



REVIEW

Autoimmunity and pulmonary hypertension: a perspective

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ABSTRACT: The association between autoimmunity and pulmonary arterial hypertension (PAH) has been appreciated for >40 yrs, but how autoimmune injury might contribute to the pathogenesis of this disease has only been examined in a case-specific manner.

It is becoming increasingly clear that a variety of diverse clinical diseases, ranging from viral infections to connective tissue disorders, can culminate in pulmonary vascular pathology that is indistinguishable. Is there a hitherto unappreciated biology that unites these seemingly unrelated conditions?

The answer to this question may come from the increasing body of evidence concerned with the central importance of regulatory T-cells in preventing inappropriate B-cell activity. Two striking similarities between conditions associated with severe angioproliferative pulmonary hypertension are a defect in the CD4 T-cell compartment and auto-antibody production. Pathogenic auto-antibodies targeting endothelial cells are capable of inducing vascular endothelial apoptosis and may initiate the development of PAH.

The present review will focus on what is known about autoimmune phenomena in pulmonary arterial hypertension patients, in order to better consider whether an early loss of self-tolerance followed by autoimmune injury could influence the early development of severe angioproliferative pulmonary hypertension.

KEYWORDS: Auto-antibodies, autoimmunity, endothelium, immune regulation, immune tolerance, pulmonary hypertension

Severe pulmonary arterial hypertension (PAH) can be a manifestation of a number of collagen vascular diseases and viral infections. For example, ~12% of patients with scleroderma will develop PAH [1]. Other disorders, such as systemic lupus erythematosus, polymyositis, Sjögren's syndrome and Hashimoto's thyroiditis have all been associated with the development of severe PAH [2]. It is also known that certain viral infections, *e.g.* HIV and human herpes virus (HHV)-8, can be associated with the development of severe PAH [3–6]. Of great interest, is that all of these conditions are either characterised by, or have a propensity to, autoimmunity. A self-directed immune attack may occur because of a relative paucity of regulatory CD4 cells. It is already known that for some of these diseases, there is a diminution of the putative regulatory subset thought responsible for peripheral immune tolerance, the CD4+CD25+ cell. The present review will discuss how a

loss of self-tolerance could initiate a process which ultimately results in PAH.

PULMONARY HYPERTENSION: PATHOLOGY AND CLASSIFICATION

The most recent clinical and pathological classifications of pulmonary hypertensive diseases were established at the Evian conference [7], and updated in 2003 in Venice, Italy [8]. Pathologically, PAH is characterised by a proliferation of endothelial cells and expansion of vascular smooth muscle and adventitial cells in pulmonary arteries [9]. There is a growing appreciation that vasoconstriction of pulmonary pre-capillary arterioles may not be the single most important factor leading to severe pulmonary vascular remodelling. Although not yet widely accepted, the authors of the current review use the term "severe angioproliferative pulmonary hypertension" (SAPPH) [10] to distinguish, categorically, between pulmonary vascular disease which develops because of endothelial cell proliferation and pulmonary vascular disease which develops

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predominantly because of increased muscularisation of vessel walls [8]. Examples of PAH arising from endothelial cell pathology include idiopathic pulmonary arterial hypertension (IPAH; formerly known as primary pulmonary hypertension) [8], HIV-induced PAH and CREST (calcinosis, Raynaud's phenomenon, oesophageal dysfunction, sclerodactyly, telangiectasis)-related SAPPH. Examples of PAH associated with severe muscularisation of the pre-capillary arterioles, which demonstrate no clear cut evidence for endothelial cell pathology, include some hypoxia-associated PAH conditions such as chronic mountain sickness [11, 12] and neonatal PAH [13, 14]. The term SAPPH is preferred for several reasons. 1) SAPPH provides a pathobiological concept, (*i.e.* angiogenesis or angioproliferation). 2) SAPPH unites both so-called primary and secondary forms of PAH, under the banner of "severe" and associates the condition with complex pulmonary vascular lesions, including plexiform lesions. 3) SAPPH likely provides prognostic and therapeutic information in that, at the present time, the treatment for these severe forms of angioproliferative

