B-cells in pulmonary arterial hypertension: friend, foe or bystander?

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B-cells are recruited to and activated in PAH lungs and contribute to vascular remodelling through production of autoantibodies targeting the lung vasculature and cytokines. B-cell targeted therapies are a promising direction for select PAH conditions. https://bit.ly/3T9mNde

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Abstract

There is an unmet need for new therapeutic strategies that target alternative pathways to improve the prognosis of patients with pulmonary arterial hypertension (PAH). As immunity has been involved in the development and progression of vascular lesions in PAH, we review the potential contribution of B-cells in its pathogenesis and evaluate the relevance of B-cell-targeted therapies. Circulating B-cell homeostasis is altered in PAH patients, with total B-cell lymphopenia, abnormal subset distribution (expansion of naïve and antibody-secreting cells, reduction of memory B-cells) and chronic activation. B-cells are recruited to the lungs through local chemokine secretion, and activated by several mechanisms: 1) interaction with lung vascular autoantigens through cognate B-cell receptors; 2) costimulatory signals provided by T follicular helper cells (interleukin (IL)-21), type 2 T helper cells and mast cells (IL-4, IL-6 and IL-13); and 3) increased survival signals provided by B-cell activating factor pathways. This activity results in the formation of germinal centres within perivascular tertiary lymphoid organs and in the local production of pathogenic autoantibodies that target the pulmonary vasculature and vascular stabilisation factors (including angiotensin-II/endothelin-1 receptors and bone morphogenetic protein receptors). B-cells also mediate their effects through enhanced production of pro-inflammatory cytokines, reduced anti-inflammatory properties by regulatory B-cells, immunoglobulin (Ig)G-induced complement activation, and IgE-induced mast cell activation. Precision-medicine approaches targeting B-cell immunity are a promising direction for select PAH conditions, as suggested by the efficacy of anti-CD20 therapy in experimental models and a trial of rituximab in systemic sclerosis-associated PAH.

Introduction

Pulmonary arterial hypertension (PAH) is a severe disease characterised by progressive thickening and obliteration of pulmonary vessels, resulting in increased vascular resistance, elevated pulmonary artery pressures, and right heart failure [1]. Current treatments mostly rely on vasodilating thickened vessels and have limited effectiveness in reversing vascular remodelling or preventing the formation of new lesions. Therefore, there is an urgent need for innovative therapeutic strategies that can modify the natural course of the disease.

Extensive research has emphasised the crucial role of immunity in the development and progression of pulmonary vascular alterations in PAH. Notably, B-cells have attracted attention due to their potential as

therapeutic targets, as they can be specifically reduced by the same treatments used for haematological malignancies and autoimmune disorders [2]. As such, it appears relevant to examine the extent to which the cellular and humoral arms of B-cell immunity contribute to the pathogenic events occurring during PAH.

This review summarises the emerging evidence regarding the involvement of B-cells in the pathogenesis of PAH, highlighting activating mechanisms, effector functions and therapeutic potential. Although all causes of PAH were included in our literature search, most of the available evidence stems from idiopathic (i)PAH and connective tissue disease (CTD)-PAH. Whether B-cell immunity is altered in other forms of PAH warrants further investigation.

Abnormal B-cell homeostasis in PAH

Normal B-cell development involves a peripheral maturation step in secondary lymphoid organs, during which naïve B-cells, after antigen encounter, activate and terminally differentiate into either antibody-secreting cells (plasmablasts and plasma cells) or memory B-cells (figure 1).

Several lines of evidence suggest an abnormal B-cell homeostasis in PAH. Although not consistently observed [3–5], most studies have reported lower circulating B-cell counts in iPAH and CTD-PAH compared to healthy controls [6–9]. B-cell subset distribution is also altered: typical modifications include an expansion of antigen-inexperienced populations (mostly mature naïve B-cells), plasmablasts and plasma cells (especially immunoglobulin (Ig)A$^+$); findings that contrast with a contraction of the memory B-cell

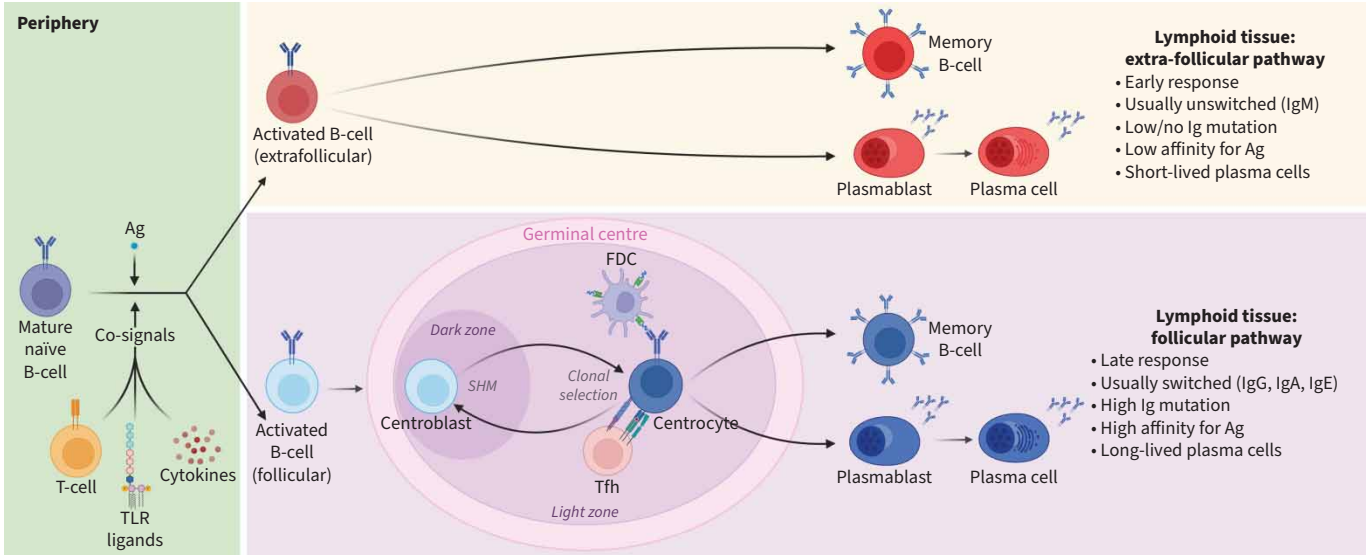

FIGURE 1 Peripheral B-cell maturation. Mature naïve B-cells are activated upon engagement of their B-cell receptor (BCR) with their cognate antigen (Ag), along with mandatory co-stimulation signals (interaction with a T-cell, Toll-like receptor (TLR) stimulation and/or cytokines). They engage into their terminal differentiation into either memory B-cells or antibody-secreting cells (plasmablasts and plasma cells) through two distinct pathways that differ in their anatomical localisations, as follows. 1) Within lymphoid follicles, follicular B-cells form germinal centres with functionally distinct dark zones and light zones. Dark zone B-cells, referred to as centroblasts, undergo a process called somatic hypermutation (SHM) (mutations in the Ag-binding region of the BCR, that increase its affinity for the Ag), mediated by an enzyme called activation-induced (cytidine) deaminase (AID), during which they stop expressing their membrane BCR. Centroblasts then migrate into the light zone, where they become centrocytes, re-express their BCR, and are selected based on their affinity with the Ag presented by follicular dendritic cells (FDCs) and interaction with cognate T follicular helper (Tfh) cells. Clones with higher Ag affinity receive stronger survival signals and move back to the dark zone for additional rounds of SHM. In the light zone, centrocytes undergo another important event called class-switch recombination (change in BCR isotype from immunoglobulin (Ig)M/IgD to either IgG/IgA/IgE, which expands their effector properties), also mediated by AID. After several rounds of migrations between dark and light zones, clones with the highest Ag affinity exit the germinal centres and terminally differentiate into either memory B-cells or antibody-secreting cells. As it requires the generation of a germinal centre reaction, the follicular response occurs later after Ag encounter, but results in the production of highly mutated, usually switched imunoglobulins, with high Ag affinity, secreted by long-lived plasma cells. 2) In extrafollicular sites, activated B-cells terminally differentiate into memory B-cells or antibody-secreting cells without occurrence of a germinal centre reaction. As such, extrafollicular responses occur more quickly, but result in the production of usually unswitched immunoglobulins with lower Ag affinity (due to lower mutations), secreted by short-lived plasma cells. Only pathways relevant to this topic are depicted.

compartment (particularly nonswitched forms) [4, 5, 10, 11]. These anomalies correlate with survival in patients with heritable and iPAH [12]. The lower levels of circulating memory B-cells may be explained by an enhanced pulmonary recruitment, as they are found in increased proportions within the lungs of hypoxic rats [13].

A recently identified B-cell population, associated with autoimmune processes and characterised by a specific membrane phenotype (CD27/IgD double negativity, low CD21 expression), is expanded in iPAH patients [11]. Their levels are similar in systemic sclerosis (SSc) patients with and without PAH [14], but correlate with clinical markers of pulmonary hypertension [15]. Other novel B-cell subsets, such as innate-like B1 B-cells, have not yet been investigated in PAH, but should be given further consideration.

