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Schistosomiasis and the pulmonary vasculature (2013 Grover Conference series)

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Abstract: Inflammation is associated with multiple forms of pulmonary arterial hypertension (PAH), including autoimmune (scleroderma) and infectious (HIV, schistosomiasis) etiologies. More than 200 million people worldwide are infected with *Schistosoma*, predominantly in Brazil, Africa, the Middle East, and South Asia. Schistosomiasis causes PAH in about 6.1% of those chronically infected and is particularly associated with the species *Schistosoma mansoni*. Treatment for schistosomiasis-associated PAH includes antihelminthic treatment, if active infection is present (although associated with little immediate benefit to the pulmonary hypertension), and then pharmacologic treatment with targeted pulmonary vascular therapies, including phosphodiesterase type 5 inhibitors and endothelin receptor antagonists. The pathophysiological mechanism by which this parasitic infection causes pulmonary hypertension is unknown but is unlikely to be simple mechanical obstruction of the pulmonary vasculature by parasite eggs. Preexisting hepatosplenic disease due to *Schistosoma* infection is likely important because of portopulmonary hypertension and/or because it allows egg embolization to the lung by portocaval shunts. Potential immune signaling originating in the periegg granulomas causing the pulmonary vascular disease includes the cytokines interleukin (IL)-4, IL-6, IL-13, and transforming growth factor β. Modulating these pathways may be possible targets for future therapy of schistosomiasis-associated PAH specifically, and study of this disease may provide novel insights into other inflammatory causes of PAH.

Keywords: pulmonary hypertension, schistosomiasis, IL-13, TGF-β.

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Pulmonary hypertension (PH) is a condition of increased pressure in the pulmonary arterial system, causing increased afterload on the right ventricle and resulting in right heart failure. Pulmonary arterial hypertension (PAH) is a subset of PH in which the pulmonary arterial vasculature becomes obstructed and prevents normal blood flow through the lungs.

There are multiple causes of PAH, including congenital heart disease and association with connective tissue diseases such as scleroderma, but worldwide, schistosomiasis is likely one of the most common causes. Heart failure due to schistosomiasis was first reported in 1932 in Egypt.[1] At the time, many parasite eggs were observed in the lungs of individuals who died of this condition, and for a long time PH and right heart failure were thought to be due to mechanical obstruction. This concept was first suggested in the 1940s[2] and continued to be the assumed mechanism through the Second World Symposium on Pulmonary Hypertension, held in Evian, France, in 1998.[3,4] However, further studies of lung tissue from patients with this disease revealed the presence of plexiform lesions and other pathologic findings that were similar to other forms of PAH (see below), including idiopathic PAH (IPAH), the classic form of PAH. Thus, in the most recent classification system, constructed at a meeting in Dana Point, California, in 2008[5] and Nice, France, in 2013,[6] schistosomiasis-associated PAH has been categorized as a World Health Organization (WHO) group 1 disease, along with IPAH and connective tissue disease–associated PAH.

PARASITOLOGY

Schistosomiasis is the third most common parasitic disease worldwide (after malaria and intestinal helminths),

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infecting 200–300 million people.[7] The disease is caused by infection with the parasite *Schistosoma*, a flatworm fluke of the Trematoda class. *Schistosoma* is endemic in 52 countries. The most common species to infect humans is *Schistosoma mansoni*, which is endemic in northeast Brazil and Africa. Other *Schistosoma* species that infect humans include *Schistosoma hematobium*, endemic in Africa, the Middle East, India and East Asia; *Schistosoma mekongi*, endemic in Cambodia and Laos; and *Schistosoma japonicum*, endemic in China and Southeast Asia. Aside from people, *Schistosoma* can also infect nonhuman primates, other mammals (including some domestic livestock), and birds.

Unfortunately, schistosomiasis remains massively undertreated relative to the disease burden, leading to its classification as one of the 6 "neglected" tropical diseases. According to a 2013 WHO report,[8] in 2010 it was estimated that 237 million individuals required treatment for schistosomiasis, which subsequently increased to 243 million individuals in 2011 (an annual increase of 2.5%). In the same time periods, 35.0 million individuals received treatment in 2010 and 28.1 million in 2011, a decline of 19.7%; currently, only 11.6% of those who require treatment receive it. Reasons for the decrease in treated individuals cited include "logistical reasons, unreliable funding for implementation, change of implementing contractors, inadequate capacity at country level, fewer countries reporting data and also fewer people treated in some countries which reported data."[9] Currently, there are approximately 110 million praziquantel tablets pledged annually, which should be adequate to treat 40 million individuals.[8] The majority of the active disease burden is in Africa (85% of active disease, and 40 of the 52 countries where the disease is endemic, are in Africa), but management is limited. The WHO report notes, for example, that "although there was an adequate supply of praziquantel in the Central African Republic and Senegal, since both countries benefit from the Merck praziquantel donation through WHO, no treatments were administered in 2011,"[8(p85)] likely due to lack of "reliable funding for implementation."[8(p86)]

Biologically, individuals are susceptible to reinfection after adequate antihelminthic treatment, for reasons that are poorly understood.[10] There is some evidence of a gradual immunity that can develop over years of infection and treatment. However, this same lack of robust adaptive immunity has also substantially limited vaccine development, and no vaccine currently exists for schistosomiasis, although this is an area of active research.

