Anti-inflammatory and Immunosuppressive Agents in PAH

Jolyane Meloche, Sébastien Renard, Steeve Provencher, and Sébastien Bonnet

Abstract Pulmonary arterial hypertension (PAH) pathobiology involves a remodeling process in distal pulmonary arteries, as well as vasoconstriction and in situ thrombosis, leading to enhanced pulmonary vascular resistance and pressure, to right heart failure and death. The exact mechanisms accounting for PAH development remain unknown, but growing evidence demonstrate that inflammation plays a key role in triggering and maintaining pulmonary vascular remodeling. Not surprisingly, PAH is often associated with diverse inflammatory disorders. Furthermore, pathologic specimens from PAH patients reveal an accumulation of inflammatory cells in and around vascular lesions, including macrophages, T and B cells, dendritic cells, and mast cells. Circulating levels of autoantibodies, chemokines, and cytokines are also increased in PAH patients and some of these correlate with disease severity and patients' outcome. Moreover, preclinical experiments demonstrated the key role of inflammation in PAH pathobiology. Immunosuppressive agents have also demonstrated beneficial effects in animal PAH models. In humans, observational studies suggested that immunosuppressive drugs may be effective in treating some PAH subtypes associated with marked inflammation. The present chapter reviews experimental and clinical evidence suggesting that inflammation is involved in the pathogenesis of PAH, as well the therapeutic potential of immunosuppressive agents in PAH.

Keywords PAH • Inflammatory mediators • Immunosuppressive agents

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Contents

List of Abbreviations

BMPRII	Bone morphogenetic protein receptor 2
CCL	C-C motif ligand
CsA	Ciclosporin A
CTD	Connective tissue disease
CYC	Cyclophosphamide
DHEA	Dehydroepiandrosterone
EC	Endothelial cells
HAART	Highly active antiretroviral therapy
HHV-8	Human herpes virus 8
HIF-1α	Hypoxia inducible factor 1 alpha
HIV	Human immunodeficiency virus

IL	Interleukin (i.e., IL-6, IL-2)
iPAH	Idiopatic pulmonary arterial hypertension
MCP-1	Monocyte chemotactic protein 1
MCT	Monocrotaline (induced PAH)
MIP-1α	Macrophage inflammatory protein 1 alpha
MMF	Mycophenolate mofetil
MTX	Methotrexate
NFAT	Nuclear factor for activated T cells
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PA	Pulmonary artery
PAH	Pulmonary arterial hypertension
PASMC	Pulmonary artery smooth muscle cells
PDGF	Platelet-derived growth factor
PH	Pulmonary hypertension
RANTES	Regulated upon activation normal T cell expressed and secreted
RV	Right ventricle/ventricular
SLE	Systemic lupus erythematosus
SSc	Systemic scleroderma
Tc	Cytotoxic T lymphocytes
Th	Helper T lymphocytes
TNF-α	Tumor necrosis factor alpha
Treg	Regulatory T lymphocytes
VEGF	Vascular endothelial growth factor

1 Introduction

Pulmonary arterial hypertension (PAH) is a progressive life-threatening disease characterized by a progressive elevation of pulmonary vascular resistance, right ventricular (RV) failure, and ultimately death (Rubin 1997). According to current classification (Badesch et al. 2009), PAH constitutes the first category of pulmonary hypertension (PH) (Simonneau et al. 2009). This specific group of pre-capillary PH includes idiopathic PAH (iPAH), heritable PAH, and PAH related to drugs and toxins, congenital heart disease, connective tissue disease (CTD), human immuno-deficiency virus (HIV) infection, portal hypertension, chronic haemolytic anemia, and schistosomiasis.