Moreover, several observations suggest that circulating B-cells are activated in PAH. B-cells from SSc-PAH patients display an increased expression of CD25 and are prone to apoptosis [7]. Genes involved in B-cell-specific processes are the most differentially expressed genes in peripheral blood mononuclear cells (PBMCs) from SSc-PAH compared to SSc-no PAH and healthy controls [8, 9]. In iPAH, B-cells display a distinct mRNA expression profile, characterised by upregulation of genes involved in inflammation and immune response, compared to healthy controls [16].

Activating mechanisms of B-cells in PAH

B-cell activation classically requires the engagement of a specific antigen to its cognate membrane B-cell receptor (BCR) [17]. Costimulatory signals are mandatory and usually provided by antigen-specific $CD4^+$ T-cells through direct cell–cell interactions and cytokine production [17]. In the case of PAH, two families of ligands and receptors could be of particular importance: the B-cell activating factor (BAFF) system, which is known to drive survival and proliferation of autoreactive B-cells in autoimmune diseases [18], and the transforming growth factor (TGF)β-receptor (R) superfamily, which plays a role in both vascular and immune homeostasis [19]. Other B-cell activating mechanisms (such as interaction of nonspecific danger-associated molecular patterns with innate immunity receptors, or costimulatory signals provided by innate lymphoid cells) have not been specifically studied in PAH.

Abnormal BCR signalling

The BCR platform is a complex signalling system consisting of a membrane Ig, a transmembrane signal transducer (the CD79A–CD79B heterodimer), a constellation of kinases that mediate downstream intracellular signalling (such as Bruton tyrosine kinase (BTK)) and several membrane co-receptors that act as positive (CD19, CD21) or negative (CD22, CD35) signal regulators [20]. Antigen engagement on its cognate BCR triggers this signalling cascade leading to B-cell activation through various intracellular pathways (notably the BTK–NF-κB axis) [20].

Gene expression studies performed on PBMCs from SSc-PAH patients and lungs from various PAH patients reveal a significant upregulation of genes involved in BCR signalling and the NF-κB pathway in the PAH groups [8, 21]. In lungs from rats with monocrotaline (MCT)-induced PAH, genes related to B-cell activation, and especially BCR signalling mediators, display the strongest correlations with haemodynamic and histological markers of PAH severity [22]. These anomalies become more pronounced with the course of the experimental disease. Pathway analysis reveals significant dysregulation of several key components of the BCR complex (such as upregulation of CD79A/B, BTK, CD19 and CD21) [22].

Among the various components involved in the abnormal BCR signalling observed during human and experimental PAH, BTK was the focus of recent attention. In iPAH and CTD-PAH patients, intracellular BTK expression is increased in total B-cells, as well as in the naïve and memory subsets, and correlates with the presence of circulating autoantibodies [5]. Moreover, B-cell-specific BTK overexpression in transgenic mice exposed to bleomycin induces a pulmonary hypertension phenotype, leads to accumulation of memory B-cells and antibody-secreting cells (ASCs) in mediastinal lymph nodes and stimulates production of circulating antibodies targeting endothelial antigens [5]. In MCT rats, treatment by a BTK inhibitor attenuates right ventricle systolic pressure (RVSP) elevation, pulmonary vascular remodelling, right ventricle hypertrophy and endothelial–mesenchymal transition, although these effects could be mediated by inhibition of macrophage BTK [23].

Abnormal costimulatory signals

In addition to antigen–BCR interaction, second costimulatory signals are mandatory to induce B-cell proliferation and differentiation. These are usually provided by direct cell–cell interactions with cognate T-helper (Th) cells, as well as by specific cytokine environments produced by various sources [17, 24].

Th2 cells, IL-4, IL-6 and IL-13

Th2 cells and their related cytokines (such as interleukin (IL)-4, IL-6 and IL-13) seem to play a major role in B-cell activation in PAH. Preferential class-switching towards IgE, a hallmark of Th2 stimulation, is frequently noted in PAH. Increased IgE serum levels and IgE^+ B-cells are observed in the blood of PAH patients and the lungs of several experimental models [25, 26].

Single-cell RNA sequencing performed on the lungs of hypoxia-induced pulmonary hypertension (HPH) mice reveals a significant infiltration of Th2 cells with upregulated genes involved in the IL-4 pathway, an increased expression of costimulatory receptors in both T-cells (CD28) and B-cells (CD86 and CD40), as well as differential expression of genes involved in B-cell activation and IgE class-switching (GLε) [26]. In a similar model induced by combined hypoxia and ovalbumin exposure, Th2 inhibition due to CD294 deficiency or IL-4/IL-13 neutralisation decreases lung infiltration by IgE^+ B-cells and attenuated pulmonary hypertension [25]. Overall, these data suggest that Th2 cells contribute to B-cell activation and IgE class-switching through direct interactions and cytokine production.

Increased IgE production leads to mast cell priming and activation through binding of FcεRI receptors [27]. Interestingly, lung infiltration by mast cells is a prominent feature of the disease in human and experimental PAH, and IL-4, IL-6 and IL-13 were identified as the main mediators produced by mast cells after IgE stimulation in animal models [26, 28–32]. Mast cell inhibition in MCT rats results in lower B-cell lung infiltration, lower circulating autoantibodies and normalised serum IgG levels [30]. Most of these effects are also observed in animals treated with anti-IL6 antibodies [30]. Overall, these data indicate that Th2 cytokines produced by mast cells also participate in B-cell activation in PAH [28, 30].

Tfh cells and IL-21

Although T follicular helper (Tfh) cells are mainly located next to germinal centres in lymphoid organs, a circulating equivalent (cTfh) to this population has been identified in human peripheral blood [33], facilitating their study in PAH.

In SSc patients, cTfh cell counts are higher in the presence of PAH and correlate with circulating plasma cell counts [34]. SSc cTfh cells produce more IL-21 and CXCL13 (a B-cell chemokine) than healthy controls. In co-culture experiments using cTfh and B-cells from SSc and healthy controls, SSc cTfh cells induce more plasmablast differentiation and IgG/M production than SSc non-cTfh T-cells and healthy control cTfh cells. This effect is neutralised when co-culture is performed with an IL-21 antagonist.

In iPAH patients, cTfh levels have been described as increased [11], or decreased but with a higher cTfh17 subset [5]. BTK intracellular levels in B-cells positively correlate with the proportion of several cTfh subsets [5]. Considered collectively, these data suggest that Tfh cells promote B-cell recruitment and activation, plasmablast differentiation and Ig production, through an IL-21-mediated mechanism.

Dendritic cells and Th17 cells

Dendritic cells contribute to B-cell activation through antigen presentation, expression of costimulatory signals (such as CD40L) and cytokine production [35]. Aside from follicular dendritic cells, which operate within germinal centres in cooperation with Tfh cells, other DC subsets, such as conventional dendritic cells and monocyte-derived dendritic cells (moDCs), have been implicated in both T-cell and B-cell priming and activation [36]. Although both subsets infiltrate the lungs of iPAH patients in perivascular areas [37, 38], their exact role in B-cell activation during PAH has yet to be investigated.

Interestingly, moDCs generated from iPAH PBMCs induce a preferential differentiation of $CD4^+$ T-cells into Th17 cells compared to healthy controls [39]. Th17 cells have been incriminated in fostering autoimmune responses in various inflammatory diseases, notably through expansion of autoantigen-specific B-cells, initiation of the germinal-centre reaction and heightened antibody production [40, 41]. As such, the implication of Th17 cells in PAH B-cell activation warrants further consideration.

Abnormal production of B-cell survival signals: the BAFF system

The BAFF system consists of several protein members of the tumour necrosis factor (TNF) superfamily: two ligands, BAFF and a proliferation-induced ligand (APRIL), and three receptors, transmembrane activator and CAML interactor (TACI), B-cell maturation antigen (BCMA) and BAFF-receptor (BAFF-R). BAFF-R is expressed on most B-cells beyond the pre-B-cell stage with the exception of ASCs, but can only bind BAFF. TACI and BCMA expression is restricted to activated B-cells, memory B-cells and ASCs, but they bind both BAFF and APRIL. Fixation of the ligands on their receptors promote the survival, proliferation and differentiation of peripheral B-cells through activation of the NF-κB pathway.

Compared with normal B-cells, the generation of autoreactive B-cells is more dependent on the BAFF system [18].

The role of the BAFF system in B-cell activation in the context of PAH has yet to be thoroughly investigated. Serum BAFF concentrations have been assessed in several SSc cohorts: while BAFF levels are similar in patients with and without pulmonary hypertension, they strongly correlate with clinical markers of pulmonary hypertension severity in SSc-PAH patients [42–45]. A pathogenic variant in the gene coding for TACI has been identified as a new causative mutation for PAH, possibly by promoting dysregulated vascular inflammation [46]. BAFF-R is one of the most upregulated gene in the lungs of MCT rats [22].

