IL-13 receptor α2-arginase 2 pathway mediates IL-13-induced pulmonary hypertension

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Submitted 13 March 2012; accepted in final form 1 November 2012

PULMONARY ARTERIAL HYPERTENSION (PAH) is a lethal disease caused by elevated vascular resistance due to lumen-obliterating processes in pulmonary arteries. Thus far, the underlying mechanisms of PAH have not been clearly understood (15). There is strong circumstantial evidence for an immune pathogenesis of PAH (33). In addition, PAH often accompanies chronic inflammatory diseases such as autoimmune disorders (systemic sclerosis) or infectious diseases (human immunodeficiency virus, human gamma herpes virus, or hepatitis C) (33). Collectively, these data indicate that a number of different inflammatory pathways are involved in the pathogenesis of PAH.

IL-13, a T-helper type 2 effector cytokine, is a critical mediator of many pulmonary diseases characterized by inflammation and tissue remodeling (39). Intriguingly, a mechanistic link between antigen-driven Th2 immune response and the development of severe pulmonary arterial remodeling without hemodynamic compromise in mice has been recently identified (8). In addition, IL-13 is reported to be an important mediator of the development of pulmonary hypertension (PH) in a murine model of Schistosomiasis (14). Furthermore, increased expression of both IL-13 and its receptors in small pulmonary arteries of patients with idiopathic PAH has been observed (16). Taken together, previous literature implicates a potential role of IL-13 in the development of PH, but a direct pathogenic role of IL-13 in the development of PH has not been explored yet. Furthermore, a potential mechanism underlying IL-13-induced PH has never been explored. Our work shows that, when IL-13 is chronically overexpressed in murine lungs using a transgenic (Tg) approach, the mice develop characteristic phenotypes of PH. In addition, we identified arginase 2 (Arg2) as a crucial mediator in the development of severe pulmonary arterial remodeling with-2-arginase 2 pathway mediates IL-13-induced pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 304: L112–L124, 2013. First published November 2, 2012; doi:10.1152/ajplung.00101.2012.—Although previous literature suggests that interleukin (IL)-13, a T-helper type 2 cell effector cytokine, might be involved in the pathogenesis of pulmonary hypertension (PH), direct proof is lacking. Furthermore, a potential mechanism underlying IL-13-induced PH has never been explored. This study's goal was to investigate the role and mechanism of IL-13 in the pathogenesis of PH. Lung-specific IL-13-overexpressing transgenic (Tg) mice were examined for hemodynamic changes and pulmonary vascular remodeling. IL-13 Tg mice spontaneously developed PH phenotype by the age of 2 mo with increased expression and activity of arginase 2 (Arg2). The role of Arg2 in the development of IL-13-stimulated PH was further investigated using Arg2 and IL-13 receptor α2 (Ro2) null mutant mice and the small-interfering RNA (siRNA)-silencing approach in vivo and in vitro, respectively. IL-13-stimulated medial thickening of pulmonary arteries and right ventricle systolic pressure were significantly decreased in the IL-13 Tg mice with Arg2 null mutation. On the other hand, the production of nitric oxide was further increased in the lungs of these mice. To our in vitro evaluations, the recombinant IL-13 treatment significantly enhanced the proliferation of human pulmonary artery smooth muscle cells in an Arg2-dependent manner. The IL-13-stimulated cellular proliferation and the expression of Arg2 in hpaSMC were markedly decreased with IL-13Ro2 siRNA silencing. Our studies demonstrate that IL-13 contributes to the development of PH via an IL-13Ro2-Arg2-dependent pathway. The intervention of this pathway could be a potential therapeutic target in pulmonary arterial hypertension.

PULMONARY ARTERIAL HYPERTENSION: Pulmonary vascular disease; arginase; inflammation; animal model

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MATERIALS AND METHODS

Animals. Constitutive IL-13-expressing Tg mice (CC10-IL-13 Tg) were generated as previously described (44). Nitric oxide synthase 3 (NOS3) null mice were purchased from the Jackson Laboratories (Bar Harbor, ME), and Arg2 null mice were a generous gift from Dr. William O'Brien, Department of Molecular and Human Genetics, Baylor College of Medicine. IL-13Ro2 null mutant mice on C57BL/6 background were generated and provided by Dr. M. Grushy of Harvard University School of Public Health (38). All of these mice have the C57BL/6 background, including the wild-type (Wt) control mice. All animals were anesthetized with ketamine/xylazine (100 mg/10 mg·kg−1) before any intervention was done. All of the animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Yale University.
Measurement of right ventricle systolic pressure and pulmonary angiography. The right internal jugular vein was catheterized for measurement of right ventricle (RV) systolic pressure (SP) using a standard pressure transducer (Millar). For pulmonary angiography, a barium-gelatin mixture was infused via the right internal jugular vein, followed by infusion of 4% ice-cold paraformaldehyde. Next, the animals were placed in iced water until used. The lungs were harvested for imaging via X-ray.

Immunohistochemistry and immunofluorescence. Formalin-fixed and paraffin-embedded sections of lung specimen were used for immunohistochemistry (IHC) and immunofluorescence (IF) staining. For immunocytofluorescence, cells seeded in a four-well chamber slide were permeabilized with Triton X-100 and fixed with 4% paraformaldehyde at room temperature for 20 min before staining. For IHC, a commercialized kit was used (Dako). The primary antibodies used for IHC include mouse α-smooth muscle actin antibody (Dako), rabbit myosin smooth muscle antibody (Biomedical Technologies), and rabbit arginase 1 (Arg1) and -2 antibodies (Santa Cruz Biotech). The primary antibodies used for IF included goat mouse thrombomodulin/CD141 antibody to detect endothelial cells (R&D Systems), mouse α-smooth muscle actin-Cy3 antibody to detect pulmonary arterial smooth muscle cells (Sigma-Aldrich), and rat F4/80 antibody to detect macrophages (Abcam).