Schistosoma has a mandatory two-host life cycle, including both a snail host and a mammalian or avian host (Fig. 1). Each *Schistosoma* species has a specific snail species that is its intermediate host; for example, *Biomphalaria glabrata* is the snail host of *S. mansoni*.

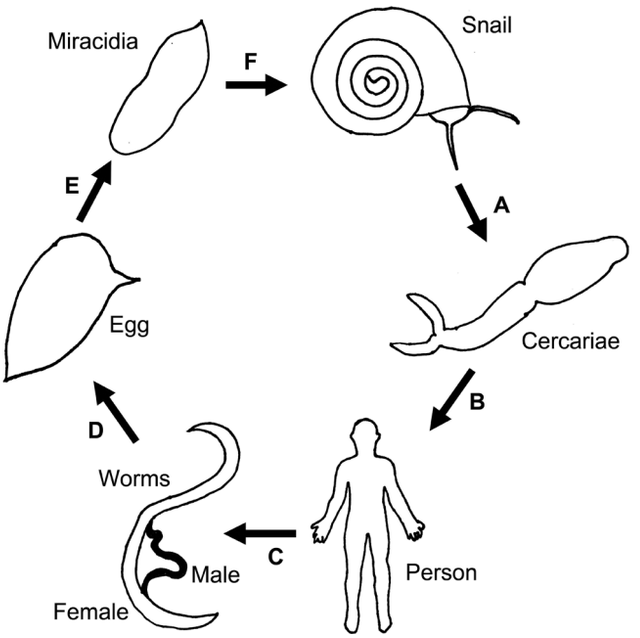

Figure 1. Schistosomiasis life cycle. *A*, The snail releases cercariae into fresh water. *B*, The cercariae penetrate the skin of individuals exposed to the water. *C*, Within the person, the worms home to the target organ (bladder venous plexus for *Schistosoma hematobium*; portal vein for all other species), *D*, The worms mate and release eggs. *E*, Most of the eggs penetrate the intestinal wall to reach the lumen (the bladder wall for *S. hematobium*), where they are excreted into fresh water and hatch into miracidia. *F*, The miracidia infect the snail host, completing the life cycle.

The life cycle (viewed from the human infection standpoint) starts when snails release the cercariae form of the *Schistosoma* parasite, which enters fresh water. Cercariae have a characteristic forked tail, can live up to 24 hours after release from the snail, have peak release from the snails in the morning (timed to correspond to human activity), and swim to the surface of the water (where humans are present). Cercariae penetrate the skin of individuals who are exposed through bathing, working, or drinking the water in as little as 5 minutes. The cercariae use proteolytic enzymes to facilitate percutaneous entry.

After entering the host, cercariae remain in the skin for 1 or 2 days, as they lose their tail and transform into schistosomula. At the site of entry, cercariae cause a punctuate erythematous rash, called cercarial dermatitis, which resolves after the parasite leaves the skin. The schistosomula enter the systemic venous circulation and pass to the pulmonary arterial circulation, where they become lodged in the lung vasculature. There, they cause an immune complex–mediated hypersensitivity reaction, called acute pulmonary schistoso-

miasis or Katayama fever, with signs and symptoms of fevers, chills, dry cough, and peripheral eosinophilia.[11,12] This syndrome self-resolves in 4–6 weeks as the schistosomula transform into adult worms, pass through the lung, and enter the pulmonary venous circulation. The parasites then home to their target organ, which is the portal venous circulation (particularly the large intestine, including the cecum) for all species except *Schistosoma hematobium*, which goes to the bladder venous plexus.

Once in the target organ, the worms mate and begin to release eggs, up to 300 per day for *S. mansoni*.[13] The eggs are metabolically very active and secrete more than a thousand proteins to facilitate passage through tissue planes.[14] About 90% of the eggs pass through the intestinal wall and enter the intestinal lumen, allowing the parasite to return to the environment. Once they contact fresh water, the eggs hatch and miracidia are released. Miracidia are the stage infectious to snails, thus completing the life cycle.