Since 1994, when Tuder et al. (1994) identified for the first time inflammatory infiltrates within PAH patients' plexiform lesions, an increasing amount of experimental and human reports supported the crucial role of inflammation in PAH development (Dorfmuller et al. 2003; Hassoun et al. 2009; Price et al. 2012). Numerous inflammatory disorders and autoimmune processes are associated with the

development of experimental and human PAH (Daley et al. 2008; Morse et al. 1997; Negi et al. 1998; Nicolls et al. 2005). Moreover, bone morphogenetic protein receptor type 2 (BMPRII), a pathway largely involved in heritable PAH (Deng et al. 2000; Lane et al. 2000; Machado et al. 2001), also modulates the inflammatory response (Hagen et al. 2007; Song et al. 2008). However, even if inflammation in PAH is well recognized to promote and/or perpetuate pulmonary vascular remodeling (Pullamsetti et al. 2011), its precise relationship with other components of the PAH pathogenesis like endothelial cells (EC) dysfunction, proliferation of pulmonary artery (PA) smooth muscle cells (PASMC) and fibroblast, and in situ thrombosis remains elusive. Furthermore, it is still unclear if inflammation really initiates vascular remodeling ("initial hit"), participates in its progression ("secondary hits") or represents a reactive response to remodeling only ("bystander phenomenon") (Price et al. 2012). From a therapeutic perspective, some reports have shown that immunosuppressive or anti-inflammatory drugs may improve PAH, both in animal models (Bonnet et al. 2007; Price et al. 2011; Sutendra et al. 2011; Suzuki et al. 2006; Wang et al. 2011; Zheng et al. 2010) and human cases (Jais et al. 2008; Karmochkine et al. 1996; Ogawa et al. 2011; Tanaka et al. 2002).

This chapter will review the relevance of inflammatory processes in experimental and human PAH, the implication of cells and other mediators in the inflammatory response observed in PAH, the potential targets for immunosuppressive treatment iPAH, and the updated clinical experience with available immunosuppressant agents.

2 Clinical Evidence of Inflammation in Human PAH

Over the past decades, many inflammation processes have been clearly associated with PAH development, including CTD (Asherson 1990; Cool et al. 1997; Fagan and Badesch 2002; Hachulla et al. 2005), HIV infection (Cool et al. 1997; Humbert 2008) and schistosomiasis infection (Butrous et al. 2008; Tuder 2009). PAH may also occur as an ultimate complication of exceptional autoimmune/inflammatory disorders such as POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammapathy, skin changes) (Lesprit et al. 1998) or Castleman disease (Montani et al. 2005a). Also, several reports indicate an association between Hashimoto thyroiditis and PAH (Thurnheer et al. 1997). Even in iPAH, inflammatory phenomenons are present, confirming the key role of inflammation in the global pathophysiology of PA vascular remodeling (Hall et al. 2009; Humbert et al. 1995; Perros et al. 2007; Soon et al. 2010).

2.1 Histological and Cytological Data in Human PAH

Histological data were the first to support the role of inflammation in PAH pathobiology. Tuder et al. (1994) described in 1994 perivascular inflammatory cell

infiltrates composed of T cells, B cells, and macrophages in seven of ten cases of iPAH with plexogenic arteriopathy. Cool et al. (1997) reported a few years later mononuclear inflammatory cells surrounding vascular sites of plexiform growth in CTD-PAH lungs. These common features between iPAH and CTD-PAH underlined a potential role of inflammation in all PAH subtypes. Since these results, many histological and fundamental reports confirmed that immune cells abnormalities are implicated in human PAH pathogenesis (Hassoun et al. 2009; Kherbeck et al. 2011; Perros et al. 2011; Price et al. 2012), including very recent data suggesting that in iPAH, the tertiary lymphoid follicle composed of B lymphocytes, T lymphocytes, and dendritic cells have connections to remodeled PA via a stromal network supplied by lymphatic channels (Perros et al. 2012).

