Extended Data Fig. 2). GO terms of ‘regulation of chromosome organization’, ‘regulation of immunoglobin-mediated immune response’ and ‘immunoglobulin-mediated immune response’ (Extended Data Fig. 2) suggested that antibody class-switch recombination may have occurred in B cells during PH development. We also found enhanced TH2 cell response (Extended Data Fig. 3a–d), upregulated

| Table 1 | Demographic and clinical characteristics of PAH patients and healthy people |
|-----------------|---------------------------------|-----------------|-----------------|
| Feature | Patients with PAH (n = 124) | Control subject (n = 116) | P value |
| Age at sampling (years) | 32.0 ± 8.1 | 33.3 ± 5.0 | 0.14 |
| IPAH:HPAH | 116:8 | | |
| Female:male (ratio) | 3.1:1 | 2.9:1 | 0.79 |
| NYHA functional class | N/A | | |
| I | 5/124 | | |
| II | 51/124 | | |
| III | 62/124 | | |
| IV | 6/124 | | |
| Hemodynamics | N/A | | |
| Mean right atrial pressure (mmHg) | 5.2 ± 4.2 | | |
| Pulmonary capillary wedge pressure (mmHg) | 71 ± 3.6 | | |
| Mean PAH (mmHg) | 55.7 ± 15.5 | | |
| Cardiac index | 2.8 ± 0.8 | | |
| Cardiac output (l min⁻¹) | 4.7 ± 1.6 | | |
| Total pulmonary resistance (dyn s cm⁻5) | 1128.7 ± 534.4 | | |
| 6-min walk distance (m) | 426.1 ± 98.7 | N/A | |
| SvO₂ (%) | 68.0 ± 7.8 | N/A | |
| NT-pro-BNP (pg ml⁻¹) | 1,230.8 ± 1149.0 | N/A | |
| Creatinine (µmol l⁻¹) | 74.7 ± 15.8 | N/A | |
| Uric acid (µmol l⁻¹) | 430.1 ± 136.4 | N/A | |
| C-reactive protein (mg l⁻¹) | 2.87 ± 2.7 | N/A | |
| PAH therapy | N/A | | |
| No therapy | 27/124 | | |
| Single therapy | 74/124 | | |
| Prostacyclin analog | 4/124 | | |
| ET-receptor antagonist | 8/124 | | |
| PDE5 inhibitor | 64/124 | | |
| Combination therapy | 21/124 | | |
| Prostacyclin analog + ET-receptor antagonist | 0/124 | | |
| Prostacyclin analog + PDE5 inhibitor | 1/124 | | |
| ET-receptor antagonist + PDE5 inhibitor | 19/124 | | |
| Three therapies combined | 1/124 | | |
| Blood cells (%) | | | |
| Neutrophils | 58.75 ± 0.89 | 57.99 ± 0.82 | 0.52 |
| Lymphocytes | 33.63 ± 0.88 | 32.90 ± 0.74 | 0.51 |
| Monocytes | 5.90 ± 0.20 | 6.21 ± 0.13 | 0.18 |
| Eosinophils | 1.47 ± 0.11 | 2.23 ± 0.16 | <0.0001 |
| Basophils | 0.25 ± 0.02 | 0.68 ± 0.03 | <0.0001 |

Values are presented by mean ± s.d. ET, endothelin; N/A, not available; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PDE5, phosphodiesterase 5; SvO₂, mixed venous oxygen saturation. Differences between groups were assessed using an unpaired, two-tailed Student’s t-test.
Fig. 1 | Serum IgE levels in PAH patients and PH animal models. a, Serum IgE levels of IPAH/HPAH patients and sex-/age-matched healthy controls (non-PAH, n = 116; IPAH/HPAH, n = 124). b, Serum IgE levels in mice with hypoxia-induced PH (n = 9). c, Serum IgE levels in rats with MCT-induced PH (n = 4). d, The t-SNE representation of aligned gene expression data of cells extracted from lung tissues of four hypoxic groups in C57BL/6 mice, showing the partition of distinct clusters. e, The expression of B cell marker gene Cd19 exhibited on a t-SNE plot (gene expression log normalized by Seurat). f, The percentage of B cells among total cells from the four hypoxic groups. g, Heatmap of key genes differentially expressed during hypoxia exposure (presented for natural log transformed). h, Flow cytometry quantification of CD21+CD23+ B cells (0 week, n = 5; 4 weeks, n = 8). i, Flow cytometry quantification of IgE+ cells in CD19+ cells (n = 4). For a and h–j, data are shown as mean ± s.e.m. and the differences between groups were evaluated by unpaired, two-tailed Student’s t-test. For b and c, data are shown as mean ± s.e.m. and the differences between groups were assessed using one-way ANOVA with Bonferroni’s post-hoc test.
transcription of genes in the IL-4 pathway in T cells (Extended Data Fig. 3e), as well as enhanced Cdt1+ T cell–B cell interactions (Extended Data Fig. 3f–h), supporting that IgE was produced by activated B cells in PH lung via a classic mechanism. By experimental confirmation, we found increased proportion of activated B cells (CD21+CD23+, Fig. 1h), enhanced germinal center B cell activation (indicated by increased Aicda messenger RNA expression in B220+GL7+IgD+IgM− cells; Extended Data Fig. 4a,b) and increased proportion of memory B cells, plasma cells and plasma blasts (Extended Data Fig. 4c–g) in hypoxic lung. Moreover, the upregulation of Gls mRNA expression in both B cells and PH lung tissues further confirmed that active B cells switched to the IgE isotype (Fig. 1i). The increased percentage of IgE+ cells in PH lungs (Fig. 1j) rather than spleen or lymph nodes (Extended Data Fig. 4h,i) potentially resulted in the elevation of serum IgE levels during PH development.
Fig. 3  |  FcεRIα deficiency attenuated hypoxia-induced PH in mice. a. Relative FCERIA mRNA expression in the lung tissues of control subjects (non-PAH) and PAH patients (n = 4). b. Representative western blot (top) and quantification of FcεRIα and β-actin (bottom) in the lung tissues from control subjects (non-PAH) and PAH patients (n = 4). c. Representative images of FcεRIα immunohistochemical staining of the lung sections. d. Relative FcεRIα mRNA expression in lung tissues of mice with hypoxia-induced PH (0 weeks, n = 4; 1 week, n = 4; 2 weeks, n = 5; 4 weeks, n = 4). e. Representative western blot (top) and quantification of FcεRIα relative to β-actin (bottom) in the lung tissues of mice with hypoxia-induced PH (n = 4). f. Relative FcεRIα mRNA expression in the lung tissue of rats with MCT-induced PH (0 weeks, n = 4; 2 weeks, n = 4; 4 weeks, n = 5). g. Representative western blot (top) and quantification of FcεRIα and β-actin (bottom) in lung tissue of rats with MCT-induced PH (n = 4). h. Schematic diagram for an experiment testing in FcεRIα+/+ (WT) and FcεRIα-/- (KO) mice in hypoxia-induced PH (control: n = 5 for WT and n = 4 for KO; hypoxia: n = 8 for each group). i. RVSP of WT and KO mice under control and hypoxia. j. RV/LV+S values in mice. k. PA AT/ET values measured by Doppler analysis. l. Representative images of α-SMA immunofluorescent staining of the lung sections. m. Quantification of the percentage of wall thickness, classified into 20–50 μm and 50–100 μm in diameter. n. Proportion of nonmuscularized (N), partially muscularized (P) or fully muscularized pulmonary (F) vessels (20–100 μm in diameter). Results are shown as mean ± s.e.m. For a and b, quantitative results were assessed using unpaired, two-tailed Student’s t-test. For d–g, quantitative results were assessed using one-way ANOVA with Bonferroni’s post-hoc test. For i–k, m and n, quantitative results were assessed using multi-group comparison by two-way ANOVA with Bonferroni’s post-hoc test.
Blocking serum IgE attenuated PH development in mice and rats. To determine the function of IgE in PH development, we used an experimental anti-IgE antibody to neutralize serum IgE in a hypoxic mouse model of PH (Fig. 2a and Extended Data Fig. 5a). In mice exposed to hypoxia, the anti-IgE antibody significantly attenuated the increase in right ventricular systolic pressure (RVSP; Fig. 2b and Extended Data Fig. 5b), reduced the extent of RV hypertrophy weight ratio (right ventricular/left ventricular + septum (RV/(LV + S)), Fig. 2c), and restored the ratio of pulmonary artery (PA) acceleration time (AT) over ejection time (ET) (PA AT/ET, Fig. 2d), compared with hypoxic mice administered with the isotype control antibody. Moreover, the anti-IgE antibody also attenuated hypoxia-induced pulmonary vascular muscularization, as indicated by the decreased pulmonary vascular wall thickness (Fig. 2e,f), increased percentage of nonmuscularized vessels (N) and decreased percentage of fully muscularized vessels (F) compared with the isotype control antibody-treated animals (Fig. 2g).