Interestingly, the BAFF system has been extensively implicated in the pathogenesis of atherosclerosis, another cardiovascular disease that involves inflammation and shares common mechanisms with PAH [47]. In humans, mutations in BAFF-R are the second most prominent candidate gene in determining cardiovascular risk [48]. In experimental models, animals that are BAFF-R-deficient or treated with anti-BAFF-R antibodies display reduced atherosclerosis [49–51]. Paradoxically, mice treated with anti-BAFF antibodies or that are TACI-deficient have increased atherosclerosis, which was explained by unexpected extra-B-cell effects: BAFF ligates to macrophage TACI and represses its production of pro-atherogenic CXCL10, and APRIL binds endothelial heparan-sulfate proteoglycans and limits the constitution of atherosclerotic plaques [52, 53]. Finally, endothelial progenitor cells isolated from PBMCs of systemic lupus erythematosus (SLE) patients express BAFF-R, TACI and BCMA. Incubation with BAFF induces their apoptosis, which is rescued by belimumab, an anti-BAFF antibody [54]. Whether these processes are similarly at play in the pulmonary vascular bed of PAH has yet to be explored. These data collectively suggest that there are pleiotropic effects of the BAFF system in vascular diseases; effects that go beyond B-cells themselves and could also involve activated signalling pathways in endothelial cells that may recapitulate certain B-cell signalling pathways.

Abnormal TGFβ-R signalling

Aside from their role in vascular homeostasis, ligands and receptors from the TGFβ superfamily are also important regulators of immune processes [19]. Specifically, bone morphogenetic protein (BMP) signalling has been shown to inhibit peripheral B-cell maturation: BMP-6 represses naïve and memory B-cell proliferation, plasmablast differentiation and Ig production; and BMP-7 induces B-cell apoptosis, especially in germinal centres [55–57]. B-cells express both BMP receptors (BMPR)1 and 2, but with a different pattern according to their maturation stage (predominance of BMPR1 in germinal centre B-cells, and of BMPR2 in naïve B-cells) [55, 57]. Their regulatory effects appear to be mediated by the canonical SMAD1/5/8 pathway and their downstream targets ID1, ID2 and ID3 [55–57].

As dysregulation in the TGFβ-R superfamily homeostasis plays a crucial role in the pulmonary vascular remodelling observed during PAH [58], especially in heritable forms associated with defective BMPR2 signalling, its impact on B-cell maturation warrants further investigation.

Effector mechanisms of B-cells in PAH

B-cell immunity encompasses both the direct cellular actions of cells and antibodies; both of these arms may be involved in PAH pathogenesis. B-cells give rise to plasmablasts, which subsequently differentiate into nondividing plasma cells. Plasma cells synthesise Igs whose effects are either direct (functional consequences of the antigen stimulation or inhibition induced by the antibody binding) or indirect (opsonisation, *i.e.* tagging antigen for phagocytes; antibody-dependent cellular cytotoxicity, *i.e.* tagging antigen for cytotoxic cells; and complement activation) (figure 2). Additionally, another effector mechanism, specific to IgEs, consists in mast cell priming and activation through FcεRI receptor fixation (figure 2).

Other important B-cell properties have been acknowledged, namely cytokine/growth factor production and direct cell–cell interactions (figure 2). Through these newly described functions, B-cells are recognised as major regulators of immune responses, but also can also potentially act on nonimmune processes [59]. We speculate on how antibodies and B-cells may contribute to pulmonary vascular pathology in PAH.

Production of autoantibodies

The importance of autoantibodies in the constitution and perpetuation of vascular lesions in PAH was highlighted by several experimental observations. Passive transfer of serum IgG from various pulmonary hypertension models to healthy animals is sufficient to induce pulmonary vascular remodelling and haemodynamic alterations [60–62]. Conversely, Ig-deficient mice are resistant to hypoxia-induced pulmonary hypertension [63].

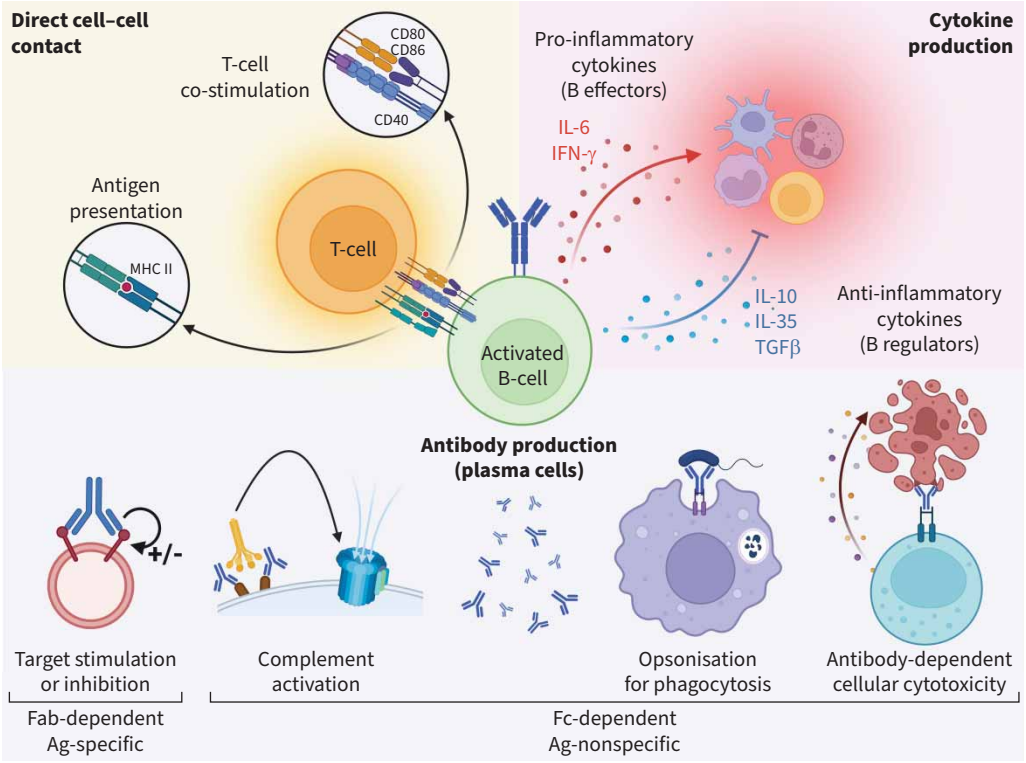

FIGURE 2 B-cell effector functions. Activated B-cells mediate their effects through three major mechanisms, as follows. 1) Antibody production: antibodies are secreted immunoglobulins that comprise an antigen-binding fragment (Fab), responsible for binding the antigen (Ag), and a crystallisable fragment (Fc), that allows interactions with complement fractions and specific membrane receptors called Fc receptors. Effects mediated by the Fab fragment are Ag-specific and consist of stimulation or inhibition of their target Ag. Effects mediated by the Fc fragment are Ag-nonspecific and consist of complement activation and Ag tagging for phagocytes (opsonisation) or cytotoxic cells (antibody-dependent cellular cytotoxicity) through their Fc receptors. 2) Cytokine production: specific B-cell subsets have been identified based on their ability to promote inflammation (effector B-cells, through production of interleukin (IL)-6 and interferon (IFN)-γ), or to alleviate immune responses (regulatory B-cells, notably through production of IL-10, IL-35 and transforming growth factor (TGF)β). 3) Direct cell–cell contact: B-cells express class II major histocompatibility complex (MHC II) and can act as professional antigen-presenting cells. They can also modulate T-cell activation by providing stimulatory or inhibitory signals through a wide range of membrane receptors (*e.g.* CD40, CD28 or CD80/86). Only functions relevant to this topic are depicted.

Serum immunoglobulins

In the United Kingdom national iPAH cohort, serum levels of IgG, IgM, IgA, IgG1, IgG2 and IgG4 are identical to those of healthy controls [11]. Only IgG3 titres are significantly increased, possibly due to preferential class-switching induced by elevated IL-21 concentrations [11]. When unsupervised clustering was applied to the whole-blood transcriptome from this cohort, three patient subgroups were delineated that display incremental risks of mortality; interestingly, the genes, whose expression allows the best discrimination between clusters, are Ig genes. Here, higher expression was associated with better survival [12]. Although not measured in this cohort, previous studies reported elevated IgE levels in iPAH patients, due to skewed isotype switching induced by Th2 cytokines [25, 26]. Few data are available regarding patients with CTD-PAH, with conflicting reports of elevated IgA [64] and decreased IgG [45] levels in SSc-PAH (compared to SSc without pulmonary hypertension).