Assessment of vascular remodeling. The vessels were classified into three groups according to their external diameter: vessels with an external diameter of <80 μm, those with an external diameter of 81–150 μm, and those with an external diameter of >151 μm. A length-to-breadth ratio of less than two was required to ensure transverse sections through the vessels. IHC against α-smooth muscle actin was performed to demarcate the media. The thickness of the media was expressed as a fraction of the external diameter of the pulmonary artery. Thirty vessels per animal (i.e., 10 vessels in each size group) were measured. In addition, pulmonary arterioles with an external diameter of <100 μm were counted and classified by circumferential staining with α-smooth muscle actin as nonmuscular, partially muscular (<75%), or completely muscular (>75%) arterioles. For each animal, at least 100 arterioles from each lung were characterized (30, 34). Image-processing software (SPOT Advanced version 4.1.1, Sterling Heights, MI) was used for measurement.

Preparation of RNA and cDNA and real-time PCR. Semiquantitative real-time PCR was performed as described previously (22). The following forward (For) and reverse (Rev) primers were used in this study: human IL-4 receptor (IL-4R) (For: TCATTGAGTACGCTTGCAGT, Rev: GTGTCGAGACATTTGTTG), human IL-13Rα1 (For: GCCTGTGTTTCTCTCCGTTGA, Rev: AGTTGGAATTGCTCTTTTGT), human IL-13Rα2 (For: GTCTAAATCCCTTGGCAG, Rev: CTCATGGACCTTTCCAAAGA), human Arg1 (For: AGTTGAGA CACTAGGAATTG, Rev: TCCAGTCCTGCTAACATCTAAAAC), human Arg2 (For: AAGTTGCACTTGGTTGTTG, Rev: CGCTGATT CTACATCGGTGG), human found in inflammatory zone (FIZZ) 1α (For: ATGGGCGCTTCTTCTCGG, Rev: GCCAGTGACAGCCATCCCAG), human platelet-derived growth factor (PDGF) B (For: TCTTCTGCTACTACCGGTGG, Rev: GTGAGGACGCCCTATGCTT), human serumotin transporter (5-HT7) (For: ATGGAGACCA GCCATCCCAG, Rev: AAGGAGAACGCCCTTTGA), human epidermal growth factor (EGF) (For: AAGGAGA CATTGCAAGAAAATGG, Rev: ACATACCTCTCTCTGCTTGGACC ACTGCTCATGTTGCTTCTAG), human survivin (For: ACCTGAAAGCTTCTTCGCA, Rev: AACCTTCACGCTACCTAC), human basic fibroblast growth factor (bFGF) (For: AGAGAGACTCTGCTCTGGTG, Rev: GACCTCCCTCTACCCTGGTG), human transforming growth factor (TGF)-β1 (For: GGGCAGATCCGTCTCAAGCG, Rev: GTGAGGGTCACACTTACAG), human endothelin-1 (For: CAGCAGT TCTTGCCTCTG, Rev: ACTTCTTATCCAGGAG), and human β-actin (For: CATGTACGGTGCTATCCAGGC, Rev: CTCC TTTAAGTGCACGAGC).

Immunoblot analysis. Western blot analysis was done using whole lung or cell lysates as described previously (22). Primary antibodies used in this study include rabbit Arg1 and -2 antibodies (Santa Cruz Biotechnology), mouse endothelial nitric oxide synthase (eNOS/NOS3) antibody (BD Biosciences Pharmingen, San Diego, CA), and rabbit phospho- eNOS (Ser1177) antibody (Cell Signaling, Danvers, MA).

Enzyme activity. Tissue levels of nitrite/nitrate as an indicator of NO production and arginase activity in lung lysates were measured using commercially available kits according to the manufacturer’s recommendations [NO quantification kit (Active Motif) and QuantiChrome Argi-nase Assay Kit (BioAssay Systems)]. Using the same samples, tissue levels of N-tyrosine as a marker of peroxynitrite were also assessed using a commercially available kit (OxiSelect Nitrotyrosine ELISA Kit; CELL BIOABS, San Diego, CA).

Cell culture. Primary hepatic pulmonary artery smooth muscle cells (hpaSMC) were purchased from Lonza (Basel, Switzerland). Cells between the 5th to 10th passages were used. Supplements and growth factors for hpaSMC include EGF, insulin, bFGF, FBS and gentamicin, and amphotericin-B.

Cell proliferation assay. Cells were seeded (4,000 cells/well) in 96-well plates. Water-soluble tetrazolium salt-1 (WST-1)-reagent (Roche) was added directly into cell culture media between 16 and 24 h after starvation to detect mitochondrial dehydrogenase enzymes. After 1 h of incubation, the plate was then read at 450 nm with the reference reading at 630 nm. Cell proliferation was further assessed using the bromodeoxyuridine (BrdU) Cell Proliferation Assay Kit (Cell Signaling Technologies) following the manufacturer’s instruction.

Small-interfering RNA transfection. In a six-well tissue culture plate, 2 × 10^5 cells/well were seeded with 2 ml of antibiotic-free normal growth medium supplemented with FBS. The small-interfering RNAs (siRNAs) for Arg2 and IL-13Rx2, and control siRNA were purchased from Santa Cruz Biotechnology. Transfection was performed using the commercially available kit according to the manufacturer’s recommendations (Santa Cruz Biotechnology). The silencing effect of each siRNA was evaluated by quantitative RT-PCR. The expression level of each gene in the cells transfected with specific siRNA was <80% lower compared with that of the cells transfected with control siRNAs. The silencing effect of each siRNA was evaluated by quantitative RT-PCR. Cells transfected with scrambled siRNA were used as a control for all of the experiments using siRNA.

The expression level of each gene in the cells transfected with specific siRNA was ~60–90% lower compared with that of the cells transfected with control siRNAs.

Assessment of tissue hypoxia in the lungs. Mice were given 60 mg/kg of pimonidazole by intraperitoneal injection 90 min before death. Next, IHC against pimonidazole was performed using formalin-fixed and paraffin-embedded tissue section according to the manufacturer’s recommendation (Hypoxyprobe-1 Kit; Hypoxyprobe).

Statistics. We used t-tests or ANOVA for continuous normally distributed outcomes to test for differences between groups. The Wilcoxon rank test or nonparametric ANOVA was used for nonnor- mally distributed outcomes to see differences between groups. SAS version 9.1 (SAS Institute, Cary, NC) was used for statistics, and statistically significant differences were accepted at P < 0.05. The bar graphs represent means ± SE.