2.2 Inflammatory Mediators and Biomarkers in Human PAH

Increased circulating levels of interleukin (IL)-1β and IL-6 were initially reported in iPAH by Humbert et al. (1995) in the early 1990s. Elevated serum cytokines have also been observed in other PAH subtypes like CTD-PAH (Gerbino et al. 2008), HIV-PAH (Humbert et al. 1995), as well as PAH associated with sickle cell disease (Niu et al. 2009) and congenital heart disease (Diller et al. 2008). Some authors even suggested inflammatory cytokines (Soon et al. 2010) and C-reactive protein (Quarck et al. 2009) levels predicted survival in PAH, although this remains controversial (Montani et al. 2011; Soon et al. 2010). Different types of chemokines are also increased in human PAH patients' serum, such as chemokine (C-C motif) ligand (CCL) 2 [known as monocyte chemotactic protein (MCP)-1] (Sanchez et al. 2007), CCL5 [known as regulated upon activation normal T cell expressed and secreted (RANTES)] (Dorfmuller et al. 2002), and CXC3CL1 (known as fractalkine) (Balabanian et al. 2002). In addition, PASMC from PAH patients demonstrate stronger migratory and proliferative response to CCL2 compared to controls (Sanchez et al. 2007). Thus, besides evidences of local inflammation in PAH, these data suggest PAH is associated with a systemic inflammatory state that may correlate with disease severity.

2.3 Inflammatory Conditions Associated with Human PAH

Clinical arguments have been accumulated supporting the role of inflammation in human PAH, especially in the spectrum of CTD and infectious diseases.

2.3.1 CTD and Inflammatory Diseases Associated with PAH

Systemic Scleroderma

The frequent association between two rare conditions is a strong argument for a link between them. Among CTD, scleroderma (SSc) is most commonly associated with PAH, occurring in 5–15 % of SSc patients (Hachulla et al. 2005; Mukerjee et al. 2003b). As in iPAH, EC activation and apoptosis, inflammatory cells recruitment, intimal proliferation, and advential fibrosis leading to vessel obliteration are observed in SSc-PAH (Cerinic et al. 2003; Dorfmuller et al. 2007; Le Pavec et al. 2011; Sgonc et al. 2000), supporting the concept that common features take part in the pathobiology of both iPAH and SSc-PAH (Terrier et al. 2008). SSc-PAH patients are also characterized as having an important number of autoantibodies (Fritzler et al. 1995; Grigolo et al. 2000; Morse et al. 1997; Mouthon et al. 2005; Nicolls et al. 2005; Okano et al. 1992; Tamby et al. 2006), including antifibroblast antibodies (Tamby et al. 2006) and anti-EC antibodies (Arends et al. 2010; Tamby et al. 2005), which activate fibroblasts and induce pro-inflammatory and pro-adhesive phenotype, promoting vascular remodeling (Chizzolini et al. 2002). Finally, the impact of the autoimmunity in PAH is further supported by the heterogeneous prevalence of PAH among SSc depending on the precise autoantibodies detected (Steen 2005).

Systemic Lupus Erythematosus

The prevalence of PAH in systemic lupus erythematosus (SLE) varies from 0.5 to 14 % depending on the diagnostic algorithm used to define PAH (Fois et al. 2010). As for other CTDs, the presence of antinuclear antibodies and rheumatoid factor, as well as abnormalities in immunoglobulin G, complement fractions, cytokines, and growth factors suggest a predominant role for immunological mechanisms in SLE-associated PAH (Quismorio et al. 1984). It is important to keep in mind that other mechanisms may lead to PH in SLE like chronic thromboembolism related to antiphopholipid syndrome, diffuse interstitial disease (Pope 2008; Torre and Harari 2011), or more rarely active pulmonary vasculitis (Asherson and Oakley 1986).

Others CTDs and Rare Inflammatory States

Other CTD inflammatory and/or autoimmune disorders such as mixed CTD (Fagan and Badesch 2002; Jais et al. 2008), polymyositis–dermatomyositis (Minai 2009), Sjogren's syndrome (Launay et al. 2007), rheumatoid arthritis (Chung et al. 2010; Hassoun 2009), Hashimoto's thyroiditis (Chu et al. 2002; Thurnheer et al. 1997), sarcoidosis (Nunes et al. 2006), and more rarely, POEMS syndrome (Jouve et al. 2007; Lesprit et al. 1998), multicentric Castleman disease (Bull et al. 2003), and systemic vasculitis (Launay et al. 2006) have been associated with PAH.