BCR repertoire diversity

Anomalies of the BCR repertoire in PAH have been highlighted by two studies, one focusing on total B-cells from SSc-PAH patients [10] and the other on plasmablasts from iPAH patients [4], that yielded similar results. First, in an analysis of the complementarity-determining regions 3 (CDR3; the Ig segment responsible for antigen specificity), no common sequence among patients was revealed; this finding argues

against a shared antigenic motif driving the humoral response. Alternatively, this result could also indicate immunological idiosyncrasy, *i.e.* that different antigens (or different epitopes of the same antigen) are driving the immune response in each patient. Second, analysis of the VDJ segments, the Ig region involved in antigen binding and supporting the diversity of the antibody repertoire, indicates over- and under-used recombinations, suggesting small clonal expansions driven by chronic exposure to specific antigens. Third, quantification of mutation loads in the VDJ segments reveals a higher number of fixed mutations, suggestive of increased selective sweeps and sustained affinity maturation. Finally, monoclonal antibodies generated from representative iPAH plasmablast clones bind multiple proteins on an autoantigen microarray, indicating that the PAH antibody repertoire is autoreactive (antibodies target self-antigens) and polyreactive (a single antibody targets multiple antigens, possibly by recognising shared post-translational modifications). These results are consistent with a persistent humoral response polarised against specific autoantigens that are probably different from one patient to another.

Conventional autoantibodies

The frequent occurrence of conventional autoantibodies in PAH has long been documented, with older studies already observing antinuclear antibody positivity in ∼40% of iPAH patients [65, 66]. In patients with CTDs, certain autoantibody specificities are associated with an increased risk of PAH occurrence (anti-centromere and anti-U1-ribonucleoprotein (RNP) antibodies in SSc [67]; anti-phospholipid, anti-sicca syndrome (SS)A/SSB and anti-U1RNP antibodies in SLE [68, 69]) and with PAH prognosis (improved survival in anti-U1RNP-positive CTD-PAH patients [70]). In the United Kingdom national iPAH cohort, several conventional antibody specificities (notably targeting cardiolipin, histones, SSB, RNP complex or thyroid antigens) occur more frequently than in healthy controls [11]. Their serum levels allow to delineate three distinct clusters of patients ("high", "intermediate" and "low" autoantibody positivity), with the "high antibody" cluster having a more severe PAH phenotype, but better survival [11].

Whether these conventional autoantibodies, that are specific to intracellular antigens, can exert a direct pathogenic role remains a controversial issue [71], although some research suggests their ability to act on endothelial cells in CTD patients. Stimulation of human umbilical vein endothelial cells (HUVECs) by immune complexes containing SSc antibodies induces endothelial activation with different characteristics according to each antibody specificity, and through distinct intracellular pathways [72]. Incubation of human pulmonary artery endothelial cells (HPAECs) with serum IgG from anti-U1-RNP-positive CTD patients specifically induces a pro-adhesive phenotype [73].

Functional autoantibodies targeting effector cells or pathways

Aside from conventional routinely tested autoantibodies, several antibody specificities have been identified subsequently (table 1), with more evidence supporting a pathogenic role in PAH [99]. These antibodies are usually designated based on the location of their putative target antigen, either on a vascular cell (anti-endothelial cell, anti-smooth muscle cell (SMC) and anti-fibroblast antibodies) or a vascular membrane receptor (members of the G-protein-coupled receptor (GPCR) or the TGFβ-R superfamilies).

Anti-endothelial cell antibodies

Prevalence: there are considerable variations in the reported prevalence of anti-endothelial cell antibodies in PAH, due to different detection methods, endothelial cell substrates and patient ethnic background. In a European population, the frequency of anti-endothelial cell antibodies is estimated at 62% (IgG) and 45% (IgM) in iPAH, 78% (IgG) and 61% (IgM) in CTD-PAH, and 33% (IgG) and 20% (IgM) in healthy controls using a HUVEC-based ELISA [74]. In Chinese cohorts, their prevalence in CTD-PAH, CTD-no pulmonary hypertension and healthy controls is 63%, 41% and 5% respectively using a HPAEC-based ELISA [75]; and 82%, 73% and 20%, respectively, using Western blotting [76]. Most studies have usually observed higher antibody titres in PAH patients compared to healthy controls, and in similar range between PAH groups; however, comparisons of CTD patients with and without PAH have yielded conflicting results [74–77, 100–102].

Targets: serum IgG from iPAH and SSc-PAH patients express distinct reactivity profiles with macrovascular and microvascular endothelial cell antigens [78], indicating different targets depending on endothelial cell types and underlying disease. If HUVECs are used, anti-endothelial cell antibodies from both patient groups recognise cytoskeletal (lamin A/C, tubulin-β chain, vimentin) and ribosomal proteins [79]. In an animal model of pulmonary hypertension, serum IgA from experimental mice specifically stains pulmonary artery intima, indicating a reactivity against endothelial antigens at the pulmonary level [103].

Effects: stimulation of various endothelial cell-lines with serum IgG from seropositive PAH patients consistently activates endothelial cells into a pro-inflammatory (increased IL-6, IL-8, C-C motif chemokine

TABLE 1 Functional autoantibodies in pulmonary arterial hypertension (PAH)

	Frequency	Targets	Effects
Vascular cells			
Anti-endothelial cell [74–81]	iPAH (62%), CTD-PAH (63–82%), HC (5–33%)	Lamin A/C Tubulin-β chain Vimentin	Induction of pro-inflammatory (increased IL-6, IL-8, CCL2 and CCL5 expression) and pro-adhesive (increased E-selectin, ICAM-1 and VCAM-1 production) properties in endothelial cells
Anti-smooth muscle cell [82, 83]	More frequent in SSc-PAH, SSc-no PAH and iPAH than in HC	STIP1 α-Enolase	Induction of contraction and proliferation of SMCs
Anti-fibroblast [84, 85]	iPAH (40%), SSc-PAH (30%), SSc-no PAH (15%), HC (3%)	Vimentin, calumenin, PI3K Tropomyosin 1 HSP-27, HSP-70, G6PD	Induction of pro-inflammatory (increased IL-1β and IL-6 secretion), pro-adhesive (enhanced ICAM-1 expression) and pro-fibrotic (production of ROS and ECM remodelling) properties in fibroblasts
Vascular receptors: GPCRs			
Anti-AT1R/ETAR [62, 86–89]	Anti-AT1R: SSc-PAH (69%), CTD-PAH (63%), iPAH (21%) Anti-ETAR: SSc-PAH (65%), CTD-PAH (55%), iPAH (11%) Positivity predicts occurrence of PAH in SSc and mortality in SSc-PAH	AT1R ETAR	Agonistic properties on AT1R/ETAR: Endothelial cells: vasoconstriction, permeability, expression of VEGF-A and PDGF-B, pro-inflammatory (IL-8 and CXCL8 production, neutrophil recruitment) and pro-adhesive (VCAM-1 expression) properties SMCs: proliferation and expression of PDGF-Rβ
Anti-ETBR [90]	Higher titres in SSc-PAH than HC (but not iPAH)	ETBR	Not investigated (hypothesised to be antagonistic, as ETBR-deficient mice develop a PAH phenotype)
Anti-α1AR [91, 92]	Pre-capillary PH (95%), with PAH (100%)	α1AR	Activation of α1AR on rat cardiomyocytes, inducing long-lasting stimulatory effects without receptor desensitisation (contrary to its natural agonist)
Anti-S1PR [93]	Anti-S1PR1: SSc-PAH (16%), SSc-no PAH (18%), HC (3%) Anti-S1PR2: SSc-PAH (26%), SSc-no PAH (15%), HC (4%) Anti-S1PR3: SSc-PAH (28%), SSc-no PAH (18%), HC (8%)	S1P receptors	Not investigated (anti-S1PR2/3 hypothesised to be agonistic, promoting PA-SMC proliferation and medial thickening)
Vascular receptors: TGFβ-Rs			
Anti-BMPR2 [11, 94, 95]	iPAH/hPAH (0–1.4%), CTD (0%), HC (0%)	Extracellular domain of BMPR2	Diminution of BMP4 signalling in PA-SMCs
Anti-BMPR1A [95]	Higher titres in SLE-PAH than in SLE-no PAH and HC	BMPR1A (=ALK3)	Not investigated (hypothesised to interfere with ligand binding)
Anti-ALK1 [94, 95]	No detection in MCTD-PAH and iPAH Higher titres in SLE-PAH than in SLE-no PAH and HC	ALK1	Not investigated (hypothesised to interfere with ligand binding)
Other			
Anti-fibrillin 1 [96, 97]	iPAH (93%), anorexigen PAH (67%), HC (2%)	N-terminal fragment of fibrillin 1	Release of sequestered TGFβ1 from fibrillin-1-containing microfibrils in the ECM, leading to fibroblast activation into a profibrotic phenotype
Anti-ACE-2 [98]	More frequent in CTD patients with vasculopathy (including PAH) than those without	ACE 2	Inhibition of ACE-2 activity (which transforms AngII into Ang(1–7), an Ang isoform with vasodilating and antiproliferative properties)

GPCR: G-protein coupled receptor; AT1R: angiotensin receptor 1; ETAR: endothelin receptor A; ETBR: endothelin receptor B; α1AR: α1-adrenergic receptor; S1PR: sphingosine-1-phosphate receptor; TGFβ-R: transforming growth factor β receptor; BMPR: bone morphogenetic protein receptor; ALK: activin receptor-like kinase; ACE: angiotensin converting enzyme; iPAH: idiopathic PAH; CTD: connective tissue disease; HC: healthy control; IL: interleukin; CCL: C-C motif chemokine ligand; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; SSc: systemic sclerosis; STIP1: stress-induced phosphoprotein 1; PI3K: phosphoinositide 3-kinase; HSP: heat shock protein; G6PD: glucose-6-phosphate dehydrogenase; ROS: reactive oxygen species; ECM: extracellular matrix; VEGF: vascular endothelial growth factor; PDGF-R: platelet-derived growth factor receptor; CXCL: chemokine (C-X-C motif) ligand; SMC: smooth muscle cell; PH: pulmonary hypertension; PA: pulmonary artery; hPAH: heritable PAH; SLE: systemic lupus erythematosus; MCTD: mixed connective tissue disease; Ang: angiotensin.

ligand (CCL)2 and CCL5 expression) and pro-adhesive (increased E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 production) phenotype [4, 75, 77, 80, 102]. No effect is observed in endothelin-1 expression [4]. Conversely to previous observations in other CTDs, serum IgG from seropositive PAH patients does not induce endothelial cell apoptosis [81, 104].