We further confirmed the role of IgE in rats with MCT-induced PH (Fig. 2h and Extended Data Fig. 5c). The results showed that MCT-injected rats treated with the anti-IgE antibody exhibited significantly reduced mPAP (Fig. 2i and Extended Data Fig. 5d), reduced RV hypertrophy (Fig. 2j) and improved PA AT/ET (Fig. 2k) compared with those administered the isotype control type antibody. Moreover, the anti-IgE antibody also decreased pulmonary vascular wall thickness (Fig. 2l,m) and attenuated vascular muscularization (Fig. 2n) caused by MCT. Taken together, these results indicate that blocking serum IgE conferred protection against experimental PH.

**FcεRIα deficiency attenuated hypoxia-induced PH in mice.** To identify the target receptor for IgE in PH development, we assessed the expression of the high-affinity receptor FcεRIα in the lung tissues from PAH patients. We observed that FcεRIα mRNA and protein levels were significantly upregulated in the patient lung (Fig. 3a,b). Immunohistochemical staining showed that FcεRIα-positive cells were located around pulmonary vessels (Fig. 3c), suggesting their potential involvement in pulmonary vascular remodeling. We next examined FCER1A mRNA levels from the published genome-wide expression data of PAH lung tissue17. In line with our findings, FCER1A mRNA levels in lung tissue from PAH patients were higher than in control subjects (Extended Data Fig. 6). In lung tissues from hypoxia-exposed mice (Fig. 3d,e) and MCT-injected rats (Fig. 3f,g), mRNA and protein levels of FcεRIα were also increased. To investigate the role of FcεRIα in IgE-mediated PH, FcεRIα−/− (wild-type (WT)) and FcεRIα−/− (knotout (KO)) mice were exposed to hypoxia to establish PH (Fig. 3h). As shown in Fig. 3i–k, FcεRIα deficiency protected against hypoxia-induced elevation in RVSP and RV hypertrophy, and decrease in PA AT/ET compared with WT mice. Histological analyses showed less pulmonary vascular thickening and muscularization in response to hypoxia in FcεRIα KO mice compared with WT controls (Fig. 3l–n). These results indicated that FcεRIα was the receptor involved in IgE-mediated PH development.

**FcεRIα expressed in MCs contributed to PH in mice.** To identify the FcεRIα-expressing cells in lung tissues, we analyzed our mouse scRNA-seq data and found that FcεRIα was mainly enriched in MCs (Fig. 4a, top). Similarly, the published scRNA-seq data18 in normal human lungs revealed FCER1A expression in MCs (Fig. 4a, bottom). These results were confirmed by the co-localization of FcεRIα+ cells and MC markers (c-Kit+ cells, Fig. 4b; tryptase+ cells, Extended Data Fig. 7a). We also found FcεRIα+ cells located proximally to α-smooth muscle actin-positive (α-SMA+) cells in pulmonary vessels (Extended Data Fig. 7b) but not airway CC10+ cells, (Extended Data Fig. 7c). Moreover, we showed an increased percentage of CD45ε−c−/Kit−FcεRIα+ cells (MCs) in hypoxic mice (Fig. 4c). This observation is consistent with the previous report of MCs infiltration in lung tissues of PAH patients and PH rats19.

To evaluate the function of MC-expressed FcεRIα in PH development, MC-specific FcεRIα KO mice (FcεRIα−/−, MCKO) were generated by crossingbreeding Fcer1agfp/fos mice (WT) mice with Mctp5-Cre transgenic mice (Fig. 4d). No immuno-inflammatory phenotype was observed at baseline for MCKO mice (Extended Data Fig. 8a–e). After hypoxia exposure, MCKO mice exhibited impaired MC accumulation and activation (Extended Data Fig. 8f,g), without affecting IgE levels (Extended Data Fig. 8h,i). Four weeks of hypoxia exposure resulted in PH in WT mice, indicated by increased RVSP and decreased PA AT/ET, which were improved in the MCKO mice (Fig. 4e,g). No significant changes were observed in RV hypertrophy, consistent with previous findings of cromolyn administration in the MCT model20 (Fig. 4f). Histological analyses showed decreased wall thickness and vascular muscularization in MCKO compared with WT mice under hypoxia (Fig. 4h–j). Together, these results suggested that FcεRIα expressed in MCs contributed to the development of PH.

**IgE-stimulated MCs to produce IL-6 and IL-13 in PH model.** To identify the factors by which IgE-activated MCs mediate PH pathogenesis, we searched for the isotype control antibody. The treatment was started from IgE treatment and revealed the IL-6 expression in lung tissues associated with inflammation and vascular cell dysfunction (Fig. 5b). Importantly, the DEGs enriched in these GO terms included many secreted factors that are known to be PH related, such as Il6, Il13, Il33, Vegfa, Vegfb, Vegfc, Fgf2, Fgf18, Hbegf, Pgfla, Ccl2 and Ccl4 (Fig. 5a). By combining these 12 genes from the RNA-seq data of IgE-stimulated MCs with our scRNA-seq data from PH patients, we found 3 candidate genes (Il6, Ccl4 and Il13) that were mainly expressed by MCs and responded to IgE stimulation (Fig. 5c). Quantitative PCR with reverse transcriptase (RT-qPCR) results confirmed the significantly upregulated expression of Il6 and Il13, but not Ccl4, in CD45ε−/Kit−FcεRIα+ MCs sorted from hypoxic lung tissues (Fig. 5d). Moreover, increased IL-6 and IL-13 expression was observed in lung tissues from hypoxic mice, and their expression was reduced under IgE–FcεRIα blockade, as shown in anti-IgE antibody-treated mice (Fig. 5e,f and Extended Data Fig. 9a–b) and FcεRIα KO mice (Fig. 5g,h and Extended Data Fig. 9c,d), as well as in MC-specific FcεRIα KO mice (Fig. 5i,j and Extended Data Fig. 9e,f). Similarly, IL-6 and IL-13 expression was also increased in MCT-injected rats and reduced by anti-IgE (Extended Data Fig. 10). Co-culture of MCs and pulmonary arterial smooth muscle cells (PASMCs) was established to study the effects of MC-derived IL-6 and IL-13 (Fig. 5k). The conditioned medium of IgE-activated MCs promoted PASMC proliferation, whereas this pro-proliferative effect of the conditioned medium was reduced with knockdown of Il6 and Il13 in MCs (Fig. 5l,m). Through a series of transcriptomic analysis and experimental validation, we have found that IgE-activated MCs produced IL-6 and IL-13, which potentially contributed to IgE-mediated PH in animal models.