Within the host, worms can live up to 30 years, continually releasing eggs. This long life span is facilitated by a thick outer tegument covering the worms, which prevents destruction by the host immune system.[15]

Schistosoma mansoni egg antigens are metabolically very active, producing a large number of proteins (termed excretory/secretory products, or ESPs) to facilitate movement through tissue planes to reenter the colonic lumen and return to the environment, thus completing the life cycle. By mass spectroscopy, there are 188 discrete ESPs, many of which are heavily glycosylated;[16] by bioinformatic ontology analysis (comparing to known proteins from other well-characterized organisms), they are predicted to have a diverse range of functions, including nucleic acid binding (histones), protein binding (heat shock proteins, 14-3-3 proteins), ion binding (calpain), and antioxidant (thioredoxin, superoxide dismutase) and metabolic (fructose bisphosphate aldolase, enolase) roles. In particular, one of the most abundant glycoprotein ESPs, termed omega-1, was subsequently identified to be the key protein that stimulates dendritic cells to promote inflammation biased toward Th2.[17]

Approximately 10% of the eggs remain within the host. Retained eggs within the portal circulation are carried with the blood flow into hepatic precapillary vessels. The short axis of the eggs is about 50 μm wide, which is the vessel diameter where the eggs lodge. The eggs elicit a vigorous localized immune response, characterized by granuloma formation that includes macrophages, eosinophils, fibroblasts, and type 2 helper T ($CD4^+$ Th2) cells. The granulomas become progressively fibrotic as the eggs are degraded, and the fibrosis persists after egg clearance. About 10%–20% of individuals with chronic *Schistosoma* infection develop particularly severe portal fibrosis, called "Symmers's clay pipestem fibrosis" because of its gross pathologic appearance[18] or more conventionally "hepatosplenic disease," which results in portal hypertension.[19] Risk factors for the development of hepatosplenic disease are unknown but may include genetic predisposition, particularly severe burden of infection, or concurrent viral hepatitis infection.[20] A major cause of death in individuals with hepatosplenic disease is rupture of esophageal varicies. However, because the disease is pathologically pre–portal fibrosis, patients with this condition do not classically have cirrhosis or end-stage liver disease.

EPIDEMIOLOGY

Almost all patients with schistosomiasis-associated PAH have preexisting hepatosplenic disease, which is relevant for understanding the pathophysiology (see below). In 2011, Ward et al.[21] published a systematic review of the schistosomiasis-associated PAH epidemiology literature. They identified 28 studies published between 1938 and 2009, of which 16 were from Brazil, 12 were prevalence studies, and 9 were autopsy studies. The mean sample size was 173 patients, with a range of 8–682 patients. All published studies were judged to be of relatively low quality (a score of 3 or less out of 6), with none randomly sampling a community (many series included only patients hospitalized or referred for severe hepatosplenic disease, for example) and many diagnosing PH by autopsy findings rather than by right heart catheterization or even echocardiogram. The prevalence of PH among patients infected with *Schistosoma* ranged from 0% to 28.3%, with a mean of 7.4%. Taking into account the sample size of each study, the authors estimated the prevalence of PH among patients with *Schistosoma* infection to be 6.1%, the prevalence of PH among patients with hepatosplenic disease to be 15.1%, and schistosomiasis to be the cause of 30.8% of all cases of PH. In general, it is thought that schistosomiasis-associated PAH is primarily attributable to *S. mansoni* infection (endemic in Brazil and Africa), but this could be due to detection and reporting bias, as there are case reports of PAH occurring as a consequence of *S. hematobium* infection,[22] for example.

CLINICAL PRESENTATION AND DIAGNOSTIC EVALUATION

Patients with schistosomiasis-associated PAH may be asymptomatic, their PH detected through screening of individuals with schistosomiasis infection or hepatosplenic disease. However, the majority of patients who present will

have signs and symptoms due to the increased pulmonary vascular resistance and consequent right heart failure, like other patients with PAH. There may additionally be concurrent signs or symptoms of prehepatic portal hypertension (cutaneous varicies or history of intestinal bleeding).

Transthoracic echocardiography can provide significant data about the severity of the disease and right ventricular function and is the primary means of diagnosis in many countries with endemic schistosomiasis-associated PAH. However, there can be significant discrepancy between the estimated right ventricular pressure and the actual pulmonary artery pressure measured on right heart catheterization.[23]

The gold standard in diagnosing and determining the severity of PH is right heart catheterization, providing direct measurement of the pulmonary artery pressure. PH is defined as a mean pulmonary artery pressure in excess of 25 mmHg; PAH is defined as PH with a normal pulmonary capillary wedge pressure (≤15 mmHg). In addition, the right atrial pressure, cardiac output, and pulmonary artery oxygen saturation can be measured as indicators of right ventricular failure. During right heart catheterization, an acute vasodilator challenge can be performed (inhaled nitric oxide or intravenous prostacyclin) to determine whether the patient is a candidate for calcium channel blocker therapy, although in a recent series by Fernandes et al.,[24] none of the 54 patients with schistosomiasis-associated PAH who underwent right heart catheterization had a significant vasodilator response, and in another study by Japyassu et. al.,[25] only 3 of 84 of patients with *Schistosoma*-associated PAH had a significant acute vasodilator response.