PAH is quite similar [10]. SAPPH is also characterised by the presence of inflammatory cells in and around affected pulmonary vessels. HEATH [15] described the presence of mast cells in plexiform lesions in patients with primary PAH >30 yrs ago. Further work by TUDER *et al.* [16], and also HUMBERT *et al.* [17] described the presence of inflammatory infiltrates in the vascular lesions of PAH. Figures 1 and 2 illustrate the immune pathology in a patient with IPAH which is notable for lymphocyte and mast cell infiltration, as well as immunoglobulin G deposition in and around the narrowed and occluded vascular lumen.

PAH: AUTOIMMUNITY AND IMMUNOREGULATION

Whether the presence of inflammatory and immune cells, such as T- and B- lymphocytes in the lesions [16] is cause or consequence of SAPPH remains unknown and may be debated for some time. However, it has been recognised for >40 yrs that there are associations between autoimmune disorders and severe PAH. Little progress has been made in the current

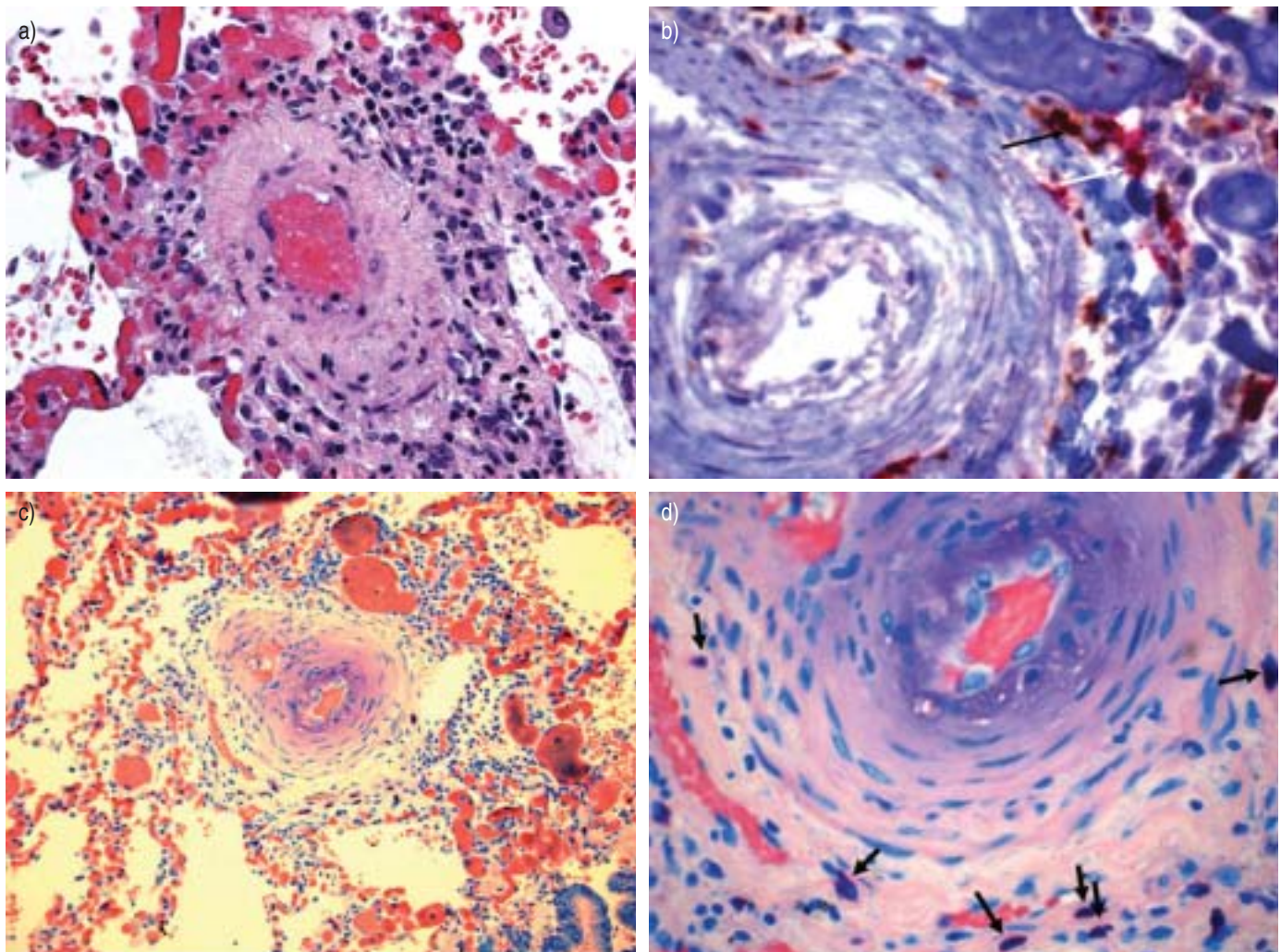