RESULTS

Chronic lung-specific overexpression of IL-13 induces vascular remodeling and hemodynamic changes. To see whether chronic overexpression of IL-13 in lungs induces PH, we evaluated the pulmonary vascular phenotypes of 2-mo-old IL-13 Tg mice and Wt littermates. We observed that 1-mo-old IL-13 Tg mice also display noticeable vascular remodeling (data not shown); however, the vascular phenotype was the most evident by the age of 2 mo (Fig. 1A). As reported previously, we also
observed macrophage- and eosinophil-dominant inflammation, emphysematous alveolar destruction, and fibrosis in IL-13 Tg lungs (44). The major infiltrating cells in the perivascular region are eosinophils in IL-13 Tg lungs (data not shown).

RV hypertrophy, assessed by RV weight normalized to the weight of the left ventricle (LV) + interventricular septum (IVS) in Wt and IL-13 Tg mice (n = 8 in each group, *P < 0.05). C: RV systolic pressures (RVSP) in Wt and IL-13 Tg mice (n = 6 in each group, **P < 0.01). D: Pulmonary barium-gelatin angiography from Wt and IL-13 Tg mice. E: α-smooth muscle actin immunostaining of the pulmonary arteries from Wt and IL-13 Tg mice. F: α-smooth muscle (SM) actin (left) and myosin (right) immunostaining of the pulmonary arteries from a IL-13 Tg mouse. G: Immunohistochemistry (IHC) against α-smooth muscle actin for the evaluation of small muscular pulmonary arteries (arrows) at the periphery of the lungs from Wt and IL-13 Tg mice. H: IHC against pimonidazole, which represents hypoxic area, in the lungs from Wt and IL-13 Tg mice. All bars represent 25 μm.

Fig. 1. Interleukin (IL)-13 induces vascular remodeling and hemodynamic changes of pulmonary hypertension. The vascular remodeling and hemodynamic changes of 2 mo-old wild-type (Wt) and IL-13 transgenic (IL-13 Tg) mice were evaluated. A: hematoxylin and eosin (H&E)-stained pulmonary arteries from Wt and IL-13 Tg mice. AW, airways; arrows, remodeled vessels. B: right ventricular (RV) hypertrophy measured by the weight ratios of RV/left ventricle (LV) + interventricular septum (IVS) in Wt and IL-13 Tg mice (n = 8 in each group, *P < 0.05). C: RV systolic pressures (RVSP) in Wt and IL-13 Tg mice (n = 6 in each group, **P < 0.01). D: Pulmonary barium-gelatin angiography from Wt and IL-13 Tg mice. E: α-smooth muscle actin immunostaining of the pulmonary arteries from Wt and IL-13 Tg mice. F: α-smooth muscle (SM) actin (left) and myosin (right) immunostaining of the pulmonary arteries from a IL-13 Tg mouse. G: Immunohistochemistry (IHC) against α-smooth muscle actin for the evaluation of small muscular pulmonary arteries (arrows) at the periphery of the lungs from Wt and IL-13 Tg mice. H: IHC against pimonidazole, which represents hypoxic area, in the lungs from Wt and IL-13 Tg mice. All bars represent 25 μm.

vascular density of IL-13 Tg mice, which may represent vascular pruning, a common finding in patients with PH (Fig. 1D) (24). Taken together, these findings demonstrate that IL-13 Tg mice develop the typical phenotype of PH at the age of 2 mo. To further define the vascular remodeling, we performed IHC against α-smooth muscle actin, and it showed that IL-13 Tg mice have prominent medial thickening of small pulmonary arteries, a hallmark of clinical PH (Fig. 1E). IHC against α-smooth muscle actin and myosin using two consecutive lung sections revealed that myofibroblasts (α-smooth muscle actin-positive and myosin-negative cells) were prominent in the
medial layer of pulmonary arteries in IL-13 Tg lung, which indicates a significant contribution of myofibroblasts to medial remodeling in these mice (Fig. 1F). Previous researchers reported that myofibroblasts, which are thought to be originated from the expansion of resident vascular smooth muscle cells, adventitial fibroblast, or vascular progenitor cells, might play an important role in the vascular remodeling process in PAH (26). In addition, increased neomuscularization of small pulmonary arteries was also observed in IL-13 Tg mice (Fig. 1G). Taken together, the smooth muscle cell-driven remodeling process appears to dominate in pulmonary arteries of IL-13 Tg mice. Given the presence of a significant nonvascular phenotype in IL-13 Tg lungs, we assessed tissue hypoxia using a commercially available kit (Hypoxyprobe-1). The dark brown-colored 3,3′-diaminobenzidine reaction, which signifies the presence of tissue hypoxia, was observed in the few airways in IL-13 Tg mice (Fig. 1H). This result suggests that tissue hypoxia is present in IL-13 Tg lungs, which may contribute to the development of PH to some extent in IL-13 Tg mice.

**IL-13 induces the expression and activity of Arg1 and -2 and NOS3 in the lungs.** Th2 cytokines, IL-4 and IL-13, induce expression of both Arg1 and -2 in various cell types (45). The role of arginase in the pathogenesis of PAH has recently gained much attention because increased activity of arginase can reduce NO synthesis by competing with NOS3 (endothelial) for the substrate L-arginine (41). NO is a pulmonary vasodilator and acts as an antimitogenic agent to prevent vascular remodeling (18), leading to mitigating the clinical phenotype of PAH. Subsequently, we confirmed increased expression and activity of arginases in IL-13 Tg mice (Fig. 2, A and B). IHC further revealed intense positivity for both arginases in alveolar macrophages (Fig. 2, C and D). As previously reported by others (4, 5, 27, 41), we also observed the expression of Arg2 in endothelial cells and vascular smooth muscle cells (Fig. 2E) but could not see any expression of Arg1 in vascular cells in mice (data not shown). Given the role of arginase in regulating the production of NO, we evaluated the expression and activity of three NOS isozymes in the lungs of IL-13 Tg mice. The level of total nitrate and nitrite, which reflects the total amount of NO generated in whole lung tissues, was increased in IL-13 Tg mice (Fig. 2F). Among three isozymes of NOS, the expression of NOS3 (eNOS) was only significantly upregulated in IL-13 Tg lungs (Fig. 2G). We could not see any expression of nitric oxide synthase 2 (NOS2, inducible nitric oxide synthase) at the protein level in IL-13 Tg lungs (data not shown). To further confirm these in vivo findings, we also evaluated the expression of NOS2 in macrophage in vitro, the main source of NOS2, after stimulation with recombinant (r) IL-13. Again, we did not see any significant induction of NOS2 in macrophages by IL-13 (data not shown). This suggests that IL-13 increases production of NO mainly through activation and induction of NOS3.