Anti-SMC antibodies

Prevalence: anti-SMC antibodies are detected by immunofluorescence on permeabilised human aortic SMCs in the serum of iPAH, SSc-PAH and SSc-no pulmonary hypertension patients, but not healthy controls. The prevalence of anti-stress-induced phosphoprotein 1 (STIP1) antibodies, one of the major anti-SMC idiotypes (described later), is estimated at 84% in SSc-no pulmonary hypertension, 76% in SSc-PAH and 24% in iPAH (compared with 3% in healthy controls) [82].

Targets: when immunoblotted against mammary arterial SMC antigens, serum IgG from SSc-PAH and iPAH patients more frequently recognises STIP1 and α-enolase than those of healthy controls. Other targets include cytoskeletal (such as tropomyosin α-1 chain) and mRNA regulation (such as KH-type splicing regulatory protein) proteins [82].

Effects: serum IgG from SSc-PAH and iPAH patients induces SMC contraction within a collagen matrix, conversely to those of healthy controls [82]. Serum IgG from an anti-α-enolase antibody-positive SLE-PAH patient promoted lamellipodia formation and migration of pulmonary artery SMCs (PA-SMCs), which was reversed by removal of the autoantibodies from the IgG fraction and by treatment with an α-enolase inhibitor [83]. As STIP1 is a co-chaperone of heat shock protein (HSP)70, a major regulator of SMC homeostasis, anti-STIP1 antibodies could also modulate SMC migration by modifying the interactions between these two proteins [82].

Anti-fibroblast antibodies

Prevalence: anti-fibroblast antibodies are detected in 40% of iPAH patients, 30% of SSc-PAH patients, 15% of SSc-no pulmonary hypertension patients and 3% of healthy controls, using a human dermal fibroblast-based ELISA assay [84]. Immunofluorescence performed on human pulmonary artery fibroblasts confirms significant staining with plasma from pulmonary hypertension patients, which is not observed with healthy controls [60].

Targets: by immunoblotting serum IgG from PAH patients against human dermal fibroblast proteome, several antigens were identified as putative targets of antifibroblast antibodies [85]. These proteins are involved in various biological processes: cytoskeletal organisation (vimentin, calumenin, phosphoinositide 3-kinase (PI3K)), cell contraction (tropomyosin 1), oxidative stress (HSP27 and HSP70) and other key cellular pathways [85]. Additionally, plasma from MCT rats labels pulmonary artery adventitia, suggesting reactivity against fibroblast antigens at the pulmonary level; and vimentin, PI3K and HSP27 are confirmed as targets of antifibroblast antibodies in this model [60].

Effects: human pulmonary artery fibroblasts stimulated by antifibroblast antibody-positive plasma from PAH patients acquire a pro-inflammatory (increased IL-1β and IL-6 secretion) and pro-adhesive (enhanced ICAM-1 expression) properties [60]. In SSc patients, antifibroblast antibodies induce a myofibroblast phenotype with production of reactive oxygen species and extracellular matrix (ECM) remodelling [105, 106]. Additionally, antibodies directed against fibrillin-1, a major component of ECM that regulates TGFβ signalling by sequestering TGFβ1 molecules, have been detected in iPAH sera [96]: these antibodies can indirectly activate fibroblasts by liberating the TGF-β1 proteins trapped within the ECM microfibrils [97].

Anti-GPCR antibodies: anti-AT1R and ETAR antibodies

Antibodies against GPCR have been described in various conditions and in health and are considered natural components of the immune system [107, 108]. They form a network of closely intercorrelated antibodies that target structurally and functionally related molecules, such as vascular, neuronal or chemokine receptors. Gender, age and diseases (such as SSc) modify antibody titres and correlations, in a way that suggests alterations to homeostasis (disease-specific modification of the overall network) rather than production driven by exposure to an autoantigen [107]. In PAH, antibodies targeting angiotensin receptor 1 (AT1R), endothelin receptor A (ETAR) and B (ETBR), α1-adrenergic receptor, sphingosine-1-phosphate receptor 2 (S1PR2) and S1PR3 were reported [62, 86, 90–93, 109]; but only anti-angiotensin and endothelin receptor antibodies were thoroughly investigated [62, 86, 90].

Prevalence: anti-AT1R and ETAR antibodies occur more frequently in patients with SSc-PAH (69% and 65%, respectively) and CTD-PAH (63% and 55%, respectively) than in iPAH (21% and 11%, respectively) [62, 86]. Anti-ETBR antibodies are also found at higher concentrations in SSc-PAH (but not iPAH patients) compared to healthy controls [90]. Anti-AT1R and ETAR antibodies show weak-to-nonexistent correlation with haemodynamics, but predict the occurrence of PAH in SSc patients and mortality in SSc-PAH patients [62, 86, 87].

Targets: the epitopes targeted by anti-AT1R, anti-ETAR and anti-ETBR antibodies have not been investigated so far.

Effects: anti-AT1R/ETAR antibody display agonistic properties on their target receptors. Indeed, stimulation of rat pulmonary artery segments with seropositive SSc-PAH serum increases endothelial cytosolic calcium concentration (a downstream signal of AT1R/ETAR activation) and amplifies vasoconstriction induced by angiotensin and endothelin exposure [62]. These effects are blocked by pre-treatment with valsartan and sitaxentan [62]. Anti-ETAR antibodies also increase HUVEC permeability and expression of vascular endothelial growth factor (VEGF)-A and platelet-derived growth factor (PDGF)-B, as well as human PA-SMC proliferation and expression of PDGF receptor-β; both of which are annulled by exposure to bosentan [86]. Transfer of anti-AT1R antibodies induce pulmonary vasculopathy in healthy mice [110]; and transfer of anti-ETAR antibodies worsen haemodynamics and vascular remodelling in MCT rats [86]. In SSc patients, anti-AT1R/ETAR antibody-positive serum induces pro-inflammatory (increased expression of IL-8 and CXCL8, increased neutrophil migration and activation) and pro-adhesive (increased expression of VCAM-1) properties in human microvascular endothelial cells [88].

The effects of anti-AT1R and anti-ETAR antibodies may extend beyond the vasculature. Indeed, AT1R and ETAR are expressed by normal human PBMCs. In SSc patients, serum IgG induce T-cell chemotaxis and PBMC secretion of IL-8 and CCL-18, both of which are reduced by exposure to AT1R and ETAR antagonists [89]. The functional properties of anti-ETBR antibodies have not been reported. As ETBR-deficient mice develop PAH, an inhibitory activity on its target receptor could be hypothesised [90].

Anti-TGFβ-R antibodies: anti-BMPR/ALK antibodies

Studies performed in Asian populations have identified specific patterns of reactivity against BMPR and activin receptor-like kinase (ALK) [94, 95]: presence of anti-ALK1 antibodies in SLE-PAH patients (conversely to SLE-no PAH and healthy controls), but not in mixed connective tissue disease (MCTD)-PAH and iPAH patients; presence of anti-BMPR1A antibodies in SLE-PAH patients (conversely to SLE-no pulmonary hypertension and healthy controls); absence of anti-BMPR2 antibodies in SLE-PAH, MCTD-PAH and iPAH. In the United Kingdom national iPAH cohort, patient sera were screened for reactivity against various members of the BMPR/ALK signalling pathway [11]. Anti-BMPR2 antibodies were detected in a small fraction of iPAH patients, and none of the healthy controls. Anti-BMPR2 antibodies recognise the extracellular domain of the protein and attenuate BMP4 signalling in PA-SMCs.

Indirect functions of autoantibodies

Among the various Fc-mediated functions of antibodies, two mechanisms appear of particular importance in PAH: IgG-mediated complement activation and IgE-mediated mast cell activation. A recent study highlighted the critical role of the complement cascade in driving perivascular inflammation in the lungs of iPAH patients and various pulmonary hypertension models [63]. Interestingly, IgM and IgG deposits co-localise with C4 and C3 staining in lungs of HPH mice; and Ig-deficient animals are protected against the complement activation, vascular changes and RVSP elevation caused by hypoxia. These effects are restored by immune reconstitution with polyvalent IgG from healthy controls, suggesting a possible contribution of physiologically occurring so-called “natural antibodies”.