FIGURE 1. Immune pathology of pulmonary arteriole from an idiopathic pulmonary arterial hypertension patient. A 38-yr-old (antinuclear antibody positive, human herpes virus 8 positive) female expired with idiopathic pulmonary arterial hypertension. a) Haematoxylin and eosin stain showing affected arteriole surrounded by palisading mononuclear cells. b) CD4+ (fast red substrate (red staining); white arrow) and CD8+ (3,3'-diaminobenzidine (brown staining); black arrow) cells around lesion. c) Giemsa stain of mast cells indicating peri-arteriole infiltration. d) A magnified view of the lesion, mast cells are indicated by the black arrows.

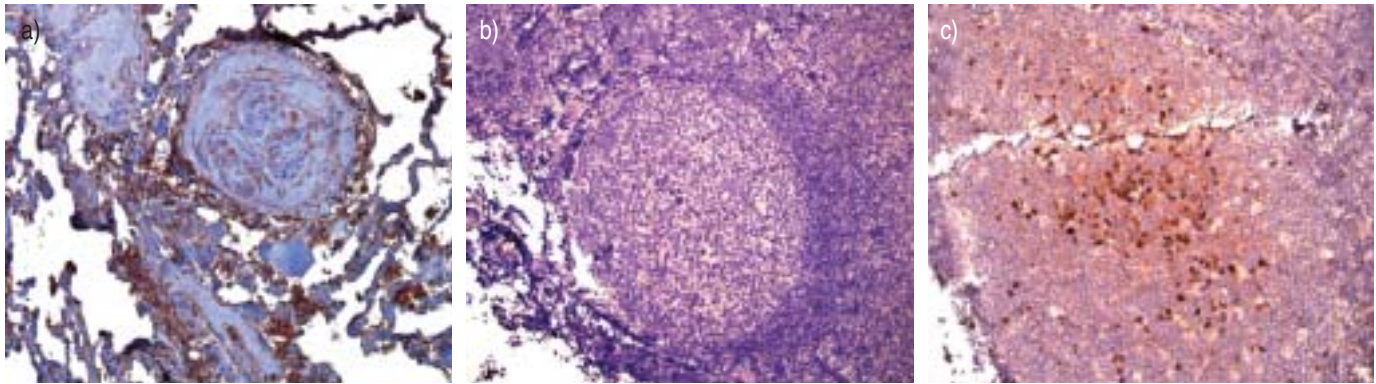


FIGURE 2. Immunoglobulin G staining of plexiform lesion from an idiopathic arterial pulmonary hypertension patient. A 38-yr-old (antinuclear antibody positive) female expired with idiopathic pulmonary arterial hypertension. a) Immunoglobulin G positivity in and around the plexiform lesion (3,3'-diaminobenzidine; brown staining). b) Negative antibody control of peripheral lymph node. c) Positive antibody control of peripheral lymph node.