If the patient is suspected of having schistosomiasis-associated PAH, it is important to determine whether active schistosomiasis infection is present, as treatment with an antihelminthic such as praziquantel is warranted. On laboratory evaluation, eosinophilia may be present, and there should be a positive serologic test for antibodies to *Schistosoma*. However, patients who have lived in an area where *Schistosoma* is endemic are likely to have a positive serologic test from prior infections, and thus it is necessary to perform a direct examination of the stool for eggs or a rectal biopsy (as well as test the urine for *S. hematobium* eggs if the patient may have been exposed to this specific species).

TREATMENT OF SCHISTOSOMIASIS-ASSOCIATED PAH

If there is concern that the patient may have active *Schistosoma* infection, the patient should be treated with an antihelminthic drug such as praziquantel. Praziquantel is very effective against mature parasites, requiring only a single dose, but it is not effective against immature parasites in patients recently infected. In patients with concern for acute infection, praziquantel can be administered in two doses, several weeks apart, to ensure clearance of all parasites,[26] or artemether can be used. Artemether is an antimalarial medication with activity against *Schistosoma* and is effective against juvenile parasites, although it is less effective against adult worms.[27] Patients with a high burden of infection can have a febrile illness after receiving praziquantel because of the release of worm antigens; a short course of corticosteroids can be administered concurrently to decrease the symptoms, but it may also lessen the efficacy of the treatment.[28] Corticosteroids should be considered particularly in patients with concern for egg deposition in the brain or spine, where localized swelling would be a significant problem.

As noted above, patients with schistosomiasis-associated PAH do not generally have substantial improvement in their disease severity after treatment with praziquantel.[29] However, there are multiple reasons for treating patients with active infection. Praziquantel will eliminate the ongoing production of eggs by adult worms, decreasing the antigenic burden in the lungs and the liver. As the preexisting eggs are degraded by the immune system, the periegg granulomas will resolve, and clearance of eggs and granulomas may decrease obstruction of the pulmonary vasculature. However, the pathologic vascular remodeling described above persists even after clearance of the eggs from the lung and resolution of the granulomas.

Small case series have documented the success of treating schistosomiasis-associated PAH with PAH-specific medications. Fernandes et al.,[30] at a referral center in São Paulo, Brazil, reported on 12 patients with schistosomiasis-associated PAH. All of the patients were from areas where *Schistosoma* is endemic and had liver ultrasounds suggestive of schistosomiasis disease. Of note, 4 patients were actively infected with *Schistosoma* at the time of initial evaluation (positive stool examination), but the clinical response to antihelminthic treatment was not specifically reported. Seven of the patients were treated with a phosphodiesterase type 5 (PDE5) inhibitor, 4 with an endothelin receptor antagonist, and 1 with combination therapy of both. The patients were followed for a mean of 35 months after the initiation of treatment. The 6-minute walk distance increased from a mean of 439 to 492 m ($P = 0.032$), and the WHO functional class distribution also improved significantly ($P = 0.002$). The pulmonary vascular resistance

on right heart catheterization improved significantly, from an average of 12.0 to 9.1 dyne-s/cm^5 ($P = 0.038$), although the mean pulmonary artery pressure did not change significantly (from 64 to 59 mmHg; $P = 0.13$). There were no significant side effects to treatment reported.

Another series, by Bandeira et al.,[31] reported the use of sildenafil in 13 patients diagnosed with schistosomiasis-associated PAH at a referral center in Recife, Brazil. The patients were followed for 6 months after initiation of treatment. The 6-minute walk distance increased from 121 to 394 m ($P < 0.001$), and the functional class of the patients also improved significantly ($P < 0.001$). The mean pulmonary artery pressure on right heart catheterization decreased significantly, from an average of 97 to 80 mmHg ($P < 0.002$). There were no significant side effects to the treatment.