**Arg2 plays a critical role in IL-13-induced pulmonary vascular remodeling.** Recent work suggests that the expression and activity of Arg2, not Arg1, is increased in the lungs of patients with idiopathic PAH, leading to the diversion of arginine away from NOS3 in endothelium (41). To further define the specific role of Arg2 in IL-13-induced PH, IL-13 Tg mice were crossed with Arg2 null mutant mice (Arg2−/−). Pulmonary vascular phenotypes were assessed in the IL-13 Tg+/Arg2−/− mice compared with IL-13 Tg+/Arg2+/+, Wt, and Arg2−/− mice. The medial thickening of pulmonary arteries was significantly decreased in IL-13 Tg+/Arg2−/− compared with IL-13 Tg+/Arg2+/+ mice (Fig. 3, A and B). Of note, no significant intimal remodeling was observed in either mouse. Arg2−/− mice did not have any significant vascular remodeling (data not shown). A systematic assessment of vascular remodeling using the imaging software further confirmed a significant reduction in the medial wall thickening of small pulmonary arteries in IL-13 Tg+/Arg2−/− compared with IL-13 Tg+/Arg2+/+ mice (Fig. 3, C and D). Furthermore, neomuscularization of small pulmonary arteries was also less frequently observed in IL-13 Tg+/Arg2−/− mice compared with IL-13 Tg+/Arg2+/+ mice (Fig. 3E). These findings indicate that a smooth muscle cell-driven vascular remodeling in IL-13 Tg lungs depends, at least in part, on the Arg2 pathway.

**Arg2 regulates IL-13-induced hemodynamic changes by modulating the production of NO.** To determine whether a significant reduction of vascular remodeling leads to improvement in hemodynamic status, RVSP of IL-13 Tg mice with or without Arg2 null mutation were measured (Fig. 4A). Null mutation of Arg2 significantly decreased RVSP, although it did not normalize pressure. We demonstrated earlier that the activity and expression of NOS3 is increased in IL-13 Tg lungs. To assess the contribution of NOS3 to the development of IL-13-induced PAH, IL-13 Tg mice were also crossed with NOS3 null mice (NOS3−/−). Null mutation of NOS3 in IL-13 Tg mice did not reduce the increase of RVSP observed in IL-13 Tg mice (Fig. 4A). Furthermore, pulmonary vascular remodeling of IL-13 Tg mice was not improved with either null mutation of NOS3 or Nω-nitro-L-arginine methyl ester (l-NAME) treatment (data not shown). This suggests that increased expression and activity of NOS3 plays a limited role against IL-13-induced hemodynamic changes. To understand the mechanism(s) of hemodynamic improvement in IL-13 Tg mice with Arg2 null mutation, the total amount of NO produced in the lungs was measured. The data showed significantly higher levels of nitrate and nitrite in IL-13 Tg+/Arg2−/− mice compared with IL-13 Tg+/Arg2+/+ (Fig. 4B). On the other hand, IL-13 also stimulated production of peroxynitrite, a nitrosative radical measured by the levels of N-tyrosine; the levels are mitigated by Arg2 null mutation with borderline significance (Fig. 4C). Thus, we conclude that null mutation of Arg2 further increased the production of NO with a decrease of peroxynitrite in the lungs of IL-13 Tg mice, which may explain the hemodynamic improvement in these mice. Interestingly, arginase activity of IL-13 Tg+/Arg2−/− mice was only slightly decreased (Fig. 4D). Because the measured activity of arginase in IL-13 Tg+/Arg2−/− is solely from Arg1, this suggests that the activity of Arg1 is preserved with null mutation of Arg2 in IL-13 Tg mice. Consistent with this result, we noted similar levels of Arg1 protein expression in IL-13 Tg+/Arg2−/− compared with IL-13 Tg+/Arg2+/+ mice (Fig. 1E).

**IL-13 induces proliferation of human pulmonary artery smooth muscle cells via an Arg2-dependent pathway.** We observed prominent medial thickening and neomuscularization of pulmonary arteries in IL-13 Tg lungs. To address whether this proliferative phenotype of vascular smooth muscle cells is due to direct effect of IL-13, we employed an in vitro system. hpaSMC were treated with rIL-13, and then the proliferation of cells was assessed using the WST-1 proliferation assay. Time- and dose-dependent kinetics confirmed that IL-13 stimulates the proliferation of hpaSMC maximally at 25 ng/ml of con-
Fig. 5. IL-13 induces the expression and activity of arginase (Arg) 1, Arg2, and nitric oxide synthase 3 (NOS3). A: the expression of Arg1 and Arg2 in Wt and IL-13 Tg lungs detected by Western blot evaluation. B: the activity of arginase in the lungs from Wt and IL-13 Tg mice assessed by a commercial kit (n = 4 in each group, **P < 0.01). C: IHC against Arg1 in the lungs from Wt and IL-13 Tg mice. D: IHC against Arg2 in the lungs from Wt and IL-13 Tg mice. E: double-labeled fluorescent IHC using antibodies against Arg2 and cell-specific markers of endothelial cells (CD141) and vascular smooth muscle cells [α-smooth muscle actin (SMA)]. Arrows, double-positive cells. F: the activity of NOS in the lungs from Wt and IL-13 Tg mice assessed by a commercial kit (n = 4 in each group, *P < 0.05). G: the expression and activation of NOS3 in the lungs from Wt and IL-13 Tg mice evaluated by Western blots. p, Phosphorylated. All bars represent 25 μm.