2.3.2 Infectious Diseases Associated with PAH

Evidence for inflammation in PAH infectious-related forms is also supported by histological, cytological, serological, and clinical data where the infective agent is commonly considered as a potential inflammatory trigger.

Human Immunodeficiency Virus and Human Herpes Virus 8

HIV infection is an independent risk factor for PAH (Opravil et al. 1997), occurring in 0.5 % of infected patients (Barnier et al. 2009; Sitbon et al. 2008). Histological features in HIV-PAH are similar to that in other PAH subtypes (Cool et al. 1997; Humbert 2008; Mehta et al. 2000). The mechanisms by which HIV leads to PA remodeling may involve chronic immune activation and upregulation of proinflammatory cytokines and growth factors (Humbert et al. 1998). Indeed, expression and production of platelet-derived growth factors (PDGF) and vascular endothelial growth factors (VEGF) are increased in lung tissue and HIV-infected T cells (Ascherl et al. 1999; Humbert et al. 1998). Viral proteins like Glycoproteine 120 and Tat are also associated with lung endothelial dysfunction in HIV infection mostly through endothelin-1 secretion (Ehrenreich et al. 1993; Ensoli et al. 1990). Controversial data also suggested Human herpes virus 8 (HHV-8) coinfection could account for the development of HIV-PAH (Hsue et al. 2008; Montani et al. 2005b). The effects of highly active antiretroviral therapy (HAART) on HIV-PAH are controversial as cases of PAH regression (Barnier et al. 2009; Speich et al. 2000) and worsening (Pellicelli et al. 1998) have been reported after initiation of therapy.

As described above, genes coding for the vasculotropic virus HHV-8 proteins have also been identified in plexiform lesions of iPAH patients by Cool et al. in the early 2000s (Cool et al. 2003). HHV-8 is known as Kaposi's sarcoma-associated herpes virus and is usually associated with angioproliferative disorders. HHV-8 could thus have a pathogenetic role in PAH, triggering vascular remodeling and plexiform lesions formation. Moreover, rare case reports documented an association between reversible PAH and HHV-8/HIV-associated multicentric Castelman's disease (Montani et al. 2005a). Although there are indirect evidences from in vitro and animal studies in favor of a link between HHV-8 and PAH pathophysiology, HHV-8 has not been detected in recent histological studies in human iPAH (Bendayan et al. 2008; Henke-Gendo et al. 2005; Valmary et al. 2011). Thus, the potential role of this virus on PA remodeling in PAH remains controversial.