A smaller study by Loureiro et al.[32] from the same group in Recife included a right ventricular imaging component. Seven patients were treated with sildenafil and followed for 3 months. At the end of the study, the patients had a significant improvement in 6-minute walk distance (from 114 to 335 m; $P < 0.001$). On cardiac magnetic resonance imaging, there was an increase in right ventricular ejection fraction from 33% to 43% ($P < 0.004$) and a decrease in right ventricular mass index (a marker of hypertrophy) from 60.9 to 48.5 g/m^2 ($P < 0.03$).[32]

In all three of these studies, the clinical improvement with medical treatment reported was better than that typically seen from the same medications used in patients with other forms of WHO group 1 disease (such as IPAH or connective tissue disease–associated PAH), as reported in larger studies, which typically show a mean improvement of 40 m in the 6-minute walk distance.

PROGNOSIS

Data regarding the prognosis of patients with schistosomiasis-associated PAH specifically are limited. Fernandes et al.,[24] at the São Paulo referral center, reported the outcome of 54 patients with schistosomiasis-associated PAH seen at their facility between 2004 and 2008, compared to 95 patients with IPAH. At baseline, the schistosomiasis-associated PAH patients were older (mean of 47 vs. 42 years; $P = 0.03$), had comparable 6-minute walk distances (442 vs. 412 m; $P = 0.20$), and had less severe disease hemodynamically, with a mean pulmonary artery pressure of 57 versus 65 mmHg ($P = 0.01$) and a pulmonary vascular resistance of 11.3 versus 16.7 dyne-s/cm^5 ($P = 0.002$). Of the patients with IPAH, 94% received PAH-specific therapy (with a PDE5 inhibitor, an endothelin receptor antagonist, or both), but none of the schistosomiasis-associated PAH patients received PAH-specific therapy (as treatment for schistosomiasis-associated PAH was not authorized at the time of the study). Despite the lack of treatment, the survival rate for patients with schistosomiasis-associated PAH was not lower than that for patients with IPAH: the survival rates for schistosomiasis-associated PAH were 95% at 1 year, 95% at 2 years, and 86% at 3 years, while the survival rates for (treated) IPAH were 95% at 1 year, 86% at 2 years, and 82% at 3 years ($P = 0.49$). When the differences in baseline demographics were corrected for, the diagnosis of schistosomiasis-associated PAH, as compared to IPAH, had a slight but not significantly increased hazard ratio for death of 1.16 (95% confidence interval: 0.29–4.68; $P = 0.84$).

PATHOLOGY

As mentioned above, schistosomiasis-associated PAH is categorized within WHO group 1 disease because of the presence of the classic findings of pulmonary vascular remodeling shared with other forms of PH, including IPAH. This includes the 4 forms of endothelial remodeling—concentric, eccentric, dilated (or angiomatoid), and plexiform—as well as medial and adventitial thickening (Fig. 2). A perivascular inflammatory infiltrate is also prominent, characterized by T cells, mast cells, and dendritic cells; the dendritic cell density is greater than that observed in IPAH tissue.[33]

Historically, lung tissue collected at autopsy from patients who died of schistosomiasis-associated PAH revealed

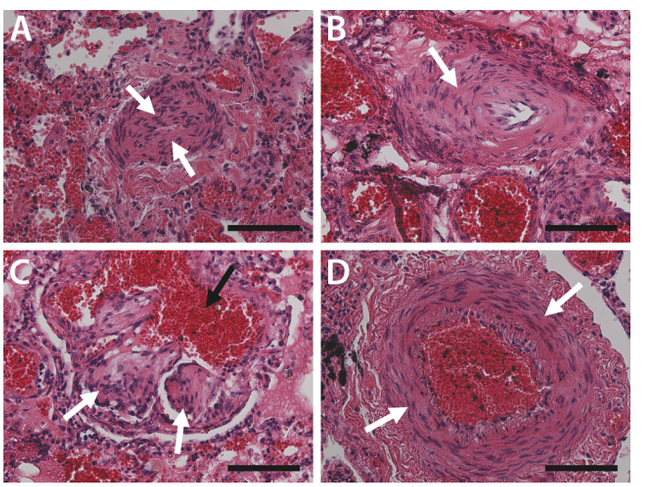

Figure 2. Pulmonary vascular pathology of schistosomiasis-associated PAH: *A*, Concentric intimal lesion (white arrows); *B*, eccentric intimal lesion (white arrow); *C*, plexiform lesions (white arrows) adjacent to an angiomatoid (or dilated) lesion (black arrow); *D*, medial thickening (white arrows). All specimens were stained with hematoxylin and eosin. Scale bars: 100 μm.

a large number of eggs and periegg granulomas. For example, in 1954, de Faria[34] reported that eggs were present in all 18 autopsy cases in his series, with a range of 4 to approximately 250 eggs seen per 2.5-cm^2 section. However, we recently completed an analysis of autopsy tissue collected from 18 patients who died of schistosomiasis-associated PH between 1980 and 2011, in which we found no eggs present in any of the lung samples.[35] We suspect that this difference is due to the modern use of antihelminthics such as praziquantal, which was developed in the 1970s. However, patients with schistosomiasis-associated PAH still have clinical worsening and death despite treatment with praziquantel, and thus the pulmonary vascular disease seems to reach "a point of no return" beyond which the disease progresses despite eradication of the worm and eggs.