understanding of how immune injury may be involved in the pathogenesis of SAPPH. In addition to the well recognised association between autoimmunity and SAPPH, there is also a link between immune insufficiency and SAPPH, because HIV+ patients and patients with AIDS develop PAH and vascular lesions which are histologically indistinguishable from IPAH. The recent description of latent HHV-8 [18] in patients with IPAH further begs the question of how the immune system modulates the development of PAH.

The answer to this question may be that most conditions associated with SAPPH are associated with a defect in the CD4 T-cell compartment, meaning that these conditions are either characterised by an absolute deficiency of CD4 cells, a decreased CD4/CD8 ratio and/or a diminished relative percentage of CD4+CD25+ cells, the putative regulatory T-cell (Treg) subset. Specifically, HIV [3], HHV-8 [4, 5] and the hepatitis C virus [6, 19–21] are all infections associated with a CD4 defect, autoimmune phenomena (including auto-antibodies) and the development of SAPPH. Similarly, other PAH-associated conditions, including connective tissue disorders, are also associated with a CD4 cell defect. For example, scleroderma [22, 23], systemic lupus erythematosus [24, 25], polymyositis [26], Hashimoto's thyroiditis [27] and Sjögren's Syndrome [28] can all exhibit selective CD4 cell defects and autoimmunity (again including auto-antibodies). More specifically, scleroderma and lupus are associated with a reduction in peripheral CD4+CD25+ cells, the putative Treg population [23–25]. Furthermore, PAH has been described following splenectomy [29]. Finally, it was recently reported that a patient with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, which is caused by a mutation leading to the loss of function of the autoimmune regulator (AIRE) protein, died with fatal IPAH [30]. The AIRE gene is of central importance in the development of thymus-dependent self-tolerance. T-cell, B-cell, macrophage and mast cell infiltration is a characteristic pathological feature of plexiform lesions from SAPPH patients [16, 31]. Furthermore, it is estimated that 30–40% of the patients with IPAH are antinuclear antibody positive, and another 10–15% of those patients may express antiphospholipid antibodies [32, 33]. A unifying hypothesis that addresses these cumulative findings is that, in the setting

of relative or absolute immunodeficiency (which includes diminished Treg activity), immune dysregulation occurs and leads to the activation of pathogenic autoreactive B-cells and T-cells.

If autoimmunity triggers the development of SAPPH, then why is the prevalence of PAH relatively low for certain autoimmune conditions such as systemic lupus erythematosus (6.2%) [34]? If the autoimmune phenomena resulting in end-organ damage are at play, why is the pulmonary vasculature not universally involved in these autoimmune connective tissue disorders? While this question can't be answered definitively at this time, there are several possibilities. It is known that in certain autoimmune conditions, a genotype can confer significantly elevated risk without complete disease penetrance. For example, in type 1 diabetes while increased risk is conferred on the basis of the HLA-DR-DQ genotype, fewer than 10% of susceptible individuals or 30–40% of identical twins of a patient with type 1 diabetes will develop the disease [35]. Environmental factors such as dietary or viral infections have been invoked as necessary "second hits" to develop this disease. Similarly, it is likely that a two-hit phenomenon may be required such that autoimmunity results in vascular injury and PAH. The current authors hypothesise, that a common factor in PAH-associated conditions is a loss of immunoregulation and that the second "permissive" factor could be the patient's genotype and/or concomitant vascular injury due to infection or temporary high shear stress. In summary, incomplete penetrance of PAH in autoimmune conditions associated with PAH may be due to the requirement of two or more additional factors that are present in a minority of the patients.

