How does inflammation contribute to pulmonary hypertension?

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Blocking inflammation could be a curative approach in pulmonary vascular diseases
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Pathogenic drivers of the pulmonary vascular disease that results in pulmonary arterial hypertension (PAH) are currently unclear, but there are many reasons to suspect that inflammation is a major contributor. Clinically, PAH is a complication of multiple autoimmune diseases including scleroderma and systemic lupus erythematosus, suggesting dysregulated immunity can trigger the vascular disease [1–3]. PAH can also complicate several infectious diseases, including HIV and schistosomiasis, suggesting there could be off-target effects of immune upregulation which inadvertently damage the pulmonary vasculature. Patients with these pro-inflammatory aetiologies often have systemic vascular diseases, including digital ischaemia in scleroderma and portal hypertension in schistosomiasis [4, 5], suggesting the same inflammatory stimuli can lead to diverse vascular pathologies.

Experimentally, pulmonary hypertension (PH) can be triggered by exposing animals to immunogenic stimuli, including HIV, schistosomiasis, ovalbumin, interleukin (IL)-6 overexpression, and Fra-2 overexpression [6–10]. In these models, blocking the inflammatory trigger, or the downstream inflammatory cascade such as Type 2 inflammation in schistosomiasis, can prevent the pulmonary vascular disease phenotype [8–13].

However, blocking inflammation has only been shown to be of clinical benefit in one specific form of highly inflammatory disease: systemic lupus erythematosus-associated PAH [14]. Despite this evidence of inflammation underlying disease pathogenesis, why has targeting inflammation in PAH been otherwise unsuccessful? There are several possibilities. First and foremost, the precise inflammation present in human PAH is complex and unclear, with probable simultaneous presence of different types of inflammation which both augment and suppress the vascular disease. Generalised immune suppression would target both deleterious and compensatory pathways, and thus not effectively inhibit pathological drivers while sparing beneficial mechanisms. Alternatively, the timing could be wrong: inflammation could trigger the disease, but no longer be pathogenic in established disease. Lastly, inflammation could be an

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epiphenomenon, which occurs in parallel but doesn't causally drive the vascular disease, or develops late as a consequence of medial and intimal pathology.

Into the knowledge gap of understanding regarding what inflammation is actually present in human pathology steps the study by MARSH *et al.* [15] in this issue of the *European Respiratory Journal*. The authors apply flow cytometry and computational techniques to generate a global view of distinct inflammatory cell signatures in the pulmonary arteries of 16 subjects with idiopathic pulmonary arterial hypertension (IPAH) and 15 lung donors as control tissue. The primary findings included the observation that in control specimens 64% of all leukocytes ($CD45^+$ cells) were of myeloid lineage (neutrophils and monocytes) and 15% were of lymphoid lineages (primarily CD4 and CD8 T-cells); in PAH the ratio shifted with the myeloid cells decreasing to 52% while the lymphoid cells enriched to 31%. Further, these PAH lymphoid cells consisted of CD4, CD8 and γδ T-cell subsets.

The study by MARSH *et al.* [15] nicely complements the prior work by SAVAI *et al.* [16], which used immunostaining approaches to characterise the inflammatory cells in IPAH lungs. Flow cytometry has the advantages of using many simultaneous markers to highly characterise single cells, and more readily quantifying relative proportions of different cell types. In contrast, immunostaining allows analysis of cells within preserved tissue architecture. The work by SAVAI *et al.* [16] thus focused on the vascular compartment, sometimes resulting in slightly different data. SAVAI *et al.* [16] also identified a significant increase in CD4 and CD8 T-cells in the adventitial space. A third study also reported an increase in the density of T-cells in the lungs of patients with schistosomiasis-associated PAH [17]. Experimental studies in animals, complementing these observational human data, have suggested CD4 T-cells are pathological in promoting hypoxia-induced PH (T-helper (Th)17 CD4 T-cells) [18, 19] and schistosomiasis-induced PH (Th2 CD4 T-cells) [11].