Another recent work demonstrated the importance of IgE-induced mast cell activation in the pathogenesis of PAH [26]. In HPH mice and MCT rats, treatment with anti-IgE antibodies or mast cell-specific FcεRI deletion normalises haemodynamics, pulmonary vascular thickening and muscularisation, as well as IL-6 and IL-13 expression in lung tissues.

Production of cytokines/growth factors and direct cell–cell interactions

Pro-inflammatory properties: effector B-cells

B-cells have been increasingly recognised as a major source of pro-inflammatory cytokines (notably IL-6 and interferon (IFN)-γ) in autoimmune diseases in recent years [111]. For instance, circulating B-cells from SSc patients produce more IL-6 than those from healthy controls [112, 113].

However, in PAH, data regarding these pro-inflammatory properties are scarce. In a novel model of SSc-PAH induced by P-selectin glycoprotein ligand-1 deficiency in female mice, IFN-γ-producing B-cells infiltrate the lungs in higher proportion than in control animals [114]. In a rat PAH model induced by a double-hit of anti-VEGF Sugen-5416 injection and ovalbumin immunisation, B-cell depletion is associated with lower IL-6 expression in lungs [115].

Recently, the NLRP3 inflammasome has emerged as an important pathway to promote inflammatory properties in B-cells, such as IL-1β production [116]. Interestingly, its activation in B-cells is regulated by the BAFF system and BCR signalling [117], which makes this inflammasome a relevant putative mechanism by which B-cells could contribute to the inflammatory milieu observed in PAH. This pathway has not yet been studied in this field, but warrants further consideration.

Anti-inflammatory properties: regulatory B-cells

The term "Bregs" (regulatory B-cells) refers to a heterogeneous group of various B-cell subsets, all involved in suppressing immune responses and maintaining immune tolerance through distinct mechanisms (secretion of IL-10, IL-35 and TGFβ, direct cellular interactions mediated by CD1d and programmed death-ligand 1) [118]. As such, defective Breg function can play a crucial role in the emergence of the autoreactivity observed in various autoimmune diseases.

In SSc-PAH patients, circulating levels of $CD24^{hi}$ $CD27^{+}$ Bregs are decreased compared to SSc-no PAH patients [14]. HPH mice have decreased Breg levels in the spleen and peripheral blood, and decreased blimp-1 (a major Breg transcription factor) expression in the lungs [119]. Adoptive transfer of Bregs improves haemodynamics and pulmonary vascular remodelling in HPH mice. In co-culture experiments, Bregs favour $CD4^{+}$ differentiation into T follicular regulator cells at the expense of Tfh cells, and inhibit hypoxia-induced PA-SMC proliferation.

Angio-active properties: angiogenic B-cells?

A novel angiogenic B-cell population have been reported in patients with melanoma and eosinophilic oesophagitis, as well as in healthy controls [120]. This subset, characterised by a specific membrane phenotype ($IgG4^{+}$ $CD49b^{+}$ $CD73^{+}$ B-cells), can produce pro-angiogenic cytokines (such as VEGF and PDGFA) and promote endothelial cell tube formation.

The presence and putative role of this subset has yet to be investigated in PAH. However, indirect evidence may suggest a direct action of B-cells on the vasculature in these patients. In SSc, B-cells produce angiogenic mediators (such as angiogenin and angiopoietin 1) in greater proportion than healthy controls, but without difference in patients with and without PAH [45]. Various plasma cell proliferative disorders are characterised by elevated VEGF levels and vascular manifestations, including PAH [121].

Local involvement of B-cells in PAH lungs: functional tertiary lymphoid organs as a model recapitulating B-cell participation in PAH

Most available data regarding B-cell alterations in PAH patients stem from experiments using peripheral blood samples, with corroborating evidence observed in the lungs of experimental models (table 2). However, several studies have evaluated B-cells and antibodies directly within explanted lungs from PAH patients undergoing transplantation.

B-cell infiltration and Ig deposits are observed in PAH lungs

B-cell infiltration has been reported in the lungs of PAH patients and several pulmonary hypertension models [6, 28, 30–32, 60, 103, 122–125]. They typically form lymphoid aggregates located in perivascular areas (within the adventitial layer, distributed throughout the whole arterial tree and around plexiform lesions) but also less frequently in peribronchial areas. Solitary infiltrating B-cells are rarely seen and, when present, usually localise within pulmonary artery intima, or between endothelial cells in plexiform lesions. B-cell lung infiltration in PAH is probably an early event, as it preceded pulmonary vascular remodelling and RVSP elevation in a model of PAH induced by Sugen-5416 administration in athymic rats [28].

Similarly, local antibody deposition in the form of immune complexes has been observed in human and experimental PAH and mostly localises in pulmonary artery intima and adventitia [6, 63]. The main target antigen of immune complexes isolated from the lungs of iPAH patients was recently identified as SAM domain and HD domain-containing protein 1, a cellular enzyme expressed by endothelial cells in reaction to the increased replication of human endogenous retrovirus-K [126]. Interestingly, other candidates include cytoskeletal and ribosomal proteins, reminiscent of the targets recognised by anti-endothelial cell and anti-fibroblast antibodies from the peripheral blood of PAH patients (described earlier). Similarly, serum Ig from two distinct PAH models labelled the pulmonary artery intima and adventitia when applied on lung samples from diseased animals [60, 103]. Overall, these data suggest that the circulating autoantibody in PAH may actually target lung-specific antigens.

TABLE 2 Comparison of B-cell anomalies in the peripheral blood from human pulmonary arterial hypertension (PAH) patients and the lungs from animal pulmonary hypertension (PH) models

	Human diseases (peripheral blood)	References	Experimental diseases (lungs)	References
B-cell homeostasis	↓ Total B-cells ↑ Naïve B-cells ↑ Plasmablasts and plasma cells ↓ Memory B-cells ↑ Activated B-cells	[10]	↑ Total B-cells (MCT, Su/Hx) ↑ Memory B-cells (Hx) ↑ Expression of B-cell activation genes (MCT)	[22, 30, 119]
BCR signalling	↑ Expression of BCR signalling genes ↑ Expression of BTK gene in total B-cells, naïve B-cells, memory B-cells ↑ BTK activity in total B-cells, correlated with ANA positivity	[8, 21]	↑ Expression of BCR signalling genes, correlated with disease severity (MCT) ↑ BTK staining, mostly colocalised with macrophages (MCT) B-cell-specific overexpression of BTK induces PH and generates anti-endothelial cell Abs in mice exposed to intratracheal BLM	[5, 22, 23]
Th2 cells, IL-4, IL-6 and IL-13	↑ IgE levels and IgE^+ B-cells	[26]	↑ IgE^+ B-cells (Hx/OVA, MCT) ↑ Expression of CD86, CD40 and GLε by B-cells, suggesting B-cell/T-cell interaction and IgE production (MCT) Th2 inhibition and IL-4/13 neutralisation decrease IgE^+ B-cell infiltration and attenuate PH (Hx/OVA)	[25, 26]
Tfh cells and IL-21	↑ cTfh cells (especially cTfh17 subset), correlated with BTK levels in B-cells cTfh cells induce preferential plasmablast differentiation and IgG/IgM production, an effect that is neutralised by IL-21 antagonist (SSc)	[5, 34]	↑ Tfh cells (Hx) ↑ Expression of IL-21 gene (MCT)	[22, 119]
BAFF system	Correlation of BAFF levels with PH markers (SSc-PAH)	[45]	↑ Expression of BAFF and BAFF-R genes, correlated with disease severity (MCT)	[22]
Anti-endothelial cell Abs	Frequent occurrence of anti-endothelial cell Abs Induction of pro-inflammatory and pro-adhesive properties in endothelial cells	[74, 80]	ICs decorate lumen-obliterating endothelial cells (Su/OVA) Serum IgA stains PA intima (PH model induced by cDC-specific A20 deficiency)	[103, 115]
Anti-fibroblast Abs	Frequent occurrence of anti-fibroblast Abs Anti-fibroblasts target vimentin, calumenin, PI3K, tropomyosin 1, HSP27, HSP70 Induction of pro-inflammatory, pro-adhesive and pro-fibrotic properties in fibroblasts	[84, 85]	Serum IgG stains PA adventitia (MCT) Anti-fibroblast Abs target vimentin, PI3K and HSP27 in lung fibroblasts (MCT)	[60]
Anti-AT1R/ETAR Abs	Frequent occurrence of AT1R/ETAR Abs Agonistic properties in endothelial cells and SMCs	[62, 86]	Passive transfer of human anti-AT1R Abs induces pulmonary vasculopathy in healthy mice Passive transfer of human anti-ETAR worsens PH in MCT rats	[86, 110]
Bregs	↓ $CD24^{hi}$ $CD27^+$ Bregs (SSc-PAH)	[14]	↓ Expression of *blimp1* (Breg transcription factor) Adoptive transfer of Bregs improves PH in Hx mice Bregs co-cultured with PA-SMCs inhibit their hypoxia-induced proliferation	[119]