**Clinically used omalizumabameliorated established rat PH.** We next evaluated the effect of anti-IgE therapy on the progression of established PH in rats using omalizumab, a clinically used recombinant human monoclonal antibody. The treatment was started from 14 d post-MCT injection, when the pulmonary vascular remodeling and mPAP elevation were already present19 (Fig. 6a). After 2 weeks of treatment, omalizumab effectively lowered the serum IgE levels (Fig. 6b), reduced mPAP (Fig. 6c) and RV hypertrophy (Fig. 6d), improved PA ejection function (Fig. 6e), improved tricuspid annular plane systolic excursion (TAPSE, Fig. 6f), and attenuated pulmonary vascular wall thickening and muscularization (Fig. 6g–i). Anti-IgE therapy by omalizumab also decreased IL-6 and IL-13 expression effectively in lung tissues of MCT-induced PH rats.
In the present study, we have discovered that IgE was elevated in effective therapy for PH patients, especially with high serum IgE.

(Fig. 6j,k). These results indicate that omalizumab attenuates the development of established PH and targeting IgE may serve as an effective therapy for PH patients, especially with high serum IgE.

Discussion
In the present study, we have discovered that IgE was elevated in PAH patients and animal models of PH, and this promoted the pathogenesis of disease. We have shown that blocking IgE effectively attenuated the development of PH in different models. Using constitutive and cell type-specific KO animals, IgE was found to promote PH by stimulating MCs to produce IL-6 and IL-13, revealing the immune-based mechanism in PH (Fig. 7). Our findings provided proof of concept that IgE–FcεRIα contributes to PH development and also provided pre-clinical evidence supporting the translational
Fig. 5 | IgE-stimulated MCs to produce IL-6 and IL-13 in experimental PH. a. Volcano plot of DEGs between BMMCs treated with and without IgE, with two samples for each group; the P value was tested using Wald’s test in DESeq2 and adjusted by false discovery rate (FDR). b. Bubble plot of GO pathway enrichment of the DEGs in response to IgE. c. Dot-plot of gene expression in cell clusters.
Fig. 6 | Anti-IgE therapy using omalizumab might prevent established PH development in MCT rats. a, Schematic diagram of anti-IgE therapy on MCT-induced rats PH model using omalizumab. The treatment of omalizumab (200 μg, subcutaneously (s.c.)) was started from 14 d post-MCT injection (n = 6 for each group). b, Serum IgE levels in saline- or omalizumab (Oma)-treated MCT rats. c, The mPAP of indicated rats. d, RV/LV+S values of indicated rats. e, PA AT/ET values of indicated rats. f, TAPSE of indicated rats. g, Representative images of Russell–Movat staining and α-SMA immunofluorescence staining of the lung sections from rats in MCT + saline group and MCT + Oma group. h, Quantification of wall thickness of pulmonary vasculature, 20–50 μm and 50–100 μm in diameter. i, Proportion of nonmuscularized (N), partially muscularized (P) or fully muscularized (F) pulmonary vasculatures 20–100 μm in diameter from indicated rats. j, IL-6 content in lung tissues from indicated rats. k, IL-13 content in lung tissues from indicated rats. All above quantitative analysis results are shown as mean ± s.e.m. For b–e, g and h, the difference between multiple groups was evaluated by one-way ANOVA with Bonferroni’s post-hoc test. For i, the difference between groups was evaluated as mean ± s.e.m. using the unpaired, two-tailed Student’s t-test.
potential of anti-IgE therapies with IgE antibody (such as omalizumab) in the treatment of PAH.

Elevation of IgE has been reported in a number of cardiovascular diseases. Our current study showed that IgE was elevated in PH, released from stimulated B cells in lung tissues, but not from spleen or lymph nodes. Germinal center B cells, plasma blasts and plasma cells contributed to lung IgE production. Moreover, infiltration of T_{H}2 cells and production of T_{H}2 cell cytokines (IL-4, IL-5, IL-13) have been reported in lung tissues from PAH patients or PH animal models. In the present study, we first revealed increased interaction between T_{H}2 cells and B cells in the lung tissues of PH mice through scRNA-seq. This process may promote antibody class switching into the IgE isotype in B cells, subsequently leading to increased IgE production. To specifically block the function of IgE, we used anti-IgE antibody and demonstrated a causative role of IgE elevation in PH development. Besides IgE antibodies, reducing IgE production by targeting B cells with rituximab may be an alternative approach. However, targeting B cells may interfere not only IgE-dependent functions, but also other B-cell-mediated and IgE-independent functions. For example, it has been shown that rituximab augmented IGHV2-5 segment usage and decreased IgD+ proportion in systemic sclerosis-associated PAH patients. Nevertheless, the effects through targeting B cells versus IgE and their differences in PH deserve to be evaluated experimentally in the future.

Exogenous antigens such as mite or pollen may also cause IgE elevation, in the context of asthma or allergic reaction. However, there is no confirmed clinical relationship between asthma and PH. IgE is not necessary for all types of asthma, because IgE elevation and reactivity are not observed in patients with IgE-independent asthma. We previously reported the relation of antigen-specific IgE with cardiovascular diseases. The present study showed that myocardial infarction was positively associated with IgE induced by endogenous antigens and inversely associated with IgE induced by exogenous antigens (such as IgE specific for oak or peanut), suggesting that endogenous antigen-induced IgE is more directly related to cardiovascular diseases. In the present study, animal models of PH were induced without exogenous antigens such as dust mites or ovalbumin; the human serum and tissue samples were collected from PAH patients without allergy, infection or autoimmune diseases. Hence, IgE was probably induced by endogenous autoantigens, which contributed to PH development. Indeed, previous studies have reported autoantigens in pulmonary vessels from PAH patients (such as lamin A/C and tubulin β-chain in endothelial cells, as well as vimentin and calumenin in fibroblasts). We propose that the exposure of autoantigens in damaged pulmonary vessels elicits a local adaptive immune response, resulting in IgE production during PH development. Studies with transfusing purified IgE from PH rats into naive rats could support the function of endogenous IgE in PH, which warrants further investigation when the purification technique has been improved.

FcεRIα is the high-affinity receptor that interacts with IgE directly. We observed increased FcεRIα expression in the lung