SCLERODERMA: A PARADIGM OF AUTOIMMUNE PAH
Scleroderma is a connective tissue disorder characterised by excessive collagen accumulation in the skin and visceral organs. As mentioned above, it is estimated that ~12% of scleroderma patients will develop SAPPH [1]. Scleroderma researchers believe that endothelial cell apoptosis may be the first event in the pathogenesis of scleroderma [36]. Anti-endothelial antibodies are found in the circulation of scleroderma patients [37, 38], and their presence correlates with the clinical

progression of this disease [38]. An instigating injury to endothelial cells in scleroderma that may trigger such auto-antibody formation may be viral infection [39]. A number of investigators have found evidence for viral infections, such as Epstein-Barr virus, parvovirus B19 and hepatitis C, E, and G in patients with scleroderma [40–45]. Although the role of cytomegalovirus (CMV) in the pathogenesis of scleroderma is debated [46], indirect evidence for a role of CMV-specific antibodies in the development of this disease has also been discussed [47, 48]. Not only are absolute lymphocyte counts reduced in scleroderma [49, 50], but scleroderma patients also have relatively fewer CD4+CD25+ cells in the peripheral circulation compared with healthy controls [23]. In this setting of diminished Tregs, a dysregulation of B-cells is also observed [51]. Plexiform lesions found in the arterial walls of scleroderma PAH patients include an inflammatory infiltrate [52] consisting of macrophages, T-cells, B-cells and mast cells [53–55]. In summary, scleroderma is an autoimmune disorder that has been associated with viral infection, endothelial damage, diminished Tregs, dysregulated B-cells, abundant mast cells and auto-antibodies. This review will subsequently discuss how Treg activity is related to B-cell activity, how mast cell infiltration is linked to the production of auto-antibodies, and finally, how these processes may culminate in PAH.

AUTOREACTIVE B-CELL ACTIVATION IN THE ABSENCE OF T-CELL REGULATION

Two findings, made nearly 30 yrs ago, strongly implicated T-cells as being responsible for the control of self-reactive T-cells and B-cells in the maintenance of self-tolerance. In 1969 NISHIZUKA and SAKAKURA [56] demonstrated that neonatal thymectomy of normal mice, most notably between day 2 and 4 after birth, led to autoimmune destruction of ovaries. In 1973, PENHALE *et al.* [57] showed that thymectomy of adult rats followed by several exposures to sublethal X-irradiation led to the development of autoimmune thyroiditis. As inoculation of normal CD4+ cells prevented these diseases, both groups suspected that depletion of suppressor T-cells was a putative mechanism for the development of autoimmunity [58, 59]. As this population was eventually narrowed down to the CD4+CD25+ cell, it became clear that depletion of this relatively small subpopulation (5–10% of CD4+ cells) was sufficient to break natural self-tolerance and incite chronic and destructive autoimmune diseases. This loss of self-tolerance was associated with the appearance of various disease-specific auto-antibodies [60]. With complete elimination of CD4+CD25+ cells, systemic autoimmunity occurs as manifested by multi-organ inflammation and auto-antibody production [61].

In addition to controlling T-cell activity, Tregs influence B-cell responses. For example, CD4+CD25+ Tregs have been shown to directly inhibit lipopolysaccharide-induced proliferation of B-cells *in vitro*. In an adoptive transfer system, CD4+CD25+ T-cells downregulate T-cell-mediated production of self-reactive antibodies. As activated B-lymphocytes produce CCL5 and attract CD4+CD25+ T-cells *in vitro*, it has been postulated that B-cell recruitment of CD4+CD25+ T-cells could limit B-cell autoimmune responses [62]. CD4+CD25+ cells prevent the activation of anti-DNA antibodies in a transgenic system [63]. Thus, Tregs regulate antibody responses against self and

nonself antigens. Tregs may exert a direct inhibitory effect on B-cells [62] or may inhibit T-cell differentiation [64]. Therefore, in the absence of appropriate regulation by T-cells, auto-antibodies can arise, and autoimmune disease can develop. Thus, a loss of Treg-mediated self-tolerance leads, not only to a loss in T-cell tolerance, but to a breakdown in B-cell tolerance as well. In the absence of these Tregs, other cells presumably provide stimulatory signals to relevant self-reactive B-cells, rescue them from apoptosis, and stimulate them to form pathogenic antibodies [65].