PATHOPHYSIOLOGY

Unfortunately, relatively little is known about the connection between *Schistosoma* parasitic infection and pulmonary vascular disease. As discussed above, it is unlikely that mechanical obstruction of the pulmonary vasculature by eggs alone is the cause of PH, as the disease continues despite worm eradication with praziquantel and modern autopsy series of patients who die of schistosomiasis-associated PAH do not show a significant egg burden.[35]

It is possible that portal hypertension due to hepatosplenic disease may contribute to schistosomiasis-associated PAH, similarly to how patients with cirrhosis can develop portopulmonary hypertension (also by an unclear mechanism). Portosystemic shunting is likely an important pathogenic mechanism for the development of schistosomiasis-associated PAH, because it allows the delivery of antigenic eggs to the lung and consequent granulomatous inflammation.

Our group had theorized that even after clearance, the eggs could leave a small amount of antigenic debris behind that would drive an ongoing immune response. However, after creating and validating an antibody to known schistosomal antigens, we were unable to detect any significant amount of antigens persistent within the lungs of patients who died of schistosomiasis-associated PAH.[35]

We and others have found that mice experimentally infected with *S. mansoni* can develop pulmonary vascular remodeling, PH, and right ventricular hypertrophy.[36,37] There are obvious limitations of such a model, primarily that the acute (1–3 months) infection in the mouse cannot fully rep-

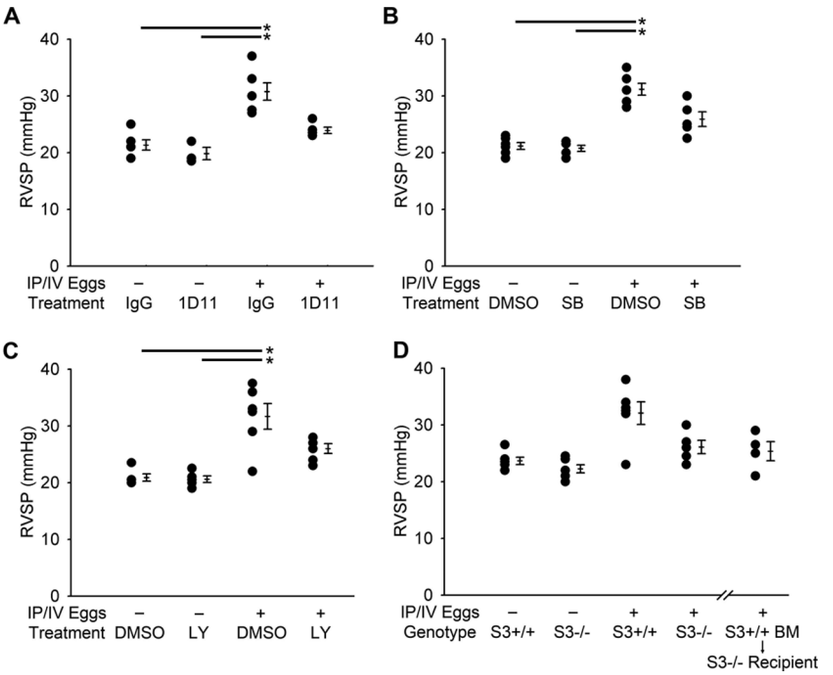

Figure 3. Transforming growth factor β (TGF-β) signaling is necessary for *Schistosoma*-induced pulmonary hypertension (PH). Blockade with the pan-TGF-β neutralizing antibody 1D11 (*A*), the TGF-β-R1/ALK5 inhibitors SB431542 (SB; *B*) or LY364947 (LY; *C*), or the absence of the TGF-β canonical intracellular signaling mediator Smad3 (S3), particularly in peripheral (non-bone marrow–derived) cells (*D*) suppresses the PH phenotype due to *Schistosoma* exposure in the mouse. All plots are from Graham et al.,[45] reprinted with permission. RVSP: right ventricular systolic pressure; IgG: immunoglobulin G; BM: bone marrow.

resent the decades of infection that occurs in people. Similarly, Crosby et al.[38] recently found that treating mice infected with *S. mansoni* with praziquantel prevents progression of experimental PH, in contrast to the continuation of disease in patients treated with praziquantel.