Fig. 7 | Graphic abstract. In the pulmonary vasculature, stimulated B cells undergo class switching to produce IgE. The increased levels of IgE bind to the FcεRIα receptor and activate MCs to release IL-6 and IL-13, which in turn induce the proliferation of smooth muscle cells and vascular remodeling in PH. Blocking IgE with antibodies such as omalizumab effectively targets this key mediator and ameliorates experimental PH.
tissues of rodent PH models and PAH patients. Perivascular FceRIα+ cells were rare in the lung tissues of non-PAH subjects, which was consistent with a previous report\(^6\). Remarkably, in PAH lung tissues, we found significant increases in FceRIα+ cells around the vascular lesions. The mechanism responsible for the increase in FceRIα expression in PH lung is still unclear. Importantly, scRNA-seq data from mouse, rat and human lung tissues all revealed FceRIα expression in MCs. Perivascular MCs (derived from bone marrow) possess high FceRIα expression\(^2\), but whether these infiltrated MCs contributed to FceRIα expression elevation warrants further investigation. Based on our results, we propose that a concomitant increase in IgE production and its effector MCs with the high-affinity FceRIα receptor in the lung triggers downstream signaling responses and ultimately results in pulmonary vascular remodeling. Although we focused on FceRIα in MCs in mouse models of PH, we also realized the species difference in FceRIα expression. In addition to MCs, FceRIα is expressed in human dendritic cells (Fig. 4a), but not in murine counterparts\(^9\). Infiltration of dendritic cells was observed in human PAH lung samples\(^5\). FceRIα in dendritic cells has been shown to promote T(H2) cell recruitment and activation\(^24\), which for IgE generation from B cells and MC-derived IL-6 was shown to contribute to the development of PH. Among many MC-released mediators, we identified and characterized FcεRIα in PH activated MCs and contributed to the development of PH. Stabilizing MCs may contribute to human PH. Thus, the anti-IgE therapy may have consistent with a previous report\(^26\). Remarkably, in PAH lung tissues from PAH patients\(^15,30\). Stabilizing MCs \(\beta 2\) cell recruitment and activation\(^4\), which may contribute to human PH. Thus, the anti-IgE therapy may have therapeutic advantage in human PH because it is able to suppress FcεRIα-mediated pathogenesis from both MCs and dendritic cells. The clinical trial with the anti-IgE antibody omalizumab in human PH is of great interest. Activation of MCs has been reported in MCT-induced PH rat and human lung tissues from PAH patients\(^15,16\). Stabilizing MCs through cromolyn in the early stage protects against MCT-induced PH development\(^16\). However, the mechanism responsible for MC activation remains unknown. Our results revealed that IgE elevation in PH activated MCs and contributed to the development of PH. Among many MC-released mediators, we identified and characterized predominant factors IL-6 and IL-13. IL-6 is widely recognized to be important in PH, because circulating IL-6 was elevated in patients with PAH\(^31,32\). Moreover, IL-6 was reported to be important for IgE generation from B cells and MC-derived IL-6 was shown to promote PH by stimulating B cells\(^32\). These results suggested that MC-derived IL-6 may reciprocally activate B cells to secrete more IgE, forming a vicious cycle in disease pathogenesis. In addition, IL-13 may also participate in PH, as transgenic mice overexpressing IL-13 in the lung spontaneously developed PH. IL-13 was reported to promote the proliferation of PA smooth muscle cells\(^33\) and migration of PA endothelial cells\(^34\). Taken together, we have shown that IgE served as an activator of MCs and the subsequent release of cytokines, in particular IL-6/IL-13, promoted pulmonary vascular remodeling. We also noticed lower eosinophil and basophil counts in peripheral blood from PAH patients than non-PAH subjects. These observations could be explained by the pulmonary recruitment for these cells. IgE, eosinophil and basophil together participate in an adaptive immune response\(^35\). IL-3 and IL-5 released by activated T cells promoted eosinophil and basophil infiltration, respectively\(^17,20\), leading to decreased cell numbers in circulating blood. Whether and how eosinophil and basophil participate in PH development warrant further investigation.

**Methods**

**PAH and control subjects.** A total of 124 patients with IPAH or HPAH and 116 age- and sex-matched non-PAH subjects were enrolled in a single-center clinical cohort from 2016 to 2018 in Fuwai Hospital. PAH patients included 94 women and 30 men, with an average age of 52.0 years. Non-PAH subjects included 86 women and 30 men, with an average age of 33.3 years. Samples were collected from Fuwai Hospital. For patients, the diagnosis of IPAH or HPAH was based on the 2015 European Society of Cardiology/European Respiratory Society\(^4\) guidelines, and was confirmed to meet the new criteria defined in the 6th World Symposium on Pulmonary Hypertension in 2018. Subjects with (1) allergy, infection or autoimmune diseases, (2) tumors or fibrosis and (3) juvenile age <18 years were excluded. Control subjects were recruited from a pool of healthy volunteers at Fuwai Hospital. A health questionnaire that included medical history and medication history was used to screen potential participants. Age-/sex-matched individuals without PH, cardiovascular diseases, pulmonary diseases, cancer, infection, allergy or autoimmune diseases were included. Individuals with abnormal body mass index and abnormal blood indices were excluded. PH lung tissues were obtained from the China–Japan Friendship Hospital Lung Transplantation Center during 2018–2019. Specimens that had (1) lung infection, (2) allergic diseases, autoimmune diseases or (3) complicated pulmonary diseases (tumor, fibrosis, and so on) were excluded. Four non-PAH lung tissue samples were collected from healthy transplant donors. All studies were first approved by the Institutional Ethics Committee of Peking Union Medical College (2018043) and Fuwai Hospital (approval no. 2017-877). No compensation was provided for participants. Informed consents to use blood sample and lung tissue for research were provided by both PAH and non-PAH participants before enrollment.

**Animal experiments.** For rodent models of PH, male mice (C57BL/6) with chronic hypoxia-induced PH (age 10 weeks, 10% O\(_2\), 4 weeks) and rats (Sprague Dawley), MCT-induced PH (200 g, 60 mg kg\(^{-1}\) of MCT, one injection, 4 weeks) were used. For pharmacological studies, hypoxic mice were treated with anti-IgE antibody (BD, catalog no. 553416) or isotype control antibody (BD, catalog no. 555839) through tail vein injection (2 μg dose per mouse saline (PBS), total four doses)\(^24\). MCT-injected rats were treated with anti-IgE antibody (BD, catalog no. 553914) or isotype control antibody (BD, catalog no. 554121) through tail vein injection (16 μg dose per rat, every week, total four doses). Omalizumab (Novartis) was injected subcutaneously, 200 μg dose per rat, every week, total two doses for each rat\(^16\). For genetic studies, FcεRIα\(^{-/-}\) (KO) mice (C57BL/6 mice) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. FcεRIα\(^{-/-}\) (Biocytogen, C57BL/6 background) and Mep15-Cre mice (Biocytogen, C57BL/6/WT) were crossed to generate FcεRIα\(^{-/-}\)Mep15-Cre\(^{flox/flox}\) mice. The number of animals used was determined by power calculation. Based on previous studies, we hypothesized a 10% change in RVSP between treated and untreated groups in PH mice and 20% changes in mPAP in PH rats. The s.d. was approximately 6% in mice PH and 10% in rat PH. The calculation showed n>7.56 in mice and n>5.24 in rats. Therefore, at least eight mice or six rats per group were used in our animal study. Animals were randomly divided into the different groups for each experiment. All animal experiments were conducted under protocols approved by the Animal Research Committee of the Institute of Laboratory Animals, Chinese Academy of Medical Sciences and Peking Union Medical College (ACUC-A01-2018-005).

**scRNA-seq and analysis.** Lung tissues from mice with hypoxia exposure for 0, 1, 2 and 4 weeks were harvested and scRNA-seq performed. The lung tissue was digested for 15 min at 37 °C in phosphate-buffered saline (PBS, total four doses) containing 200 U ml\(^{-1}\) of collagenase I (Sigma-Aldrich), 0.05 U ml\(^{-1}\) of elastase (Sigma-Aldrich), 5 μM of neutral protease (Worthington) and 0.3 U ml\(^{-1}\) of deoxyribonuclease I (DNase I, Promega). The digested tissues were neutralized with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS) and filtered through a 40-μm cell strainer (Invitrogen) to obtain single-cell suspensions. Live lung cells were sorted by PBS containing 0.5% bovine serum albumin (BSA) using a 100-μm nozzle on a MoFlo-XDP cell sorter (Beckman). Single-cell suspensions of the four groups (hypoxia 0, 1, 2 and 4 weeks) were loaded on a Chromium Single-Cell Controller (10X Genomics) to generate single-cell and gel bead emulsion. The single-cell sequencing library was prepared according to the instructions of the Chromium Single-Cell 3' Library & Gel Bead Kit v2.1 (10X Genomics). The libraries were sequenced on an Illumina HiSeq X Ten in paired-end reads to enable approximately 50,000 reads per single cell (Novo Generation Bioinformatics Technology Co., Ltd.).\(^5\)