Parasites: Schistosomiasis-Related PAH

Among the estimated 200 million people infected by schistosomiasis worldwide (King 2010), between 2 and 5 % are believed to develop PAH (Graham et al. 2010), but their survival remains, in any case, better than in iPAH (dos Santos Fernandes et al. 2010). PAH occurs almost exclusively in patients with hepatosplenic
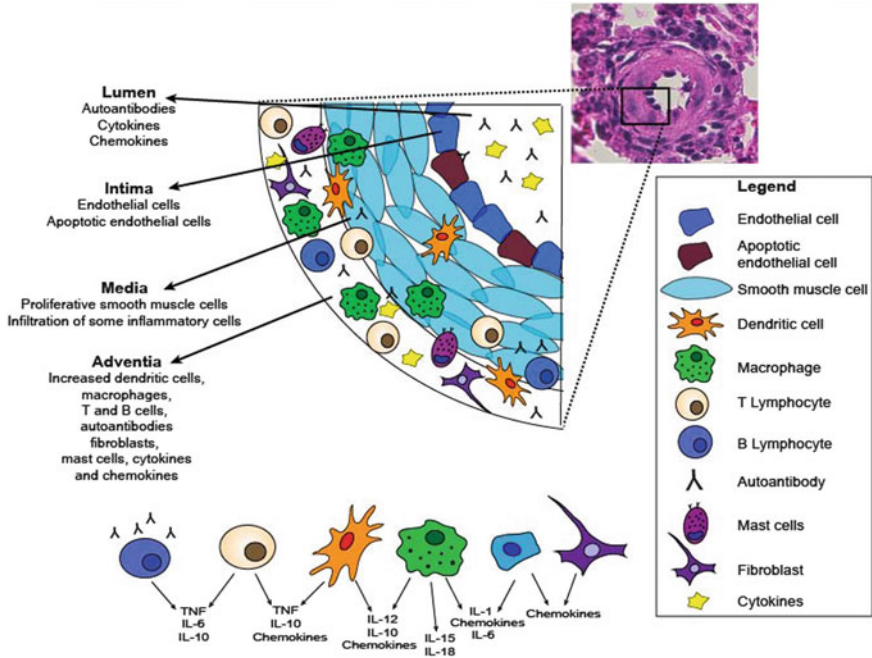
Fig. 1 Large amounts of inflammatory cell infiltrate pulmonary arteries in PAH. There is also vascular wall hypertrophy and presence of apoptotic endothelial cells. Cytokines (interleukins, TNF, MCP, etc.) and autoantibodies (anti-endothelial cells and antifibrolast for example) are present in the vascular wall and blood circulation. These autoantibodies can bind to endothelial cells and fibroblast and enhance cell modification (i.e., apoptosis and increase collagen production). Infiltration of T and B cells as well as macrophages, dendritic cells, mast cells, and fibroblast is observed in PAH. These inflammatory cells produce different cytokines as shown on the *bottom part* of this figure

infection by *Schistosoma mansoni* (Lapa et al. 2009) with subsequent portal hypertension. Histologically, plexiform lesions as well as concentric medial hypertrophy and thrombosis similar to those seen in iPAH are observed in schistosomiasis-related PAH (Tuder 2009). The mechanisms leading to PAH remain elusive, but may be related to mechanical impaction of pulmonary vessels by eggs, focal arteritis, and inflammation and increased pulmonary blood flow as consequence of portocaval shunts. Contribution of inflammation to vascular remodeling is not well understood but could be mediated by the modulation of regulatory T lymphocytes (Treg) activity resulting in the overexpression of a specific transforming growth factor superfamily (Freitas et al. 2007) or by the upregulated IL-13 signaling (Graham et al. 2010). In chronically infected animals without PH, increases in perivascular CD68-macrophages and CD45-lymphocytes have also been reported, suggesting an important switch from a Th1 to a Th2 immune response (Crosby et al. 2010). However, very few data allow extrapolating these experimental findings to human schistosomiasis-related PAH. Taken together, these non-exhaustive data suggest that increased levels of inflammatory mediators are common in human PAH (Fig. 1). Initial or latent inflammatory disorders may be an initial trigger for pulmonary vascular remodeling

or act as a "second hit." Although the relationship between inflammation and PAH is most obvious for PAH related to autoimmune, inflammatory, and infectious diseases, this association is not limited to a particular type of PAH.

3 Cells and Inflammatory Mediators Implicated in Pulmonary Vascular Remodeling

Different cell types, chemokines, cytokines, and antibodies play a role in the pathogenesis of PAH. This inflammatory process is not specific to a PAH subgroup, suggesting a central role of inflammation in PAH pathology.