A conventional understanding of autoimmune disorders implicates an inappropriately vigorous T-cell compartment in autoimmune disorders such as diabetes and multiple sclerosis [66]. Conversely, autoimmune diseases such as Sjögren's disease and systemic lupus erythematosus, are distinguished by pathogenic auto-antibody production in the setting of apparently compromised Treg function. Autoimmunity in PAH may more closely resemble the latter group of diseases. As discussed above, Treg activity is normally responsible for preventing auto-antibody production. Antibodies directed against the vascular endothelium could certainly promote endothelial apoptosis. It is possible that endothelial apoptosis, secondary to autoimmune injury, could initiate dysfunctional endothelial cell proliferation that culminates in PAH in the same manner that endothelial apoptosis, induced by vascular endothelial growth factor antagonism, results in endothelial cell proliferation and PAH [67]. Anti-endothelial antibodies are present in autoimmune disorders associated with PAH including systemic lupus erythematosus [68], mixed connective tissue disease [69] and scleroderma [38]. In lupus and Sjögren's syndrome, antibody and complement deposits are localised in the walls of pulmonary arteries of patients with PAH [70, 71]. So, in short, the effector cells in this form of autoimmunity may be B-cells which (following differentiation into plasma cells) produce anti-endothelial antibodies. Although the role of autoreactive B-cells is emphasised here, the presence of T-cells in the inflammatory lesion suggests that dysregulated T-cells also contribute to autoimmune injury in PAH.

It is helpful to consider the antiphospholipid syndrome which may be a clinical scenario that has many elements of the PAH model described above. Patients with the antiphospholipid syndrome have altered T-lymphocyte subsets in the periphery, most notably a significantly reduced CD4+CD25+ population [72]. The antiphospholipid syndrome is often associated with viral syndromes associated with PAH, such as HIV and hepatitis C, which can have immunomodulatory effects [73]. The hypothesis posited here states that defects in the Treg population (which could occur after a viral infection) will lead to a loss of Treg activity with subsequent production of auto-antibodies and associated vascular endothelial injury. The antiphospholipid syndrome is associated with antiphospholipid antibodies that bind and activate endothelial cells. This antibody engagement ultimately leads to apoptosis of vascular endothelial cells [74, 75]. Finally, unmanipulated athymic nude mice, that also have an isolated deficiency of T-cells, have been demonstrated to spontaneously develop antiphospholipid antibodies, whereas severe combined immunodeficiency mice, which lack both T- and B-cells, do not have antiphospholipid antibodies [76]. Thus, in the antiphospholipid syndrome and other autoimmune disorders, the effector cell of greatest

importance may be the dysregulated B-cell that produces auto-antibodies to vascular endothelium because normal regulatory T-cell activity is decreased or absent.