10X Genomics single-cell transcriptome sequencing data were filtered by removing bases with a mass <3 at the start and end of the reads, using the Cell Ranger software v3.1.3 pipeline.\(^4\) The filtered reads were aligned to the MM10 mouse reference genome by STAR. For further analyses and statistics, based on the barcode and gene expression matrix, single-cell data were log(normalized) and filtered by the R package Scran 2.9.2 (refs. \(^5,6\)), with the following parameters: unique gene count per cell >150, cell counts per gene >2 and percentage of mitochondrial genes <13%. We next ran a canonical correlation analysis (CCA) to identify the common sources of variation across the four groups. The four groups of data were combined for the next analyses. The t-stochastic neighbor embedding (t-SNE) and automated cluster detection algorithms were performed stepwise based on the results of the CCA, during which the significant CCA dimensions (n) and the number of subjects (n\(_{\text{row}}\)) were considered. The resolution for cluster identification was set as 1. DEGs were identified using the Wilcoxon's test with P<0.05. DEGs from each cluster were sorted by log(fold-change) (log(FC)) relative to the other clusters. Then, the DEGs of which log(FC) was >0 were selected and GO analyses were performed by ClusterProfiler 3.14 (ref. \(^3\)). The enriched function or pathway was further clustered to predict likely cell–cell interactions. Alternatively, to detect the possible cell–cell interactions among different clusters, the Python package CellPhoneDB 2.1 (ref. \(^5\)) was used for the cell–cell communication analyses.
Echocardiographic assessment. At the endpoint of experiments performed on the experimental models, mice or rats were anesthetized with isoflurane (1.5%) and subjected to echocardiography. The body temperature was maintained at 37°C using a thermally controlled pad on a surgical table. For the heart rate, it was kept at 500–500 beats min⁻¹ and the echocardiography was performed with a 30-MHz probe. For rats, the heart rate was kept at 400–500 beats min⁻¹ and a 10-MHz ultrasonic probe was used (Vevo 770 system, Visual Sonics). The PA acceleration and PA ejection times were obtained in both mice and rats from the parasternal short axis view at the aortic valve level using the pulsed Doppler mode. Tricuspide annular plane systolic excursion was obtained from an apical four-chamber view using the M-mode. The results were calculated using Visual Sonics Vevo 2100 analysis software (v.1.6) with a cardiac measurement package and were based on the average of five cardiac cycles.

Histological analyses. Animal samples were perfusion fixed at 4 °C for 24 h, the lung tissues were embedded in paraffin. The fixed samples were sectioned at 5-μm thickness. Embedded lung tissues were prepared by repeated rounds of enzymatic digestion and antigen retrieval (by citric acid buffer, pH 6.0), the slices were incubated with primary antibody against tryptase (catalog no. ab118981, 1:200) and FcRn (catalog no. ab54411, 1:100) were obtained from Abcam. Primary antibody against CC10 (catalog no. 10490-1-AP, 1:200) and CD138 (catalog no. 10593-1-AP, 1:250) were obtained from Proteintech and antibody against tryptase (catalog no. A19801, 1:100) from ABClonal.

For the assessment of the wall thickness in experimental PH models, 15 fields from 3 lung sections per mouse or rat were randomly selected and captured. All images taken from the lung sections were coded so that the experimenters were blinded during the assessment. Small vessels (<100 μm in diameter) were selected from those accompanied by respiratory bronchioles or more distal airways. Approximately 20–40 small vessels were selected and categorized into two groups (20–30 μm and 50–100 μm). The wall thickness of each group was measured by NIS-Elements BR 5.11.00 and was calculated using the formula (2 × wall thickness/external diameter) × 100⁻¹.

For the assessment of small pulmonary vessel muscularization (<100 μm in diameter), the extent of circumferential α-SMA-positive staining was categorized as nonmuscularization (N), partial muscularization (P) and full muscularization (F). α-SMA was defined as a distinctly α-SMA-positive staining throughout the vessel cross-section (positive in double elastic lamina). α-SMA was measured by IHC analysis using Image-Pro Plus 6.0 software (Media Cybernatic). The positive area content was shown as a ratio of positive area to total area. The positive cell number was shown as a ratio of positive cell number to field area (mm²).

All the assessment and analyses were completed by experimenters blinded to the experiments.

Cell culture and lentiviral infection. To prepare bone marrow mononuclear cells (BMMCs), bone marrow cells from C57BL/6 male mice were cultured in RPMI-1640 medium (Solarbio) for 5–6 weeks with 10 ng ml⁻¹ mouse recombinant IL-3 (PeproTech) and 10 ng ml⁻¹ stem cell factor (PeproTech). Human PASMCS were obtained from ScienCell and cultured in smooth muscle cell medium (ScienCell). Human laboratory of allergic diseases 2 (LAD2) MCs were purchased from American Type Culture Collection (ATCC) and cultured in RPMI-1640 medium (for LAD2) containing 10% FBS. The cells were cultured at 37°C with 5% CO₂ in humidified conditions. For IgE treatment, LAD2 was starved in media with recombinant mouse IgE (BioLegend, catalog no. D6406) at a concentration of 10 μg ml⁻¹. To knock down IL6 and IL13, short hairpin (sh)IL6 lentivirus and shIL13 lentivirus were packaged and transduced into LAD2 cells. The knockdown efficiency was determined by RT-qPCR and western blotting. The target sequences were 5′-GCGAGACATGAAACTCATCT-3′ for IL6 and 5′-GGTCTGTCAGAACATCCACGAGA-3′ for IL13.

Cell growth assay. The growth of PA endothelial cells was determined using a Cell Counting Kit-8 (CCK8; Dojindo) according to the manufacturer’s protocol. Briefly, 1 × 10⁴ cells were seeded in 96-well plates for the different treatments. After 24 h, fresh culture medium containing 10 µl of CCK8 solution was added and the plate was incubated for 1 h at 37°C. The absorbance was measured at 450 nm.

RNA-seq. BMMCs stimulated with or without IgE were collected for RNA-seq analysis. An NEB Next, Ultra TM RNA Library Prep kit was used to prepare the ribosomal RNA-depleted RNA-seq library, according to the manufacturer’s instructions. Subsequently, the library was sequenced by Novogene (Beijing Novogene Bioinformatics Technology Co., Ltd.) with Illumina HiSeq 3000.

RNA-seq analyses. All paired-end reads were processed using the Trimmomatic software (v.0.37) with -q 25 -stringency 5 length 50 paired -phred33 parameters to remove the adapters and low-quality sequences. Then, the filtered reads were mapped to the reference genome using HISAT2 software (v.2.0.5) with default parameters⁴⁴. The reference genome sequences and the gene annotation files (GTF format) of both rat and mouse were downloaded from the website of the Ensembl Project (ftp://ftp.ensembl.org/pub/release-92/). To obtain the expression level of the annotated genes, the counts per million (CPM) of each gene were calculated by using Kallisto package⁴⁵. To calculate the read counts of each gene, the DESeq2 package (v.1.18.1)⁴⁶ was used to normalize the read counts among samples and calculate the fragments per kilobase of exon per million reads mapped (FPKM) of the genes. The DEseq2 package was used to determine the DEGs that showed significant change between the samples with or without IgE stimulation with a P value of 0.05. Furthermore, genes with upregulated or downregulated expression were chosen for further analyses and those with an FPKM < 1 were eliminated. The DEGs were presented as heatmap figures drawn by the ‘pHeatmap’ R package.

Total RNA isolation and RT-qPCR. Total RNA was extracted from the frozen lung tissues or cultured cells using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The concentration and purity of the RNA samples were evaluated with a Nanodrop. RT of the first chain was performed using a reverse transcriptase-mgRNA. The primer sets were designed for specific genes and the qPCR data were analyzed using the ΔΔCt method. Mean ± standard deviation (SD) was indicated for each experiment.

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36. Stone, K. D., Prussin, C. & Metcalfe, D. D. IgE, mast cells, basophils, and proinflammatory remodeling in pulmonary hypertension. Eur. Respir. Rev. 270–280 (2018).

37. Siracusa, M. C. et al. Basophils and allergic inflammation. J. Allergy Clin. Immunol. 132, 789–801 (2013). quiz 788.

38. Ramirez, G. A. et al. Eosinophils from physiology to disease: a comprehensive review. BioMed. Res. Int. 2018, 9095275 (2018).

39. Galié, N. et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPc), International Society for Heart and Lung Transplantation (ISHLT). Eur. Respir. J. 49, 903–975 (2015).

40. Ge, W. et al. The role of immunoglobulin E and mast cells in hypertension. Cardiovasc. Res. https://doi.org/10.1093/cvr/cvaa100 (2022).