3.1 Cell Types Implicated in Inflammatory Processes

3.1.1 T Lymphocytes (or T cells)

T cells are part of the adaptive immune response. They can differentiate into many subtypes according to different stimuli. Major subtypes found in lungs are T helper CD4^{+} (Th), Treg, and T cytotoxic CD8^{+} (Tc) cells. CD4^{+} Th cells are further divided in Th1, Th2, and Th17 according to their activation and cytokine production. Th1 play a role in cellular immunity and clearance of intracellular pathogen by producing interferon-γ. Th2 produce pro-inflammatory cytokines and are mainly implicated in humoral immunity, inflammation, and allergy. Th17 regulate tissue inflammation and autoimmunity by producing IL-17. CD4^{+} Th cells are important regulators of adaptive immune response since they stimulate B cell differentiation and macrophage activation, which are critical in triggering the immune response. CD8^{+} Tc are responsible of killing viral infected cells and tumor cells by binding to major histocompatibility complex class I molecules. Treg suppress autoreactive T cells and, thus, control self-tolerance and autoimmunity by balancing Th1 and Th2 responses. Different evidences support the role of T cells in PAH development. For instance, Treg are able to limit vascular endothelial injury and prevent PAH (Tamosiuniene et al. 2011). In animal models, athymic rats (no mature T cells) develop PAH more rapidly than rats with intact T cell production (Taraseviciene-Stewart et al. 2007), giving a protective role to T cells in PAH. Conversely, depletion of Th cells ameliorates the extent of PAH in other models (Sutendra et al. 2011). Thus, certain subtypes of T cells may confer beneficial effect in PAH, whereas others like Th2 may promote pulmonary vascular remodeling (Daley et al. 2008). Overall, infiltrated T cells within PA wall are increased in iPAH patients. Some studies have shown that Tc are decreased and Treg increased in PAH (Ulrich et al. 2008a) and others showed no difference in

Treg between control and PAH patients (Huertas et al. 2012). The precise role of these T cell subtypes is not yet defined. Studies in cancer demonstrated that T cells inhibit tumor growth, but their functions are often suppressed in tumor microenvironment (Koebel et al. 2007; Zitvogel et al. 2008). A similar pattern could be observed in PAH, as leptin can modulate the hyporesponsiveness of Treg (Huertas et al. 2012). Overall, it is clear that inflammation plays an important role in PAH pathogenesis and that T cells are implicated but the exact mechanism has not yet been completely elucidated.

3.1.2   Dendritic Cells

Dendritic cells are professional antigen-presenting cells, displaying antigen to activate the adaptive immune response (T cell activation). They are responsible for the initiation of the inflammatory response. Recent studies documented the role of dendritic cells in the inflammatory process in different disorders including SLE (Palucka et al. 2005). Furthermore, these cells are present in pulmonary vascular lesions and blood of PAH patients (Wang et al. 2009). Perros et al. (2007) demonstrated that immature dendritic cells are involved in pulmonary vascular remodeling and thus could be involved in PAH immunopathology. Finally, dendritic cells have the ability to differentiate into other cell phenotypes, including EC, expanding their potential role in PAH pathogenesis (Conejo-Garcia et al. 2004).

3.1.3   Mast Cells

Mast cells are derived from bone marrow precursors and reside in tissues adjacent to blood vessels. They are major effector cells of immediate hypersensitivity reactions (allergy). They contain numerous mediator-filled granules, containing histamine and heparin, and a cross-linking of their IgE to a receptor stimulates granules release as well as synthesis and secretion of other mediators leading to hypersensitivity reaction. Accumulation of mast cells is seen in different PAH types (Hamada et al. 1999; Heath and Yacoub 1991). The exact role of mast cells in PAH pathobiology is not yet well established, but they seem to be implicated in direct vasoactive effects (Heath and Yacoub 1991) and in vascular remodeling by releasing matrix metalloproteinases (Vajner et al. 2006). Conversely, mast cells can also produce IL-10, which has important anti-inflammatory and immunosuppressive effects.