Human data usually refer to idiopathic (i)PAH and connective tissue disease (CTD)-PAH patients, except when otherwise stated. Animal data are gathered from several animal models, mostly monocrotaline (MCT) rats and variations of hypoxia (Hx) and Sugen (Su) mice (sometimes with additional exposure to ovalbumin (OVA)). BCR: B-cell receptor; Th: T-helper cell; IL: interleukin; Tfh: T follicular helper cell; BAFF: B-cell activating factor; Ab: antibody; AT1R: angiotensin receptor 1; ETAR: endothelin receptor A; Breg: regulatory B-cell; BTK: Bruton tyrosine kinase; ANA: antinuclear antibody; BLM: bleomycin; Ig: immunoglobulin; CD: cluster of differentiation; GL: germline; cTfh: circulating Tfh; SSc: systemic sclerosis; BAFF-R: BAFF receptor; IC: immune complex; PA: pulmonary artery; cDC: conventional dendritic cell; PI3K: phosphoinositide 3-kinase; HSP: heat-shock protein; SMC: smooth muscle cell; *blimp1*: B-lymphocyte-induced maturation protein 1.

Lung B-cells organise as functional tertiary lymphoid organs and are the main source of local and circulating autoantibodies targeting pulmonary vascular antigens in PAH

B-cell aggregates in PAH lungs adopt a classic conformation of tertiary lymphoid organs (TLOs), characterised by a central B-cell follicle that contains follicular dendritic cells, and a well-segregated $CD4^+$

T-cell peripheral zone embedded in a network of fibroblastic reticular cells and supplied by high-endothelial venules [6, 30, 60, 103]. Lung TLOs in PAH appear to provide an adequate model that recapitulates the involvement of B-cells in the pathogenesis of PAH (figure 3).

TLO generation is usually initiated by the interaction of lymphoid tissue inducer cells (hypothesised to have a mast cell phenotype in PAH) and lymphoid tissue organiser cells (typically follicular dendritic cells and fibroblastic reticular cells) through the lymphotoxin (LT)α/β-LTβR couple [6]. This process results in the local production of lymphorganogenic chemokines (especially CXCL13, CCL19, CCL20 and CCL21 in PAH) by fibroblastic reticular cells and follicular dendritic cells, that recruit circulating $CXCR5^+$ and/or $CCR7^+$ T-cells and B-cells (which accounts for the decreased B-cell levels and altered subset distribution

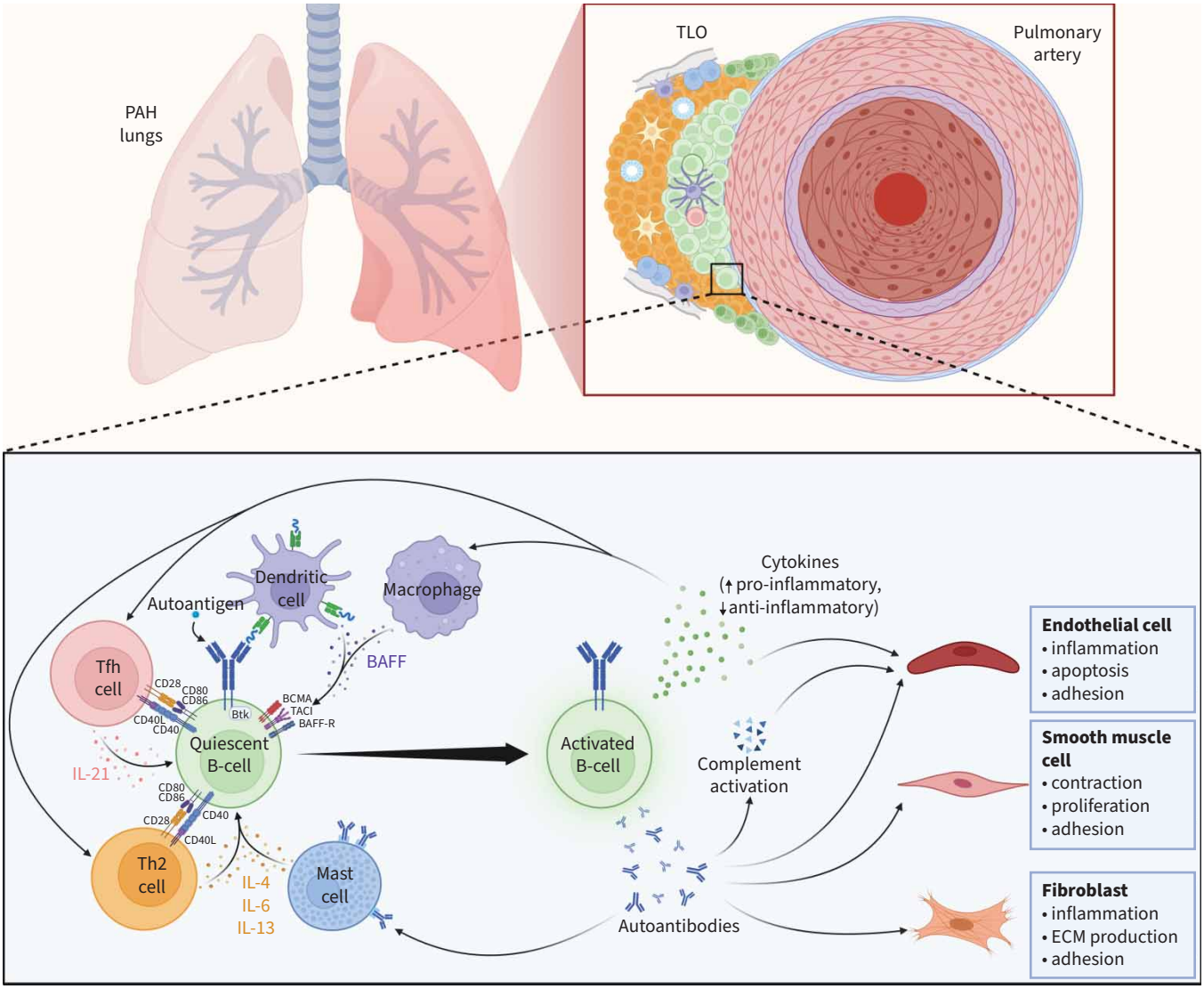

FIGURE 3 B-cells in pulmonary arterial hypertension (PAH): a proposed model. In PAH patients, B-cells are recruited to the lungs and activated by several mechanisms: interaction of lung vascular autoantigens with their cognate B-cell receptor (BCR); costimulatory signals provided by T follicular helper cells (Tfh) (interleukin (IL)-21), type 2 T helper (Th2) cells and mast cells (IL-4, IL-6 and IL-13); and increased survival signals provided by the B-cell activating factor (BAFF) system. This process results in the formation of germinal centres within perivascular tertiary lymphoid organs (TLOs) and in the local production of pathogenic autoantibodies targeting the pulmonary vasculature (endothelial cells, smooth muscle cells, adventitial fibroblasts). In addition, B-cells mediate their effects through enhanced production of pro-inflammatory cytokines, reduced anti-inflammatory properties by regulatory B-cells, immunoglobulin (Ig)G-induced complement activation, and IgE-induced mast cell activation. BAFF-R: BAFF receptor; BCMA: B-cell maturation antigen; Btk: Bruton tyrosine kinase; CD: cluster of differentiation; TACI: transmembrane activator and CAML interactor.

observed in these patients) [6]. A local expansion in lymphatic vasculature is also observed [6], which allows pulmonary self-antigens from damaged vessels to circulate and activate B-cells through interactions with their cognate BCR. Strong costimulatory signals are locally provided by Th2 cells located in T-cell zones, mast cells surrounding lymphatics and IL-21-producing Tfh within B-cell follicles [6]. Although not specifically studied in PAH, the BAFF system is also involved in B-cell activation during tertiary lymphoid neogenesis in autoimmune diseases [127]. As such, it appears that the main factors incriminated in B-cell activation in PAH can be locally available within TLOs.

B-cell activation in TLOs leads to a germinal-centre reaction, as suggested by the presence of B-cell lymphoma (Bcl)2^{-} $Bcl6^{+}$ $Ki67^{+}$ B-cells (characteristic of germinal-centre B-cells) within follicles, the strong over-expression of activation-induced cytidine deaminase (AID) (supporting the occurrence of somatic hypermutations and class-switch recombinations), and the presence of $CD138^{+}$ plasma cells and IgG^{+}/IgA^{+} cells (indicating generation of ASCs and memory B-cells, both terminal products of the germinal-centre reaction) [6, 60, 103]. These data suggest that TLOs are functional and act as local producers of autoantibodies, which mainly target autoantigen expressed by pulmonary artery endothelial cells and fibroblasts. Interestingly, experimental interventions preventing lung TLO generation in MCT rats diminish serum autoantibody titres, identifying them as the source of the circulating autoantibodies observed in PAH [60].