41. Zhao, H. et al. Role of IgE-FcepsilonRI in pathological cardiac remodeling and dysfunction. Circulation 143, 1014–1030 (2021).

42. Xie, T. et al. Single-cell deconvolution of fibroblast heterogeneity in mouse pulmonary fibrosis. Cell Rep. 22, 3625–3640 (2018).

43. Zheng, G. X. et al. Massively parallel digital transcriptional profiling of single cells. Nat. Commun. 8, 14049 (2017).

44. Butler, A. et al. Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat. Biotechnol. 36, 411–420 (2018).

45. Satija, R. et al. Spatial reconstruction of single-cell gene expression data. Nat. Biotechnol. 33, 495–502 (2015).
46. Yu, G. et al. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics J. Integrat. Biol.* 16, 284–287 (2012).
47. Vento-Tormo, R. et al. Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature* 563, 347–353 (2018).
48. Wang, L. et al. Targeting IL-17 attenuates hypoxia-induced pulmonary hypertension through downregulation of beta-catenin. *Thorax* 74, 564–578 (2019).
49. Kim, K. et al. Transcriptome analysis reveals nonfoamy rather than foamy plaque macrophages are proinflammatory in atherosclerotic murine models. *Circ. Res.* 123, 1127–1142 (2018).
50. Gallagher, M. P. et al. Detection of true IgE-expressing mouse B lineage cells. *J. Vis. Exp.* https://doi.org/10.3791/52264 (2014).
51. Tu, L. et al. selective BMP-9 inhibition partially protects against experimental pulmonary hypertension. *Circ. Res.* 124, 846–855 (2019).
52. Kikuchi, N. et al. Selenoprotein P promotes the development of pulmonary arterial hypertension: possible novel therapeutic target. *Circulation* 138, 600–623 (2018).
53. Kuklina, E. M., Nekrasova, I. V. & Valieva, Y. V. Involvement of semaphorin (Sema4D) in T-dependent activation of B cells. *Bull. Exp. Biol. Med.* 163, 447–450 (2017).
54. Zhu, J. et al. GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. *Cell Res.* 16, 3–10 (2006).
55. Lawrence, M. et al. Software for computing and annotating genomic ranges. *PLoS Comput. Biol.* 9, e1003118 (2013).
56. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550 (2014).

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**Author contributions**

T.S. carried out investigations, methodology and visualization, and wrote the original draft. Y.L. carried out investigations, methodology, visualization and formal analysis. Y.Z. carried out investigations, created software, and did a formal analysis, data curation and visualization. Z.Z. provided resources, did a formal analysis and administered the project. P.Y. supervised the writing, review and editing of the paper. J.L. carried out investigations, validation and visualization. Y.X. carried out investigations and writing (review and editing). X.S. carried out validation and visualization. B.L. and J.P. created software and curated data. X.N. and X.Q. carried out investigations and validation. C.X. provided resources and carried out a formal analysis. H.Y. and Q.C. provided resources. J.C. supervised the project and provided resources. Y.Y. supervised the project and wrote, reviewed and edited the paper. J.W. and C.W. conceived and supervised the project, wrote, reviewed and edited the paper, administered the project and acquired funding.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | Characterization of the hypoxia-induced PH mouse model and sample collection for scRNA-seq. a. Schematic diagram for the hypoxia-induced PH mouse model in C57BL/6 mice and sample collection for scRNA-seq. b. RVSP of C57BL/6 mice before (H0w) and after 4 weeks of hypoxia (H4w). c. RV/LV + S of these mice. d. PA AT/ET of these mice. e. Representative image of H&E and α-SMA staining, scale bar=25μm. 
f. Quantification of wall thickness. g. Proportion of non-muscularized (N), partially muscularized (P), or full muscularized (F) pulmonary arterioles. All above quantitative results are shown as mean±SEM, and difference between groups was evaluated by unpaired two-tailed t-test.
Extended Data Fig. 2 | Bar plots of enriched GO pathways between pairs of time points. Differentially enriched genes (DEGs) were calculated between two adjacent time points by Seurat and used to perform GO enrichment analysis employed by clusterProfiler package, whereas over representation analysis (ORA) was used for statistic test. P values was calculated by hypergeometric distribution and adjusted by FDR with Benjamini-Hochberg procedure controlled. The color in bar plots was coded by log-transformed P values.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | ScRNA-seq revealed Th2 response and T cell-B cell interaction in hypoxia-induced mice PH model. **a.** Cell cluster identification by Cd4S, Cd3d and Cd4 enrichment on t-SNE plot. **b.** GO pathway enrichment bar plot of DEGs in Cd4+ T cells during hypoxia exposure. **c.** Flow cytometry quantification of CD3+ CD4+ T cells. \( n = 5 \) mice in 0-week hypoxia and \( n = 8 \) mice in 4-week hypoxia. **d.** Flow cytometry quantification of CD4+ IL4+ T cells. \( n = 5 \) mice in 0-week hypoxia and \( n = 8 \) mice in 4-week hypoxia. **e.** Relative mRNA expression of Il4 and Gota3 in CD4+ T cells \( (n = 4) \). **f.** Dot plot of the interaction of gene pairs between B cells and CD4+ T cells \( (Cd40lg\_CD40, Cd28\_Cd86) \) in four hypoxia groups. **g.** Dot plots of the expression of Cd86, Cd40 in B cell (left), and Cd28, Cd40lg in Cd4+ T cell (right) in four hypoxia groups. **h.** Relative mRNA expression of Cd28 in CD4+ T cells and relative mRNA expression of Cd40 and Cd86 in CD19+ B cells \( (n = 4) \). For **b**, \( p \) value was calculated by hypergeometric distribution and adjusted by FDR with Benjamini-Hochberg procedure controlled. For **c-e** and **h**, quantitative results are shown as mean ± SEM and compared by unpaired two-tailed t-test.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Proportion changes in B cells subtypes from control and hypoxic lung. a. Flow cytometry gate strategy for germinal center B cells, memory B cells, plasma cells and plasma blasts in lung tissues. b. Relative Aicda mRNA expression in sorted germinal center B cells (GC B cells) from control and hypoxic lungs. c. The proportion of memory B cells from control and hypoxic lungs. d. The proportion of IgE+ cells in memory B cell population. e. The proportion of plasma cells from control and hypoxic lungs. f. The proportion of plasma blasts from control and hypoxic lungs. g. Immunochemistry staining of CD138 in bronchovascular space from control and hypoxic lung. h. The proportion of IgE+ cells in B cell population in spleen from control and hypoxic mice. i. The proportion of IgE+ cells in B cell population in lymph node from control and hypoxic mice. All values are presented as the mean ± SEM. Difference between groups were evaluated by unpaired two-tailed t-test. n = 5 for each group. PA, pulmonary artery; Br, bronchus.
Extended Data Fig. 5 | The efficiency of anti-IgE-neutralizing antibody in mice and rats. 

a. Serum IgE levels in isotype control antibody- and anti-IgE antibody-treated mice under control or hypoxia. n = 5 for control groups, n = 8 for hypoxia groups. 

b. The representative pressure tracing waveform of RVSP in isotype control or anti-IgE treated mice.

c. Serum IgE levels in isotype control antibody- and anti-IgE antibody-treated rats after saline or MCT injection. n = 6 for saline groups, n = 6 for MCT rat treated with isotype control, n = 8 for MCT rats treated with anti-IgE.