When Treg activity is diminished, mast cells, rather than T-cells, may potentiate B-cell activation *via* interleukin (IL)-4 (*i.e.* mast cells may substitute as a source for IL-4 to increase local B-cell activation [77]). Of note, mast cells are a significant source of non-T-cell IL-4 [78], and IL-4 has been implicated as an expander of autoreactive B-cells [79] and a cytokine of central importance in an experimental model of scleroderma [80–82]. The presence of mast cells in and around plexiform lesions has long been observed [15, 31]. Mast cells were originally thought to be pathogenically important in PAH [83], and were discounted as it was subsequently demonstrated that a dearth of mast cells did not prevent experimental PAH [84], while a surfeit of mast cells [85, 86] was not associated with clinical PAH. However, mast cells are indeed present in both the inflammatory lesions of PAH patients [31] and monocrotaline-induced PAH in athymic nude rats lacking T-cells [87]. The latter group is interesting because monocrotaline-induced PAH is exacerbated by an absence of Tregs (*i.e.* when no T-cells are present) and is notable for mast cell infiltration in the inflammation around plexiform lesions. The present authors propose that the presence of mast cells may not be required for all subtypes of PAH, but that, as in other autoimmune diseases, mast cells may be an important link between the innate and adaptive immune responses [88, 89]. Rather than being absolutely required for the development of PAH, mast cells may be important facilitators of the immune response by potentiating autoreactive B-cells. Given the putative importance of cytokines and chemokines in the generation of an adaptive immune response, it is important to also note that IL-1 and IL-6 serum levels are markedly elevated in severe PAH and may significantly contribute to the inflammatory milieu of this disease [90].

BONE MORPHOGENETIC PROTEIN RECEPTOR II: A NEW INTERPRETATION OF ITS ROLE IN THE DEVELOPMENT OF PAH

A genetic basis has recently been determined for some cases of familial PAH, *i.e.* the involvement of germline mutations of bone morphogenetic protein receptor II (BMPR2) [91]. BMPR2 is a member of the tumour growth factor (TGF)- β receptor family and is a ligand for bone morphogenetic proteins (BMPs) 2, 4, 6 and 7, but not TGF- β [92]. Of pertinence to the current model is that BMP2 and -4 have roles in the development, growth potential and apoptosis of T- and B-cells. BMP2 and BMP4 (and likely its receptor, BMPR2) are also essential for thymocyte differentiation [93–95], and proper Treg development in the thymus is critical to avoid autoimmune disease [60]. BMP2 mediates growth arrest and apoptosis of B lineage cells [96, 97]. Finally, BMP4 is essential for the generation of B-cell progenitors (in addition to erythro-myeloid colony forming cells and natural killer progenitors) [98]. It is conceivable that as a receptor for these BMPs, BMPR2 is intimately involved in these immune effects. This has already been suggested for the BMP effects on thymic maturation [93]. Thus, it is possible that BMPR2 mutations could deleteriously affect the normal development, maturation, growth arrest and death of lymphocytes. In a manner analogous to patients developing

PAH because of AIRE gene mutations [30], germline BMPR2 mutations may, in part, result in PAH because they lead to a fundamental defect in peripheral immune tolerance (*i.e.* inappropriate Treg development and/or abnormal ability to delete B-cells). This idea opens interesting avenues of research focusing on the role of BMPR2 in immune tolerance rather than simply on its effects on smooth muscle cell growth.

AN INTEGRATED MODEL OF SAPPH DEVELOPMENT

The fact that PAH generally does not respond to immunosuppression may explain why investigators have not comprehensively explored a causal link between autoimmune injury and PAH. However, it is quite possible that a maladaptive response to an initial inflammatory injury results in a delayed tissue injury response that has a biology entirely distinct from the triggering immune insult and is no longer responsive to immunotherapy, which may have been responsive in the “initiation phase”. In the case of PAH, endothelial cell destruction by immune-mediated injury may result in the generation of apoptosis-resistant endothelial cells which share features with malignant cells [99–101]. In this scenario, the “law of the monolayer” is broken, and these endothelial cells proliferate, become “heaped up” and eventually obscure the vessel lumen [16]. These ideas are represented in figure 3.