centration at the 48-h time point (Fig. 5A). Interferon-γ, which was used as a control reagent, did not proliferate hpaSMC (Fig. 5B). IL-13-stimulated proliferation of hpaSMC was further confirmed again using the BrdU assay (Fig. 5C). Next, studies were undertaken to see whether IL-13-induced proliferation of hpaSMC is mediated through Arg2, since null mutation of Arg2 significantly decreased the medial thickening of pulmonary arteries of IL-13 Tg mice. First, we confirmed the expression of Arg2 in these cells, which was further induced with IL-13 treatment using double-labeled fluorescent IHC (Fig. 5D). In addition, IL-13 strongly induced the mRNA and protein expression of Arg2 in hpaSMC that peaked at 48 h...
However, we could not see any significant expression of Arg1 in these cells (data not shown). Next, the cells were transfected with Arg2-specific and control siRNAs and then stimulated with IL-13. We observed that knocking Arg2 gene expression down by gene-specific siRNA significantly inhibited the IL-13-stimulated proliferation of hpaSMC (Fig. 5G). To explore the role of other well-established mitogens in the IL-13-induced proliferation of hpaSMC, we also examined the mRNA expression of PDGF, 5-HTT, survivin, endothelin-1, EGF, bFGF, hypoxia-induced mitogenic factor...
Fig. 4. Arg2 and NOS3 regulate IL-13-induced hemodynamic changes by modulating the production of nitric oxide. IL-13 Tg mice were crossed with Arg2<sup>/−/−</sup> and NOS3<sup>/−/−</sup> mice, and the 2-mo-old pups from these crosses were evaluated. A: RVSP in Wt, Arg2<sup>/−/−</sup>, and NOS3<sup>/−/−</sup> mice in the absence (−) and presence (+) of Tg expression of IL-13 (n = 6 in each group, *P < 0.05). B–D: tissue levels of nitrite/nitrate and N-tyrosine, and arginase activity in the lungs from Wt and Arg2<sup>/−/−</sup> mice in the absence (−) and presence (+) of Tg expression of IL-13 (n = 4 in each group, *P < 0.05). E: Arg1 expression detected by Western analysis using total lung lysates from Wt (W), IL-13 Tg (T), and IL-13 Tg/Arg2<sup>/−/−</sup> (TA).

(HIMF), TGF-β1, and IGF-I in hpaSMC in response to IL-13 stimulation (1, 15, 19). None of the genes of these mitogens were significantly induced in response to IL-13 treatment (data not shown). We also did not note any significant changes in the expression of type 2 response markers, such as YM1, FIZZ1, or acidic mammalian chitinase, in these cells with IL-13 stimulation (data not shown). When viewed in combination, these findings imply that Arg2 plays a pivotal role in IL-13-stimulated proliferation of hpaSMC.

IL-13-induced proliferation of hpaSMC by Arg2 is signaled through IL-13Rα2. The expression of IL-13Rα2 at the mRNA and protein levels is increased in the lungs of IL-13 Tg mice (43). Recently, Hecker et al. reported that IL-13Rα2 is prominently expressed in the medial layer of human pulmonary arteries, and the expression of IL-13Rα2 is significantly upregulated in patients with idiopathic PAH (16). Consistent with these studies, IL-13 receptor α1 (Rα1) and IL-13Rα2 were significantly induced in IL-13 Tg lungs (Fig. 6A). However, the Arg2 did not modulate the expression of these receptors in the lung (Fig. 6A). To see whether these findings are reproducible in our in vitro model, the mRNA expression of IL-13 receptors was examined using quantitative real-time PCR with or without IL-13 treatment. We observed that only IL-13Rα2, but not IL-13Rα1, was significantly upregulated in response to IL-13 treatment among all of the IL-13 receptors (Fig. 6B and data not shown). Interestingly, when the gene expression of IL-13Rα2 was ablated with IL-13Rα2-specific siRNA, hpaSMC did not proliferate in response to IL-13, suggesting IL-13Rα2 mediates the IL-13 effect on hpaSMC proliferation (Fig. 6C). Furthermore, knocking down IL-13Rα2 also significantly inhibited the IL-13 induction of Arg2 gene in hpaSMC (Fig. 6D). On the other hand, the IL-13-stimulated increase of IL-13Rα2 was not changed with Arg2 knockdown, suggesting that Arg2 is a downstream mediator of IL-13Rα2 in IL-13-stimulated responses (Fig. 6E). These findings suggest that IL-13 stimulate the proliferation of hpaSMC through an IL-13Rα2-Arg2-dependent pathway.
IL-13Ra2 regulation of IL-13-induced vascular remodeling in the lung. Because IL-13Ra2 plays an important role in the proliferation of hpaSMC, we further investigated the in vivo role of IL-13Ra2 by crossing IL-13 Tg mice with IL-13Ra2 null mutant mice. As reported previously in our laboratory, IL-13 induced macrophage- and eosinophil-dominant inflammation in the lung (44). Consistent with the previous findings from our laboratory (43), we also observed null mutation of IL-13Ra2 further enhanced IL-13-stimulated inflammatory and airway-remodeling responses in the lung (data not shown). On the other hand, IL-13-stimulated vascular remodeling was less in IL-13 Tg+/IL-13Ra2−/− mice compared with IL-13 Tg+/IL-13Ra2+/+ mice (Fig. 6F), consistent with the in vitro observation that IL-13 α2 mediates IL-13-stimulated proliferation of hpaSMC. Consistent with these vessel changes, we also noted significant decreases in left ventricular hypertrophy measured by the RV-to-LV + IVS weight ratio in IL-13 Tg+/IL-13Ra2−/− mice compared with IL-13 Tg+/IL-13Ra2+/+ mice (Fig. 6G).

IL-13Ra2 and Arg2 regulation of IL-13-stimulated signal transducer and activator of transcription and TGF-β signaling pathways. To further understand the mechanism of IL-13Ra2 regulation of IL-13-stimulated vascular smooth muscle remodeling, we investigated the signaling events regulated by IL-13Ra2 and Arg2 in hpaSMC. Because signal transducer and activator of transcription (STAT) signaling is the major pathway that IL-13 uses for its effector function (13, 17), we evaluated the role of IL-13Ra2 in IL-13-stimulated STAT6 and STAT3 signaling in hpaSMC. In Western evaluation, siRNA silencing of IL-13Ra2 significantly reduced IL-13-stimulated STAT3, but not STAT6, activation (Fig. 7A). Because TGF-β was shown as a downstream mediator of IL-13-IL-13Ra2 (10), we
evaluate the levels of active TGF-β in the lungs from IL-13 Tg mice with and without Arg2 null mutation. As shown in Fig. 7B, we noted significant increases of TGF-β in the lungs from IL-13 Tg mice, and Arg2 null mutation abrogate the IL-13 induction of TGF-β. These studies strongly suggest that Arg2 plays a critical role in the specific IL-13 signaling and effector functions mediated by IL-13Rα2.