POTENTIAL ROLE OF INTERLEUKIN (IL)-13 SIGNALING

The advantage of the experimental mouse model is that it allows a mechanistic analysis of the connection between the host immune response and the pulmonary vascular disease. Mouse models can use modern scientific techniques of pharmacologic inhibitors and gene knockouts to test the role of candidate cytokines and signaling molecules. Working with a mouse model also allows comparisons to be drawn with the scientific data exploring the inflammation and fibrosis in the liver after *Schistosoma* infection in the mouse, where it has been shown that the cytokines IL-4 and IL-13 are critical.[39] The requirement of eggs embolizing into the lungs was also supported by the mouse model, as of the mice chronically infected with *S. mansoni*, only those with eggs present in the lung had PH.[38] We have found that in the mouse, hepatic infection is not a prerequisite for the development of PH.[37]

One of the potential mechanisms underlying the pathogenesis of schistosomiasis-associated PAH is inflammation, although the relationship between vascular remodeling and inflammatory mediators remains poorly understood. IL-13 is a key inducer of several type 2 cytokine-dependent pathologies. IL-13 regulates inflammation, mucus production, tissue remodeling, and fibrosis. IL-13Rα1 is the canonical IL-13 signaling receptor, whereas IL-13Rα2 is thought to be a competitive nonsignaling decoy receptor. Other studies have shown that dysregulation of IL-13 signaling is present in human PAH, although whether IL-13 is pro- or antiproliferative remains unclear.[36,40,41] Consequent to these findings, we found evidence of enhanced PH in *S. mansoni*–infected mice lacking IL-13Rα2, suggesting that IL-13 signaling is an important mediator of the granulomatous and vascular response to schistosomiasis infection.[37] Thus, the IL-13 ligand and receptors may serve as novel biomarkers in PH and potential targets for receptor-directed therapeutic agents.

POTENTIAL ROLE OF TRANSFORMING GROWTH FACTOR B (TGF-B) SIGNALING

A second, related, potentially pathogenic signaling pathway is TGF-β. TGF-β signaling controls a plethora of cellular responses and contains a large family of multifunctional cytokines that play critical roles in embryogenesis, growth, wound repair, and inflammation and have an important role in vascular homeostasis.[42] Recent studies have revealed significant insight into the mechanisms of the activation of TGF-β receptors through ligand binding; the activation of Smad proteins through phosphorylation; the transcriptional regulation of target gene expression; and the control of Smad protein activity and degradation.[43,44] Abnormal TGF-β family signaling has been extensively linked to human PAH,[45-47] and we and others have observed that blockade of TGF-β signaling suppresses PH due to monocrotaline or hypoxia in rodent models.[45-47] Recently, we found the Th2 cytokines IL-4 and IL-13 to be necessary for TGF-β activation in mice exposed to *Schistosoma*; previously, we observed IL-13 gain of function to be sufficient for TGF-β activation.[37,45] We also found upregulation of the TGF-β signaling pathway manifested by increased Smad2/3 phosphorylation in areas of vascular remodeling in both the mouse model and human tissue from subjects who died of schistosomiasis-associated PH.[37,45] In mice exposed to *Schistosoma*, the PH phenotype was partially prevented by blockade at the level of the TGF-β ligand, type 1 receptor, or intracellular signaling molecule Smad3 (Fig. 3). Coupled with the finding of IL-4 and IL-13 suppression by TGF-β signaling blockade, there may be a positive feedback loop of IL-4/IL-13 and TGF-β propagating the disease.[45] Thus,

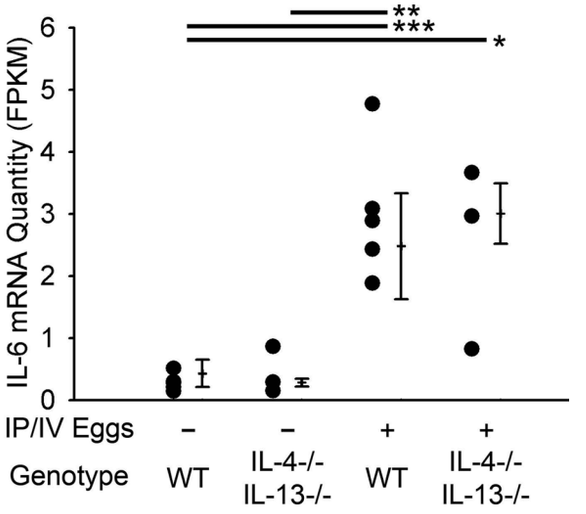

Figure 4. Interleukin (IL)-6 is upregulated in both wild-type (WT) and $IL\text{-}4^{-/-}/IL\text{-}13^{-/-}$ mice exposed to *Schistosoma mansoni*, as measured by whole-lung RNA sequencing. FPKM: fragments per kilobase of exon per million fragments mapped. The data have been deposited in National Center for Biotechnology Information's Gene Expression Omnibus (GEO) and are accessible through GEO series accession no. GSE49116. A single asterisk indicates $P < 0.05$, a double asterisk $P < 0.01$, and a triple asterisk $P < 0.005$. mRNA: messenger RNA; IP/IV: intraperitoneal/intravenous.