d. The representative pressure tracing waveform of PAP in isotype control or anti-IgE treated rats. All above quantitative results are shown as mean ± SEM. Difference between multiple groups was evaluated by two-way ANOVA with Bonferroni's post hoc test.
Extended Data Fig. 6 | Bar plot of FCER1A mRNA levels for individual patients’ data. Data were analyzed from published genome-wide expression data (GSE117261) through GEO2R. Donor, n = 23; PAH, n = 54, logF<sub>c</sub>=0.338, adj. p value=0.00706 adjusted by Benjamini & Hochberg (False discovery rate).
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Immunofluorescence staining of FcεRIα in human lung tissues. **a**, Representative images showing staining of tryptase (green) and FcεRIα (red) in lung sections of control subjects (non-PAH) and PAH patients. Scale bar=50 μm. **b**, Representative images showing staining of α-SMA (green) and FcεRIα (red) in lung sections of control subjects and PAH patients. Scale bar=50 μm. **c**, Representative images showing staining of CC10 (green) and FcεRIα (red) in lung sections of control subjects and PAH patients. Scale bar=50 μm. The arrows indicate examples of positive-staining cells. PA, pulmonary artery; AE, airway epithelium. For **a-c**, the same results were observed in 4 individual samples each group.
Extended Data Fig. 8 | Immune cell proportions in WT and MCKO mice at basal level. a. The proportion of CD45^+ cells from indicated lungs. b. The proportion of CD19^+ cells from indicated lungs. c. The proportion of CD3^+ cells from indicated lungs. d. The proportion of CD11b^+ cells in CD45^+ population from indicated lungs. e. The proportion of CD11c^+ cells in CD45^+ population from indicated lungs. f. Quantification of tryptase^+ cell numbers in lung tissues from indicated mice. g. Serum histamine levels from indicated mice. h. Serum IgE levels in KO mice model. i. Serum IgE levels in MCKO mice model. All values are presented as the mean ± SEM. For a-e, n = 4 for each group. Differences between groups were evaluated by unpaired two-tailed t-test. For f and g, n = 4 for control group, n = 7 for hypoxia group, for h and i, n = 4 for control groups, n = 6 for hypoxia groups. Differences between groups were evaluated by two-way ANOVA with Bonferroni's post hoc test.
Extended Data Fig. 9 | mRNA expression of Il6 and Il13 in mouse lung tissues. a. Relative Il6 mRNA expression from lung tissues in anti-IgE treated mouse model. b. Relative Il13 mRNA expression from lung tissues in anti-IgE treated mouse model. c. Relative Il6 mRNA expression from lung tissues in KO mouse model. d. Relative Il13 mRNA expression from lung tissues in KO mouse model. e. Relative Il6 mRNA expression from lung tissues in MCKO mouse model. f. Relative Il13 mRNA expression from lung tissues in MCKO mouse model. All values are presented as the mean ± SEM and compared by two-way ANOVA with Bonferroni’s post hoc test. n = 4 for control groups, n = 6 for hypoxia groups.
Extended Data Fig. 10 | mRNA and protein levels of IL6 and IL13 in rat lung tissues. **a.** Relative *Il6* mRNA expression from lung tissues in anti-IgE treated rat model. **b.** Relative *Il13* mRNA expression from lung tissues in anti-IgE treated rat model. **c.** IL6 protein levels from lung tissues in anti-IgE treated rat model. **d.** IL13 protein levels from lung tissues in anti-IgE treated rat model. n = 4 for control groups, n = 6 for MCT groups. All values are presented as the mean ± SEM and compared by two-way ANOVA with Bonferroni’s post hoc test.
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N5 Elements EF 5.11.00, AllDOC xECL 2.0, Bio-Rad CFX Maestro 4.1, Lab Chart 7.3.8, ED accuri CE 1.0, CytoFLEX 2.4, SH0003.2

Data analysis

Prism5, R 3.6, Flowjo 10.4.0, Image-Pro Plus 6.0, CellRanger 3.1, Securit 3.2, ClusterProfiler 3.14, Cell Phone DB 2.1, Trim Galore software (v0.3.7), HISAT2 (v2.0.5), DESeq2 (v1.16.1)

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| Sample size | The power calculation determining the number of animals used is as follows: \( n > 2 \cdot (Z_\alpha + Z_\beta) \cdot \delta^2 / s^2 \), where \( n \) is sample size, \( Z_\alpha = 1.96 \), \( Z_\beta = 1.28 \) for a study with 90% power and \( \alpha = 0.05 \) significance level, \( s \) is standard deviation, \( \delta \) is minimum difference. Based on previous studies, we hypothesized a 10% change in RVSP between treated and untreated groups in PH mice and a 20% change in mPAP in PH rats. The SD was approximately 6% in mice PH and 10% in rat PH. The calculation showed \( n > 7.56 \) in mice and \( n > 5.24 \) in rats. Therefore, at least 8 mice or 6 rats per group were used in our animal study. |
| Data exclusions | No data was excluded from analysis. |
| Replication | The cell studies were carried out with at least three independent replicates. The animal studies were replicated in two independent experiments. |
| Randomization | Animals were divided randomly through a single sequence of simple randomization assignments for each group. For cell study, cells were seeded randomly before cell stimulation. |
| Blinding | All the assessment and analyses were completed by experimenters blinded to the experiments. |

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| [x] Dual use research of concern | |

**Antibodies**

Primary antibodies against FcERIa (10980-1-AP, Proteintech, 1:1000), β-ACTIN (AC026, Abclonal, 1:10000), PCNA (BM38888, Boster, 1:1000), secondary antibodies anti-mouse IgG HRP-linked antibody (62-6520, invitrogen, 1:5000), anti-rabbit IgG HRP-linked antibody (65-6120, invitrogen, 1:10000) were used for western blot. Primary antibodies against α-SMA (ab5694, Abcam, 1:400), c-Kit (ab115801, Abcam, 1:200), and FcERIa (ab54411, Abcam, 1:100), α-C10 (10490-1-AP, Proteintech, 1:200), trypase (A19801, Abclonal, 1:100) antibodies, CD138 (SG93-1-AP, Proteintech, 1:250); Alexa Fluor 488 goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody (A11008, invitrogen, 1:500), Alexa Fluor 594 goat anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody (A11005, invitrogen, 1:500), Alexa Fluor 594 goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody (A11037, invitrogen, 1:500) were used for IHC and IF stainings. PE-CD3 (100206, Biolegend, 1:100), FITC-CD4 (11-0451-85, ebioscience, 1:100), PE-CD21 (552957, BD, 1:100), FITC-CD23 (101604, Biolegend, 1:100), FITC-CD3 (11-0193-82, ebioscience, 1:100), PE-IL4 (12-7041-82, ebioscience, 1:100), PE-CD45 (103106, Biolegend, 1:100), FITC-FCERIa (11-5898-82, invitrogen, 1:100), APC-CD117 (105812, Biolegend, 1:100), PE-CD138 (406907, Biolegend, 1:100), PerCP-IgD (405736, Biolegend, 1:100), PE-B220 (103208, Biolegend, 1:100), Alexa Fluor 488-GL7 (144612, Biolegend, 1:100), PE-CD3 (102705, Biolegend, 1:100), APC-CD138 (142506, Biolegend, 1:100) and Brilliant Violet 421-CD19 (115537, Biolegend, 1:100) were used for flow cytometry.

**Validation**

The applications of primary antibody were validated as described in product datasheets:

- Antibodies for western blotting:
  - Rabbit anti-mouse/human FcERIa (10980-1-AP, Proteintech), https://www.ptglab.com/products/FCER1A-Antibody-10980-1-AP.htm;
  - Rabbit anti-mouse/human/rat/chicken/zebrafish/pig/qryza sativa β-ACTIN (AC026, Abclonal), https://abclonal.com.cn/catalog/AC026,
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
Human PASMCs were obtained from Sciencell. Human LAD2 mast cell was purchased from ATCC. BMMC was primary cultured.

Authentication
Primary cultured BMMC was authenticated by flow cytometry.