**DISCUSSION**

IL-13, a pleiotropic Th2 effector cytokine, is released not only by type 2 T-helper lymphocytes but also by other inflammatory and structural cells in the lungs, such as macrophages and epithelial cells (39). IL-13 has been shown to play an essential role in the pathogenesis of various lung diseases, such as bronchial asthma, pulmonary fibrosis, and chronic obstructive pulmonary disease (22). However, the potential role of IL-13 in the pathogenesis of PH has not been explored until recently. Daley et al. demonstrated that antigen-driven Th2 immune response caused severe pulmonary arterial muscularization but without hemodynamic compromise in mice (8). IL-13 was elevated in the plasma of patients with systemic sclerosis with PAH compared with patients without PAH (6). In addition, IL-13 is reported to be an important mediator of the development of PH in a murine model of Schistosomiasis (14). Most excitingly, increased expression of both IL-13 and its receptors in small pulmonary arteries of patients with IL-13-induced proliferation of hpaSMC by Arg2 is signaled through IL-13 receptor α2 (Rα2). A: the mRNA expressions of IL-13 receptors in Wt and Arg2−/− in the absence of presence of IL-13 transgene (n = 4 mice/group, **P < 0.01). B: the expression kinetics of mRNAs of IL-13 receptor α1 (Rα1) and IL-13Rα2 in hpaSMC in the absence (−) or presence (+) of rIL-13 stimulation (25 ng/ml, n = 4 at each time point, *P < 0.05 compared with Wt). C: the effect of silencing of IL-13Rα2 gene on rIL-13-stimulated proliferation of hpaSMC at the 48-h time point (25 ng/ml, n = 8, *P < 0.05). D: the effect of silencing of IL-13Rα2 gene on rIL-13-induced expression of Arg2 mRNA in hpaSMC (25 ng/ml, n = 4, *P < 0.05). E: the effect of silencing of Arg2 on rIL-13-induced expression of IL-13Rα2 mRNA in hpaSMC (25 ng/ml, n = 4, *P < 0.05). F: representative IHC against α-smooth muscle actin (SMA) in the lungs from IL-13 Tg mice with and without IL-13Rα2. Arrows, SMA-positive vascular smooth muscles. Bar, 100 μm. G: weight ratio of RV vs. LV + IVS of the lungs from IL-13 Tg mice with and without IL-13Rα2 (n = 3 in each group, *P < 0.05).
idiopathic pulmonary arterial hypertension has been reported (16). Although these studies suggest that IL-13 plays a role in the pathogenesis of PAH, no studies have proven a direct causal relationship between IL-13 and PAH. Furthermore, Hecker et al. observed that IL-13 signaling suppressed proliferation of human pulmonary artery smooth muscle cells in vitro (16). This is somewhat contradictory to what is expected, considering that the proliferative phenotype of vascular smooth muscle cells in patients with PAH is well established (16). Therefore, the role of IL-13 in the pathogenesis of PAH is still not clear. In this study, we provided, for the first time, direct evidence of a pathogenic role of IL-13 in the development of PH and elucidated novel mechanisms of IL-13-induced PH using lung-specific IL-13-overexpressing Tg mice and primary human pulmonary artery smooth muscle cells. In this study, we took advantage of the Tg approach to address the effector function of IL-13 on the development of the vascular phenotype, since it provides sufficient and homogenous expression of IL-13 across the pulmonary vascular bed compared with other animal models of allergic inflammation or Schistosomiasis.

In this study, we further demonstrated that Arg2 is a critical downstream mediator of IL-13-induced PH. Arginases are the key enzymes in \( \text{L-arginine} \) metabolism that convert \( \text{L-arginine} \) to \( \text{l-ornithine} \) and \( \text{urea} \). Two isozymes of arginase with different expression patterns are known to play a role in the urea cycle (27). Th2 cytokines, IL-4 and IL-13, induce expression of both Arg1 and Arg2 in various cell types (45). The expression of Arg1 by Th2 cytokines is through Janus kinase/STAT pathways. Arg2 is induced by these cytokines via a yet unidentified pathway (29, 36, 45). We observed that alveolar macrophage is the major source of both arginases in the lungs of IL-13 Tg mice. Vascular endothelial and smooth muscle cells are reported to express both Arg1 and Arg2, but the distribution appears vessel and species dependent (27). We observed that Arg2, not Arg1, is expressed in vessels of mice. We confirmed no expression of Arg1 protein in hpaSMC, consistent with the previous studies that showed only Arg2 is expressed in human pulmonary artery endothelial cells (41). Therefore, Arg2 appears to be the major isozyme expressed in the human and murine pulmonary vasculature. Based on previous literature, arginase may contribute to the development of PAH through several mechanisms. First, arginases compete with NOS for the mutual substrate, arginine, leading to decreased bioavailability of NO (41). Second, the end products of enzymatic reactions of arginases include polyamines and \( \text{l-proline} \). Polyamines are known to stimulate cell growth and differentiation, and \( \text{l-proline} \) is an essential component of collagen synthesis (27). Thus, arginases can contribute to vascular remodeling by proliferating vascular cells and expanding extracellular matrix. IL-13 Tg mice have increased activity and expression of both arginases and NOS3. This implies that there might be a significant competition for \( \text{l-arginine} \) between these enzymes in IL-13 Tg lungs. The Michaelis constant for \( \text{l-arginine} \) is 1,000-fold higher for NOS3, but the maximum velocity is 1,000-fold lower for arginase, which implies equal avidity of both arginases and NOS3 in \( \text{l-arginine} \) use (46). In the setting of substrate (\( \text{L-arginine} \)) and/or the key cofactor deficiency (tetrahydrobioptein), electron transfer in the reductase domain of NOS3, which is a major determinant of enzymatic activity, is uncoupled to generate NO. Subsequently, NOS3 generates more reactive oxygen species rather than NO, leading to increased oxidative tissue injury (40). This phenomenon, known as NOS3 uncoupling, can paradoxically contribute to the development of the PAH (20). Given the possibility of extreme arginine depletion in IL-13 Tg lungs as a result of utilization by NOS3 and both arginases, NOS3 uncoupling can theoretically be the underlying mecha-
nism of PAH in IL-13 Tg mice. To address this intriguing question, we crossed IL-13 Tg mice with NOS3 null mutant and found that IL-13 Tg mice with null mutation of NOS3 did not reduce RVSP compared with IL-13 Tg mice. In addition, pulmonary vascular remodeling of IL-13 Tg mice was not improved with either null mutation of NOS3 or l-NAME treatment (data not shown). Because NOS2 expression was not noted in IL-13 Tg lungs or in the macrophages stimulated by rIL-13, NOS3 is likely to be the major NOS responsible for the production of NO or peroxynitrite, the critical mediators for the maintenance of vascular homeostasis, in the lungs of IL-13 Tg mice. However, either genetic or chemical ablation of NOS did not provide a significant improvement of PH phenotypes of IL-13 Tg lungs; we conclude that a significant NOS3 uncoupling does not appear to take place in IL-13 Tg lungs.