MARSH *et al.* [15] also identified an increase in dendritic cells in the lungs of IPAH patients, of both myeloid and plasmacytoid subtypes, as well as an increase in circulating plasmacytoid dendritic cells. SAVAI *et al.* [16] also reported an increase in dendritic cells. The function of dendritic cells here is unknown. Employing the power of flow cytometry, MARSH *et al.* [15] found an increase in CD1a expression in some of these cells, consistent with an activated phenotype that can secrete IL-12 and present antigen to activate CD4 T-cells. These dendritic cells could thus causally contribute to the increase in T-cells noted earlier.

Both MARSH *et al.* [15] and SAVAI *et al.* [16] reported an increase in mast cells in PAH lungs, as have prior publications [16, 20]. In animal studies, blocking mast cells is protective in models of PH [21, 22], implicating these cells as likely to be pathological.

Both studies also reported a change in macrophage populations in patients with IPAH. SAVAI *et al.* [16] noted a substantial increase in the density of adventitial $CD68^+$ macrophages and $CD14^+$ macrophages/monocytes, whereas MARSH *et al.* [15] report an increase in activated ($CD1a^+HLA^+$) macrophages [23]. Experimentally, depleting macrophages, such as *via* clodronate, protects against hypoxia-induced PH [24, 25], and blocking the recruitment of circulating monocytes (by CCR2 deficiency) protects against schistosomiasis-induced PH [12]. Interstitial macrophages are prominent sources of pathological leukotriene B_4 in PH [26], and probably have other deleterious functions [27].

Along with these increases in pro-inflammatory cell density, there may be a reduction in anti-inflammatory cells, particularly regulatory T-cells (Tregs). SAVAI *et al.* [16] reported a decrease in anti-inflammatory $FoxP3^+$ monocytes in IPAH lung, of which some were Tregs. MARSH *et al.* [15] now indirectly support these data with the finding of a higher number of plasmacytoid dendritic cells, which may decompensate for a decreased number of Tregs [28]. Experimentally, absence of Tregs (such as in the athymic rat) increases susceptibility to vascular endothelial growth factor receptor 2 blockade by SU5416, and reconstituting Tregs is protective in this model [29]. One of the potential mechanisms of Treg-induced protection is upregulation of bone morphogenetic protein type 2 receptor expression in vascular endothelial cells and macrophages, which is mutated in many heritable cases of PAH and suppressed in idiopathic disease [29]. Thus, future anti-inflammatory therapies probably need to at least spare and ideally augment Tregs.

What are the next steps towards the long-term goal of developing effective treatments for PAH by targeting inflammation? We suggest first deepening our understanding of the role of inflammation in the pathology of PAH, to permit more precise targeting of causal mechanisms. In particular, answering the following questions will be helpful.

1) Beyond describing the density and localisation of immune cells in human PH, what are the phenotypes of these cells? For example, are the CD4 T-cells activated, and if so, what phenotype are they (Th1/Th2/Th17)?

2) What are the critical interactions between the different cell populations? For example, are the $CD1a^+$ dendritic cells actually presenting antigen to CD4 T-cells, and if so, what is the antigen?

3) Does the increased density of leukocytes arise from recruitment of circulating cells, or proliferation of endogenous cells? For example, it has recently been reported that there are distinct subsets of interstitial macrophages in rodents [30]: do these subsets have parallels in human tissue, and if so, which are involved in PH?

4) The actual rise in pulmonary vascular resistance results from intima and media proliferation, hypertrophy and vasoconstriction: do inflammatory cells, generally located in the adventitia [16], directly induce medial and intimal pathology, and if so, how? Or do they function through a third party, such as the fibroblast?

5) Do inflammatory animal models of PH adequately recapitulate critical immune drivers of clinical disease?

6) Does delayed targeting of the inflammation reverse established disease in animal models of inflammatory-PH?

7) How does inflammation evolve as the disease progresses, from at-risk and preclinical into early and then late pulmonary vascular disease?

Despite these uncertainties, the field has moved ahead with attempting to target inflammation clinically. One example of more selective targeting of pathological inflammation is the ongoing study of rituximab in scleroderma-associated PAH (ClinicalTrials.gov identifier: NCT01086540). In the future, the field may need to consider preventative approaches, suppressing inflammation in at-risk individuals (such as those with scleroderma) to prevent the development of pulmonary vascular disease. Overall, blocking inflammation remains an attractive target in PH, as it may actually underlie the disease pathology, and thus could eventually be a truly curative approach.

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