CONCLUSION

National Institutes of Health registry data indicated that the median survival of patients with sporadic PAH was 2.8 yrs, with survival rates at 1, 3, and 5 yrs being 68, 48 and 34%, respectively [102]. While prostanoid therapy is improving survival for certain forms of PAH [103], PAH remains a frequently lethal disease of relatively mysterious origins. For >40 yrs it has been recognised that autoimmune phenomena are associated with PAH, but it has never been previously demonstrated that autoimmunity, itself, may be a root cause for certain forms of PAH. The human and financial toll of PAH is significant. For example, the prevalence of scleroderma in the USA was ~9,000 patients in 1996 [104], and, as noted, 12% of scleroderma patients will develop PAH [105–108]. The 1997 USA cost of caring for scleroderma patients was \$1.5 billion/year with the majority of money going towards the treatment of PAH [109]. Current treatment strategies for subtypes of PAH known to be associated with autoimmune disorders are currently no different than management of PAH without an established autoimmune association [110]. If autoimmunity is truly important in the pathogenesis of PAH, then at risk patients (such as those with scleroderma or PAH family members with BMPR2 mutations) can potentially be targeted with immunotherapy designed to prevent the establishment and propagation of autoimmune injury. Developing an understanding of how autoimmunity can trigger PAH would be invaluable in forming models of PAH pathogenesis and could promote the design of novel therapeutics which specifically address immune dysregulation. The concepts outlined in the present review attempt to explain some common clinical observations. Most notably, the hypothesis advanced here unites the concept of immunodeficiency and autoimmunity which is frequently observed in conditions associated with PAH. Furthermore, the presented model can explain cell populations (such as mast cells) that are strongly associated

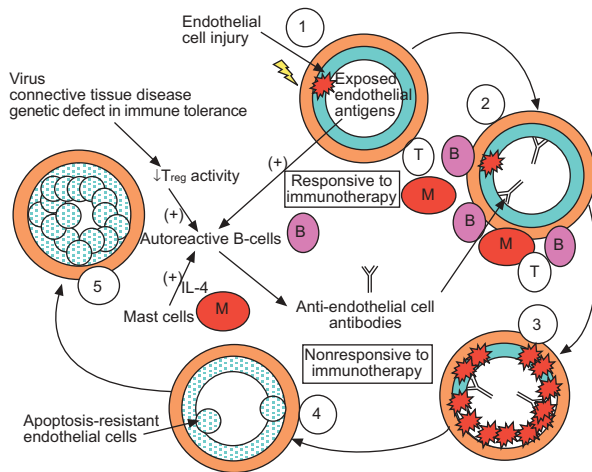


FIGURE 3. A schematic diagram displaying autoimmunity in the evolution of pulmonary arterial hypertension (PAH). 1) Injury to vascular endothelium (e.g. from a vascular trophic virus or shear stress) exposes endothelial antigens. In viral infection, connective tissue diseases or genetic defects in immune tolerance, diminished regulatory T-cell (Treg) activity may be observed. When the "two hits" of vascular injury and diminished peripheral immune tolerance occur simultaneously, this may lead to a loss of the normal control exerted on autoreactive B-cells. As part of the immediate innate host defence to vascular injury, mast cells infiltrate the perivascular space near injured endothelium. Mast cells are a rich source of interleukin (IL)-4, as well as other factors, which are capable of stimulating autoreactive B-cells to secrete auto-antibodies including anti-endothelial cell antibodies. 2) Clinical PAH is characterised by B-cells (B), T-cells (T) and mast cells (M) infiltrating plexiform lesions, and antibody-complement deposits that are located in the pulmonary arteries of patients with PAH. It is possible that during this initial inflammatory period, the lesion could be reversed with immunotherapy. 3) Antibody deposition may contribute to ongoing endothelial apoptosis. 4) As a tissue repair response, ongoing endothelial apoptosis results in the generation of apoptosis-resistant endothelial cells that have a malignant phenotype. This may be a period of injury that is no longer amenable to immunotherapy. 5) Apoptosis-resistant endothelial cells become "heaped-up". The resulting vascular remodelling leads to vascular occlusion, an increased vascular resistance and worsening of PAH.

with the disease, but have not previously been pathogenetically integrated.

In summary, by identifying a critical immune basis for many forms of pulmonary arterial hypertension, a rational design of therapeutic targets for this group of frequently fatal diseases will be strongly facilitated.

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