These results suggest that NOS signaling (including dysfunctional NOS) plays only a limited role in the pathogenesis of PH in IL-13 Tg mice.

One of the characteristic features of the IL-13-induced pulmonary vascular remodeling is the impressive medial remodeling without significant intimal change. Previous human studies reported deregulated endothelial Arg2 expression was associated with prominent intimal thickening in human PAH patients (41). In IL-13 Tg lungs, we did not note any significant intimal thickening. In the current studies, we do not know whether IL-13 has a different effect on endothelial cells vs. vascular smooth muscle cells. Inherent differences in endothelial cells between human and murine lungs might result in a different intimal remodeling response. The role and effects of IL-13 on endothelial cells in association with Arg2 expression warrant further investigation in future studies.

Our work suggests that Arg2 drives the medial thickening by direct proliferating vascular smooth muscle cells in IL-13 Tg lungs. Initially, we expected HIMF (also known as FIZZ1) to play an important role in IL-13-induced PAH, since HIMF is one of the mediators in various experimental animal models of PAH and is induced by Th2 cytokines in macrophages through an alternative activation pathway (1, 25). Although expression of FIZZ1 is upregulated in IL-13 Tg lungs, we could not detect any significant induction of FIZZ1 gene in hpaSMC after IL-13 treatment (data not shown). In the current studies, we could not rule out the paracrine effects of FIZZ1 or other factors in vascular smooth muscle cell proliferation. Because the genetic ablation of Arg2 did not completely rescue the IL-13-induced vascular phenotype, we speculate that other mediators, including FIZZ1, may contribute to the vascular remodeling process in IL-13 Tg lungs. The exact role of Arg2 in FIZZ1 or other growth factor-stimulated vascular smooth muscle proliferation warrants further investigation in future studies.

IL-13 has three receptors, Ro1, Ro2, and IL-4R. IL-13 signaling is conveyed by phosphorylation of STAT6 through R Ho2 signaling. The decoy function of IL-13Ro2 in IL-13 signaling leads to modulation of proinflammatory Th1 and Th17 responses in an animal model of colitis (37). However, a number of recent studies also strongly support that IL-13Ro2 has signaling functions especially in the area of tissue remodeling and proliferation of cancer cells (3, 9-12, 21, 31). The implication of these bifunctional roles of IL-13Ro2 as a decoy and also as an active signaling molecule in the pathogenesis of specific disease is still largely undefined. In this regard, our studies suggest that IL-13Ro2 plays a different role in IL-13-stimulated inflammatory responses and in vascular smooth muscle proliferation. Consistent with previous studies in our laboratory (42), null mutation of IL-13Ro2 significantly enhanced IL-13-induced inflammation, supporting a decoy function of IL-13Ro2. On the other hand, IL-13-stimulated vascular smooth muscle cell proliferation was significantly reduced by siRNA silencing of IL-13Ro2, suggesting an active stimulatory role of IL-13Ro2. Thus, it is intriguing to speculate that the difference in IL-13 effector function depending on the cellular compartments could result from functional dichotomy of IL-13Ro2 signaling. The decoy function of IL-13Ro2 might be mediated through inhibition of STAT6 signaling, whereas the cell stimulatory function of IL-13Ro2, such as vascular smooth muscle cell proliferation, could be mediated through STAT3 signaling. Our studies showed that IL-13Ro2 and Arg2 significantly affect the IL-13-stimulated STAT3, but not STAT6, signaling pathways. Because it has been reported that the IL-13-stimulated induction of TGF-β was mediated by IL-13Ro2 (10), the significant reduction of the levels of TGF-β in IL-13 Tg lungs with null mutation of Arg2 further supports that Arg2 plays an important role in the effector function of IL-13 mediated through IL-13Ro2. In this regard, the contribution of STAT3 signaling to the pathogenesis of PH and a proliferative function of the STAT3 pathway has been reported by several independent studies (7, 28, 32). Interestingly, our study also demonstrated that IL-13-induced proliferation of hpaSMC by Arg2 is signaled through IL-13Ro2. The contribution of IL-13 signaling through IL-13Ro2 to the development of PAH is further supported by a recent study that demonstrated increased expression of IL-13 and IL-13Ro2 in pulmonary arteries of patients with idiopathic PAH (16). However, this study reported that IL-13 suppressed proliferation of hpaSMC in vitro, which was enhanced by knocking down the IL-13Ro2 gene using siRNA (16). This is contradictory to our observation. We speculate that differences in the in vitro experimental settings might have caused the discrepancies between their study and ours. However, this needs to be further verified by additional studies. The in vivo effects of chronic exposure to IL-13 cannot be easily recapitulated by simple external administration of recombinant proteins. In this regard, our Tg mice, which constitutively express IL-13, provide us with better insight into the chronic effector function of IL-13 in the pathogenesis of PH. Therefore, it is more reasonable to conclude that IL-13 stimulates, rather than inhibits, the proliferation of vascular smooth muscle cells, since both our in vivo and in vitro models demonstrate a proliferative phenotype.