3.1.4   Macrophages

Macrophages constitute the first line of immune defense and are part of the innate immune system. In normal lungs, macrophages are mainly present in alveolar airspaces to protect against inhaled pathogens. They play a crucial role in the inflammatory and immune response: they have an antigen-presenting role and

display different antigens to activate T cells. Macrophages are increased in pulmonary vascular lesions and around remodeled PA in human and experimental PAH (Tuder et al. 1994). Macrophages are also responsible for producing a large spectrum of inflammatory mediators such as tumor necrosis factor (TNF), endothelin-1, different interleukins, and chemokines (Hassoun et al. 2009; Humbert et al. 2004). Recent studies demonstrated an interaction between macrophages and T cells in PAH (Gerasimovskaya et al. 2012). Indeed, macrophage migration is suppressed by activated T cells but not quiescent T cells, whereas activated macrophages partly block T cells antitumor growth effect. Activated macrophages are also able to suppress T cell activation, thus inhibiting the anti-migratory effect of T cells on macrophages, sustaining macrophage migration, and inflammation in PAH (Gerasimovskaya et al. 2012; Zitvogel et al. 2008).

3.1.5 B Lymphocytes

B cells are responsible of generating antibodies to specific antigenic epitopes which bind to antigens and tag cells for degradation by complement cascade and phagocytosis. In addition to increased antibody production observed in PAH, B cells are increased in PAH lung vasculature and in plexiform lesions and play a critical role in cell-mediated immune regulation though cytokines production (IL-6, IL-10, TNF), antigen presentation, and lymphoid organogenesis (Ulrich et al. 2008b).

3.2 Role of Antibodies

In iPAH, antinuclear antibodies are increased up to 40 % (Rich et al. 1986), whereas antoantibodies such as anti-Scl70, anticentromere, anticardiolipin, and anti-annexin C antibodies are also observed in CTD patients. The specific role of these autoantibodies in PAH pathogenesis is not yet elucidated. More recently, other autoantibodies have been detected in PAH patients.

3.2.1 Antifibroblast Antobodies

Antifibroblast antibodies are detected in 40 % of iPAH and in 30 % of SSc-PAH patients (Tamby et al. 2006). These antibodies enhance fibroblast differentiation and adhesion molecules production. Fibroblast are implicated in collagen production, which plays an important role in both CTD and PAH. Antifibroblast antibodies target different heat shock proteins, glucose-6-phosphate dehydrogenase, PI3-kinase, calumenin, and α-enolase (Terrier et al. 2008, 2009). These targets are implicated in oxidative stress-induced apoptosis resistance

(Efferth et al. 2006), cell energy metabolism, cell growth, and cytoskeleton organization (Shibasaki et al. 1994), leading to increased contractility of myofibroblasts as observed in SSc and PAH.

3.2.2 Anti-endothelial Cell Antibodies

Anti-EC antibodies are detected in over half of patients with SSc and their levels are increased in SSc-PAH compared to SSc patients without PAH (Salojin et al. 1997). Anti-EC antibodies are also present in iPAH patients' serum. A recent study identified lamins, beta tubulins, vinculin, and calumenin as anti-EC antibody targets (Dib et al. 2011). It has been suggested that these antibodies activate EC and induce apoptosis. Further studies would be needed to fully understand their role in the pathogenesis of PAH.