Mycoplasma contamination
Cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines
Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Rabbit anti-human/mouse/ rat PCNA (BM3888, Boster), http://www.boster.com.cn/home/product/anti-pcna-antibody_bm3888.html;
Goat anti-mouse IgG-HRP-linked antibody (62-6520, invitrogen), https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Secondary-Antibody-Polyclonal/62-6520;
Goat anti-rabbit IgG HRP-linked antibody (65-6120, invitrogen), https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Secondary-Antibody-Polyclonal/65-6120;

Antibodies used for IHC/IF stainings:
Rabbit anti-mouse/human α-SMA (ab5694, Abcam), https://www.abcam.com/alpha-smooth-muscle-actin-antibody-ab5694.html;
Rabbit anti-human c-Kit (ab115801, Abcam), https://www.abcam.com/c-kit-antibody-ab115801.html;
Mouse anti-human FcεRI (ab54411, Abcam), https://www.abcam.com/fc-epsilon-ri-fcer1a-antibody-9e1-ab54411.html;
Rabbit anti-human/mouse CC10 (10490-1-AP, Proteintech), https://www.ptgcn.com/products/SCGB1A1-Antibody-10490-1-AP.htm;
Rabbit anti-mouse/rat/human Tryptase (A19801, Abclonal), https://abclonal.com.cn/catalog/A19801;
Rabbit CD138/Syndecan-1 Polyclonal antibody (10593-1-AP, Proteintech), https://www.ptgcn.com/products/SDC1,CD138-Antibody-10593-1-AP.htm;
Goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 488 (A11008, Invitrogen), https://www.thermofisher.com.cn/zh/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11037;
Antibodies used for flow cytometry:
PE anti-mouse CD3 Antibody (100206, Biolegend), https://www.biolegend.cn/en-us/products/pe-anti-mouse-cd3-antibody-47;
FITC anti-mouse CD4 Antibody (11-0041-85, eBioscience), https://www.thermofisher.cn/cn/antibody/product/CD4-Antibody-clone-GK1.5-Monoclonal/11-0041-85;
PE anti-mouse CD21 Antibody (552957, BD), https://www.bdbiosciences.com/zh-cn/products/medical-products/flow-cytometry/monoclonal-primary-antibodies/pe-rat-anti-mouse-cd21-cd35.552957;
Biotin anti-mouse CD23 Antibody (101604, Biolegend), https://www.biolegend.com.cn/en-us/products/biotin-anti-mouse-cd23-antibody-217;
FITC anti-mouse CD19 Antibody (11-0193-82, eBioscience), https://www.thermofisher.cn/cn/antibody/product/CD19-Antibody-clone-eBio1D3-1D3-Monoclonal/11-0193-82;
PE anti-mouse IL4 Antibody (12-7041-82, eBioscience), https://www.thermofisher.cn/cn/antibody/product/IL-4-Antibody-clone-11B11-Monoclonal/12-7041-82;
PE anti-mouse CD45 Antibody (103106, Biolegend), https://www.biolegend.cn/en-us/products/pe-anti-mouse-cd45-antibody-100;
FITC anti-mouse FceRIa Antibody (11-5898-82, Invitrogen), https://www.thermofisher.cn/cn/antibody/product/FceR1-alpha-Antibody-clone-MAR-1-Monoclonal/11-5898-82;
APC anti-mouse CD117 Antibody (105812, Biolegend), https://www.biolegend.com.cn/en-us/products/apc-anti-mouse-cd117-c-kit-antibody-72;
PE anti-mouse IGE Antibody (406907, Biolegend), https://www.biolegend.com.cn/en-us/products/pe-anti-mouse-ige-3267;
PerCP anti-mouse IgD Antibody (405736, Biolegend), https://www.biolegend.com.cn/en-us/products/percp-anti-mouse-igd-9574;
PE anti-mouse/human B220 Antibody (103208, Biolegend), https://www.biolegend.com.cn/en-us/products/pe-anti-mouse-human-cd45r-b220-antibody-447;
Alexa Fluor 488 anti-mouse/human GL7 Antibody (144612, Biolegend), https://www.biolegend.com.cn/en-us/products/alexa-fluor-488-anti-mouse-human-gl7-antigen-t-and-b-cell-activation-marker-antibody-9579;
FITC anti-mouse CD38 Antibody (102705, Biolegend), https://www.biolegend.com.cn/en-us/products/fitc-anti-mouse-cd38-antibody-182;
APC anti-mouse CD138 Antibody (142506, Biolegend), https://www.biolegend.com.cn/en-us/products/apc-anti-mouse-cd138-syndecan-1-antibody-7572;
Brilliant Violet 421 anti-mouse CD19 Antibody (115537, Biolegend), https://www.biolegend.com.cn/en-us/products/brilliant-violet-421-anti-mouse-cd19-antibody-7160.

Human PASMCs were obtained from Sciencell. Human LAD2 mast cell was purchased from ATCC. BMMC was primary cultured.
Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Male C57BL/6 mice, Fcer1a-/- mice, Fcer1aflox/flox-Mcpt5Cre/+ mice (C57BL/6 background, 10-week old) were used for mice experiments. Male Sprague Dawley rats (8-week old, weighing 200 g) were used for rat experiments. For normoxia exposure, animals were housed in room air for 4 weeks. For normobaric hypoxia exposure (10% O2), animals were housed in an airtight plexiglass chamber for 4 weeks. Animals were maintained on a 12-h-light-dark cycle with a regular unrestricted diet. |
| Wild animals | This study did not involve wild animals. |
| Field-collected samples | No field-collected samples were used in the study. |
| Ethics oversight | All animal experiments were conducted under protocols approved by the Animal Research Committee of the Institute of Laboratory Animals, Chinese Academy of Medical Sciences and Peking Union Medical College (ACUC-A01-2018-005). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

| Population characteristics | PAH samples were obtained from 124 patients, including 94 females and 30 males, with an average age of 32.0 years. Non-PAH samples were obtained from 116 patients, including 86 females and 30 males, with an average age of 33.3 years. Samples were collected from Fuwai Hospital. |
| Recruitment | For IPAH/HPAH patients, the diagnosis was based on the 2015 ESC/ERS guidelines, and was confirmed to meet the new criteria defined in the 6th World Symposium on Pulmonary Hypertension, 2018. Subjects with (1) allergy, infection or autoimmune diseases; (2) tumors or fibrosis; and (3) juvenile age under 18 were excluded. Control subjects were recruited from a pool of healthy volunteers at Fuwai Hospital. A health questionnaire that included medical history and medication history was used to screen potential participants. Age/sex-matched individuals without pulmonary hypertension, cardiovascular diseases, pulmonary diseases, cancer, infection, allergy, or autoimmune diseases were included. Individuals with abnormal BMI and abnormal routine blood indices were excluded. No self-selection bias in recruitment. |
| Ethics oversight | All studies in this report were first approved by the Institutional Ethics Committee of Peking Union Medical College (2018043) and Fuwai Hospital (Approval NO.:2017-877). Informed consent to use lung tissue for research was provided by PAH and non-PAH participants before enrollment. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.
Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No  Yes

☐ Public health
☐ National security
☐ Crops and/or livestock
☐ Ecosystems
☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern

No  Yes

☐ Demonstrate how to render a vaccine ineffective
☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☐ Increase transmissibility of a pathogen
☐ Alter the host range of a pathogen
☐ Enable evasion of diagnostic/detection modalities
☐ Enable the weaponization of a biological agent or toxin
☐ Any other potentially harmful combination of experiments and agents

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4 FITC).
☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a group is an analysis of identical markers).
☐ All plots are contour plots with outliers or pseudocolor plots.
☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Single cell suspensions of mouse lung tissue were prepared by repeated rounds of enzymatic digestion and trituration with collagenase type I, neutral protease and deoxyribonuclease I, according to a previously published protocol.

Instrument

BD accuri C6 plus, cytoflex

Software

Assessed by BD accuri C6 or cytoflex, analyzed by Flowjo.

Cell population abundance

More than 50000 events were collected. After sorting the live cells and single cells, at least 20000 events were ready for further analyzed.

Gating strategy

Selecting the live cells in FSC, SSC dot plot. Choosing the single cells in FSC, A, FSC H dot plot. Events in single cell gate were analyzed.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary information.