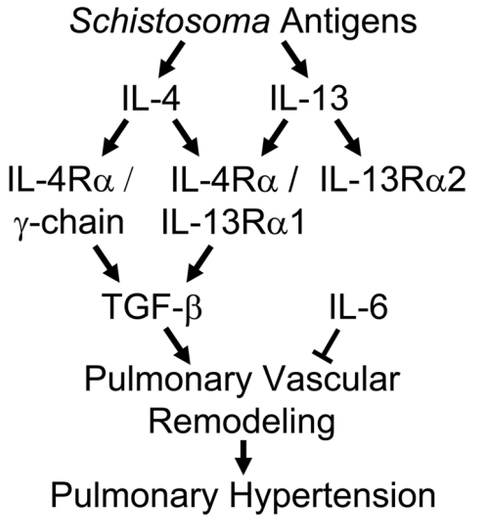

Figure 5. Proposed signaling pathway. *Schistosoma* antigens trigger the release of interleukin (IL)-4 and IL-13 by early-responding leukocytes in the periegg granuloma, including dendritic cells, type 2 helper T ($CD4^+$ Th2) cells, macrophages, and basophils. The IL-4 is received by the two IL-4 receptors: receptor 1 consists of the IL-4Rα protein and the common γ-chain protein, and receptor 2 consists of the IL-4Rα protein and the IL-13Rα1 protein. The IL-13 is received by the two IL-13 receptors: the first is the same as the IL-4 type 2 receptor, and the second is IL-13Rα2, which is thought to be a decoy (nonsignaling) receptor. Potential cells receiving the IL-4 and IL-13 stimulus includes macrophages, eosinophils, and pulmonary vascular cells. These cells, in turn, release transforming growth factor β (TGF-β), which leads to pulmonary vascular remodeling in pulmonary vascular smooth muscle and endothelial cells. IL-6 signaling, via the IL-6 receptor (IL-6R) and phospho-STAT3 and not regulated by IL-4/IL-13, has a suppressive effect on pulmonary hypertension due to *Schistosoma* exposure. Solid lines indicate relatively direct effects, and dashed lines indicate relatively indirect effects.

another potential target for patients with PAH is blockade of TGF-β signaling.

POTENTIAL ROLE OF IL-6 SIGNALING

Other investigators have observed an increase in IL-6 in patients with PAH, and in rodents overexpression of IL-6 is adequate to cause experimental PH, while blockade of IL-6 suppresses hypoxia-induced PH.[48-50] We observed a significant increase in IL-6/STAT3 (signal transducer and activator of transcription 3) signaling in both mice exposed to *S. mansoni* and the lung tissue of patients who died of this condition.[51] However, when we blocked IL-6 signaling by IL-6 genetic deficiency or with a pharmacologic STAT3 inhibitor, we observed, in fact, a more severe PH phenotype, including more severe remodeling and RV hypertrophy.[51] This finding suggests that in mice exposed to *Schistosoma*, IL-6/STAT3 signaling is upregulated, but in a compensatory manner, and that blockade of this pathway is detrimental. By whole-lung RNA sequencing, IL-6 is not modulated by IL-4/IL-13 signaling (Fig. 4), suggesting an entirely separate signaling pathway.

The overall signaling pathway elucidated to date in the mouse model is summarized in Figure 5. Despite the novel advances in the field, the exact underlying mechanisms and contribution of inflammation to the pathogenesis of *Schistosoma*-PAH are far from resolved, and future studies are needed.

CONCLUSIONS

Schistosomiasis is a major cause of PAH in patients worldwide and is important to consider in the evaluation of patients with otherwise unexplained dyspnea who have lived in areas where *Schistosoma* is endemic. Although antihelminthic treatment does not appear to significantly improve the clinical disease, it likely has some benefit and may slow the rate of progression. The use of PAH-specific medications in patients with schistosomiasis-associated PAH will likely significantly improve the prognosis, as they have done for patients with other forms of PAH.

There are many unanswered questions about why the disease occurs. Additional basic science studies using mouse models will help determine possible connections between the host immune response and pulmonary vascular disease, but going forward it will be essential to use patient samples whenever possible to ensure that the animal studies remain relevant to the human condition. With time, ongoing research will thus be able to identify new treatments specifically targeting this disease.

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