IL-13 Tg mice also have prominent airway fibrosis along with emphysematous alveolar destruction (44) with tissue hypoxemia after 2–3 mo of age. Therefore, IL-13 Tg mice may represent an animal model of PH secondary to chronic lung diseases. However, we observed that 1-mo-old IL-13 Tg mice without significant parenchymal changes also display noticeable vascular remodeling (data not shown) and that IL-13 directly induces the proliferation of pulmonary artery smooth muscle cells. Furthermore, patients with idiopathic PAH have increased expression of IL-13 in their pulmonary arteries (16). Thus, we believe that IL-13 may play a pathogenic role in the
arteriopathy of either primary or secondary PH. To establish the
diagnosis of PH, we assessed RVSP and the remodeling of
pulmonary vessels and RV and performed angiography. Al-
though these are the standardized approaches to diagnose PH in
animals, evaluations of a series of defined parameters, includ-
ing left ventricular end-diastolic pressure, would have been
more helpful to further characterize the PH phenotype that
IL-13 Tg mice have given the lack of obligate neointimal
remodeling in these mice (15).

Although Arg1 is not expressed in hpaSMC in vitro, expres-
sion of Arg1 is induced in IL-13 Tg lungs. Considering that
both arginases have similar biological effects, we believe that
Arg1 also contributes to the pulmonary vascular phenotype of
IL-13 Tg mice. Thus, the specific contribution of Arg1 in the
development of IL-13-induced PH remains to be elucidated.
Furthermore, one of the major sources of both arginases is
alternatively activated alveolar of macrophages (45). Consid-
ering the emerging interest in the role of alternative activa-
tion of macrophages in the pathogenesis of PAH (35), future studies
to address this will be interesting.

In summary, we provide, for the first time, direct evidence of
a pathogenic role of IL-13 in the development of PH. Our study
demonstrates that IL-13 enhances the expression and activity of
Arg2 in the lungs, leading to deregulation of NO homeo-
stasis, muscularization of pulmonary arteries, and hemody-
namic changes. This study also highlights that an IL-13-IL-
13R alpha 2-Arg2 pathway could be a potential therapeutic target in
patients with PAH.

ACKNOWLEDGMENTS

We appreciate Susan Arditto for reviewing the manuscript.

GRANTS

This study was supported by National Institutes of Health Grant 804225 to
C. G. Lee. W.-K. Cho is the recipient of the 2011 ATS/Pulmonary Hyperten-
sion Association/Pfizer Research Fellowship in Pulmonary Arterial Hyperten-
sion Grant.

DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: W.-K.C., J.A.E., and C.G.L. conception and design of
research; W.-K.C., J.A.E., and C.G.L. and Y.H. performed experiments; W.-K.C.,
and C.-M.L. edited and revised manuscript; W.-K.C. and P.J.L. drafted manuscript;
interpreted results of experiments; W.-K.C. and P.J.L. performed surgery.

REFERENCES

HC, Crow MT, Johns RA. Hypoxia-induced mitogenic factor (HIMF/
FIZZ1/RELMalpha) induces the vascular and hemodynamic changes of
pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 296:

2. Balabanian K, Foussat A, Dorfmann P, Durand-Gasselin I, Capel F,
Bouchet-Delbos I, Portier A, Marfaing-Koka A, Krzysiek R,
Rimaniol AC, Simonneau G, Emilie D, Humbert M. Hypoxia
promotes human pulmonary cells proliferation through induction of
2009.

3. Benk AH, Hall B, VanDemark RP, Tschudy MO, Green SA, Chen Q,
Cibulli B, Szabo C, Sessa WC, Engleman EG, Wynn TA. Opposing
roles for IL-13 and IL-13 receptor alpha2 in pancreatic cancer invasion and metastasis.

4. Gonzalez-Moreno O, Calvo A, Joshi BH, Piekarz RL. Arg1 also
contributes to the pulmonary vascular phenotype of
IL-13 Tg mice. Thus, the specific contribution of Arg1 is induced in IL-13
Tg lungs. Considering that
5. Balabanian K, Foussat A, Dorfmann P, Durand-Gasselin I, Capel F,
Bouchet-Delbos I, Portier A, Marfaing-Koka A, Krzysiek R,
Rimaniol AC, Simonneau G, Emilie D, Humbert M. Hypoxia
promotes human pulmonary cells proliferation through induction of
2009.

6. Benk AH, Hall B, VanDemark RP, Tschudy MO, Green SA, Chen Q,
Cibulli B, Szabo C, Sessa WC, Engleman EG, Wynn TA. Opposing
roles for IL-13 and IL-13 receptor alpha2 in pancreatic cancer invasion and metastasis.

7. Kneidinger N, Eickelberg O, Morty RE. Interleukin-13 receptor alpha2
chain: a potential biomarker and molecular target for ovarian cancer therapy.

Kurup VP, Hogaboam C, Bouchet-Delbos L, Portier A, Marfaing-Koka A, Krzysiek R,
Kunsthek W, Zonnenberg B, Wynn TA, Eickelberg O, Morty RE. Interleukin-13
receptor alpha 2 in health and disease.

via IL-13R alpha2 induces major downstream fibrogenic factors mediating fibrosis in chronic TNBS colitis.

10. Garcia BM, Mentink-Kane MM, Wynn TA. IL-13 receptors and

11. Fichet-Feigl S, Young CA, Geissler EK, Schlitt HJ, Strober W. IL-13
signaling in the context of systemic sclerosis-muscle cell proliferation.

HC, Crow MT, Johns RA. Hypoxia-induced mitogenic factor (HIMF/
FIZZ1/RELMalpha) induces the vascular and hemodynamic changes of
pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 296:

13. Benk AH, Hall B, VanDemark RP, Tschudy MO, Green SA, Chen Q,
Cibulli B, Szabo C, Sessa WC, Engleman EG, Wynn TA. Opposing
roles for IL-13 and IL-13 receptor alpha2 in pancreatic cancer invasion and metastasis.

Z, Montueenga L, Puri RK, Green JE. Gene expression profiling iden-
tifies IL-13 receptor alpha 2 chain as a therapeutic target in prostate tumor

15. Gordon S, Martinez FO. Alternative activation of macrophages: mech-