B-cells could contribute to right heart remodelling during PAH

Although B-cells have not been investigated as a potential player in right heart remodelling during PAH, a growing body of evidence has emerged supporting their implication in the pathogenesis and progression of left heart diseases [128]. In this setting, a B-cell infiltration is observed after acute heart injury in rodents and humans, mediated by the CXCL13–CXCR5 axis, located in the myocardium and pericardial fat, and often organised in small clusters [129–131]. Mediastinal lymph nodes also show increased germinal-centre reactions [131], supporting an enhanced local production of antibodies.

Functional circulating autoantibodies targeting cardiac autoantigens, including GPCRs (β1-adrenergic receptors) and cytoskeletal proteins (troponin, myosin), have been identified in patients with dilated cardiomyopathy [132–134], and myocardial deposition of Igs are consistently seen in explants from end-stage heart failure [135]. These antibodies could mediate their action through direct membrane binding (disrupting normal cardiomyocyte functioning) [136], complement activation [137] and interaction with FcγR located on cardiomyocytes and fibroblasts [138]. They are associated with poor clinical outcomes, such as decreased left heart function [139], increased occurrence of ventricular arrhythmias [140] and increased mortality [141].

B-cells also participate to myocardial inflammation and fibrosis through enhanced production of pro-inflammatory and pro-fibrotic cytokines (TNF-α, TGFβ) [131, 142], reduced production of anti-inflammatory IL-10 [143] and recruitment of monocytes through a CCL7-mediated mechanism [144].

As the observations of B-cell activity in myocardium parallel those made within PAH lungs, the putative contribution of B-cells to right heart remodelling in PAH is a compelling possibility that warrants further examination.

B-cell-targeted therapies in PAH

As evidence supporting their pathogenic role have accumulated, several works have tried to assess the potential of B-cells as a therapeutic target in PAH. Various approaches were investigated in both human patients and experimental models of the disease, the most studied so far being B-cell depletion induced by rituximab, an anti-CD20 monoclonal antibody.

Rituximab

Preliminary evidence suggesting an efficacy of rituximab in PAH originated from animal studies. Indeed, anti-CD20 therapy improves haemodynamics, right ventricle hypertrophy and pulmonary vascular remodelling in several models of pulmonary hypertension [30, 115, 119]. Additionally, treatment limits immune complex deposition and IL-6, VEGF and hypoxia-induced factor-1α expression in the lungs of Sugen-5416/ovalbumin rats [115]; and annulled the increase in survival and proliferation observed in pulmonary artery endothelial cells incubated with MCT plasma [30]. Concurrently, several case reports described a clinical improvement in CTD-PAH and iPAH patients treated with rituximab for indications other than the pulmonary vascular disease [145–149].

More recently, a National Institutes of Health multicentre randomised placebo-controlled trial tried to assess the safety and efficacy of rituximab as an add-on therapy in SSc-PAH patients [150]. In the primary analysis based on a model using longitudinal data through week 24, the adjusted mean change in the 6-min walk distance (6MWD) from baseline to week 24, the primary outcome measure of this study, does not differ significantly between arms (p=0.12). However, in a secondary analysis using a model with 6MWD data through week 48, the rituximab arm is superior to placebo (p=0.03). Treatment appears to be safe and well tolerated. Machine learning identified a subgroup of patients, characterised by low levels of rheumatoid factor, IL-12 and IL-17, who gain the greatest benefit from rituximab.

Interpreting these results is challenging because of several unexpected methodological issues that hindered the conduct of this trial. First, the study was underpowered because of insufficient recruitment, due to stringent inclusion criteria and competing industry-sponsored protocols. Second, the primary outcome measure was modified from haemodynamic improvement to 6MWD variation during the course of the study, because baseline pulmonary vascular resistance was lower than expected in SSc-PAH patients, rendering the original primary outcome measure much less useful. Interestingly, an independent re-analysis of the trial data focused on the subgroup that displayed the biomarker signature predictive of rituximab efficacy and revealed noteworthy findings. Specifically, patients receiving rituximab exhibited a 6MWD variation of +80 m (compared to −21 m with placebo) and a pulmonary vascular resistance variation of −1.06 Wood Units (compared to +1.5 Wood Units with placebo) [151].

Finally, these equivocal clinical findings may appear contradictory to experimental observations of rituximab efficacy. However, it should be noted that, in studies using animal models of pulmonary hypertension, anti-CD20 therapy was started at the time of disease induction, making it more of a prophylaxis than a curative treatment [30, 115, 119]. As such, rituximab could be an interesting option to prevent PAH occurrence in high-risk individuals such as CTD patients, or, alternatively, as an adjunctive therapy in select patients with the biomarkers suggesting rituximab-responsiveness. At the very least, rituximab treatment appears to be safe for patients who are being appropriately monitored for hypersensitivity reactions. Further studies are warranted to further determine the relevance and place of B-cell depletion within PAH therapeutic arsenal.

Other B-cell-oriented strategies

Proteasome inhibitors

Proteasome inhibitors (such as bortezomib) were initially developed to target plasma cells in multiple myeloma, but their use has since then been extended to autoimmune diseases [152]. In several animal models of pulmonary hypertension, proteasome inhibition improves haemodynamics, right ventricle hypertrophy, pulmonary vascular remodelling and survival [153–159]. Although a direct effect on pulmonary artery endothelial cells and PA-SMCs can explain these outcomes, a possible mediation through plasma cell inhibition was not investigated.

BTK inhibitors

BTK inhibition improves haemodynamics, right ventricle hypertrophy, pulmonary artery remodelling and fibrosis and endothelial-to-mesenchymal transition in MCT rats [23]. BTK expression mostly co-localises with macrophages in this model; and the effects of its inhibition seem mostly mediated through their action on macrophage BTK [23]. However, as intracellular BTK is increased in B-cells from iPAH and CTD-PAH patients and correlate with serum autoantibody titres [5], it is possible that BTK inhibition may also alleviate PAH through their action on B-cells, at least partly. In SSc patients, ibrutinib reduces the production of pro-inflammatory cytokines and autoantibodies by peripheral B-cells without modifying their IL-10 secretion [160].

Anti-CD22 antibody

In HPH mice, treatment by anti-CD22 antibody worsens haemodynamics, right ventricle hypertrophy and pulmonary vascular remodelling, by inducing a specific depletion of Bregs [119].

TLO-targeting strategies

Several experimental strategies targeting TLO generation were tested in MCT rats [60]: inhibition of immune cell homing to the lung (CCR7 antagonism), inhibition of TLO organising signal (LTβR antagonism) and inhibition of lymphatic vascularisation (VEGFR3 antagonism). All were able to reduce mean pulmonary artery pressure elevation, pulmonary artery remodelling and autoantibody production when administered in a preventive setting; and with a milder effect when given in a curative modality. Inhibition of somatic hypermutations through AID neutralisation have also shown interesting results in

suppressing autoreactive germinal-centre responses in lupus-prone mice [161] and should be given further consideration in the field of PAH.

Vaccination against endothelin receptors

Vaccine immunisation against the second extracellular loop of ETAR leads to the generation of inhibitory antibodies in MCT rats (conversely to naturally occurring anti-ETAR antibodies, which are agonistic) [162]. Anti-ETAR vaccination is associated with improved clinical (haemodynamics, right ventricle hypertrophy) and pathophysiological (pulmonary artery thickening, proliferation, inflammation and fibrosis) outcomes with no obvious side-effects [162, 163].

Immunoadsorption

The rationale for an effect of immunoadsorption in PAH relies on the removal of pathogenic Igs, notably anti-ETAR and anti-α1-adrenergic receptor antibodies [91]. Its efficacy is suggested by two small studies which report modest improvements in haemodynamics, right ventricle function, exercise capacity and patient-reported outcomes [91, 92].

Conclusion

In conclusion, B-cells are emerging as potential key immunological players in the pathogenesis of PAH. Their aberrant activation contributes to the inflammatory milieu and vascular remodelling characteristic of this devastating disease through various mechanisms, including autoantibody production, cytokine release, and direct cell interactions. Targeting B-cells and their associated pathways may offer promising therapeutic avenues for the treatment of PAH.

Although anomalies in B-cell immunity have been observed consistently in most PAH subsets and experimental models, the extent of their contribution to the disease pathophysiology remains insufficiently appreciated. Do they have an actual pathogenic action (“foe”), either directly by providing an endothelial insult, or indirectly by amplifying an ongoing vascular inflammation (supporting the activation of other immune cells implicated in PAH, such as T-cells and mast cells)? Are they simple spectators, passively impacted by a chronic inflammatory process (“bystander”)? Or could they contribute to mitigate vascular damage, for instance through the actions of Bregs (“friend”)? Addressing these unanswered questions would help better refine the place of B-cell targeted therapies in PAH management.

Ultimately, a deeper understanding of the role of B-cells in PAH may pave the way for personalised and targeted approaches that improve patient outcomes and quality of life.

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