3.3 Chemical Pro-inflammatory Mediators

3.3.1 Cytokines and Chemokines

A large number of cytokines and chemokines (soluble cytokines acting as chemoattractants) are elevated in PAH (Balabanian et al. 2002; Soon et al. 2010). They are mainly produced not only by the innate immune system, i.e., dendritic cells and macrophages, but also by cells which form the vascular wall and adventia (Nathan 2002). Increased IL-1 and IL-6 levels are found in severe PAH (Humbert et al. 1995). Other cytokines such as IL-2, IL-4, IL-8, IL-12p70, and TNF-α are also increased in PAH patients' serum (Fig. 1). Some of these cytokines, like IL-1b, IL-8, and TNF-α, predict outcome in PAH patients (Soon et al. 2010). The implication of these cytokines in PAH development has also been shown in animal models. For example, knock-out mice for IL-6 are resistant to hypoxia-induced PAH (Savale et al. 2009) and mice overexpressing IL-6 develop severe PH (Steiner et al. 2009), suggesting a central role of IL-6 in PAH. Furthermore, circulating IL-6 levels are predictive for the presence of associated PAH among SSc patients (Gourh et al. 2009). IL-6 is known to have many effects on inflammatory and vascular cells (Steiner et al. 2009). In fact, IL-6 stimulates T lymphocytes accumulation, chemokine production (such as CXCL3) by EC (Imaizumi et al. 2004), as well as PASMC and EC proliferation (Savale et al. 2009; Steiner et al. 2009). C-reactive protein is a marker of inflammation and tissue damage and is an active player in vascular wall damage and atherosclerosis (Scirica and Morrow 2006). C-reactive protein levels predict cardiovascular events (Labarrere and Zaloga 2004). Recently, Quarck et al. (2009) demonstrated that C-reactive protein levels are also increased

in PAH patients and correlate with long-term outcomes. Moreover, normalization of its levels with therapy is associated with improved functional capacity and survival in PAH (Quarck et al. 2009; Sztrymf et al. 2010).

An important chemokine potentially involved in PAH pathogenesis is fractalkine (CX3CL1 for C-X3-C motif ligand 1). Fractalkine, which can be detected as a soluble form, is anchored in EC membrane and is overexpressed in severe PAH (Balabanian et al. 2002). This chemokine may be responsible for leukocyte capture from the blood and recruitment (if they express C-X3-C motif receptor) (Balabanian et al. 2002). Circulating levels of monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) are also increased in PAH. MCP-1 is secreted by EC, macrophages, and fibroblasts and stimulates leukocytes and monocytes migration. As described earlier, cytokines can also act as chemokines. Thus IL-8, which is produced by hematopoietic cell and part of the C-X-C class, is chemoattractant for T cells and neutrophils (Schall et al. 1990). Macrophage inflammatory protein-la (MlP-lα, also known as CCL3) promotes migration of monocytes and T and B cells through the endothelial junctions and underlying tissue (Schall et al. 1993). MlP-lα mRNA expression is increased in PAH lung biopsy specimens compared to control (Fartoukh et al. 1998). RANTES is another important chemoattractant for T cells and monocytes and is increased in PAH (Dorfmuller et al. 2002; Luster 1998; Schall et al. 1990). Dorfmüller et al. (2002) demonstrated that RANTES mRNA levels are increased in EC from small remodeled PA and in plexiform lesions, thus increasing the amount of inflammatory cells in remodeled arteries of PAH patients.

3.3.2 Other Growth Factors Implicated in Inflammation

Growth factors including PDGF, epidermal growth factor, VEGF, and fibroblast growth factor 2 are implicated in the proliferative and apoptosis-resistant phenotype contributing to pulmonary vascular remodeling in PAH. There is an overlap between certain cytokines and growth factors. For example, IL-6 stimulates PASMC proliferation through an increase in VEGF and its receptor VEGFR2 (Steiner et al. 2009). PDGF-like molecules are secreted by many cell types, including PASMC, EC, and macrophages (Heldin 1992) and their expression is increased in PAH patients (Humbert et al. 1998). It has been shown that PDGF is able to induce smooth muscle cell proliferation and migration, which explains its implication in various fibroproliferative disorders such as hypoxic PH (Katayose et al. 1993; Schermuly et al. 2005).

4   Inflammation in PAH: Beyond the Pulmonary Vasculature

Right ventricular failure is an important component of the PAH pathophysiology. Indeed, RV failure is the leading cause of death in PAH. Not surprisingly, many circulating biomarkers currently used in PAH prognosis assessment are in fact cardiac-derived biomarkers (Barrier et al. 2012), such as brain natriuretic peptides (Blyth et al. 2007; Gan et al. 2006; Nagaya et al. 2000), troponins (Hoeper et al. 2004; Torbicki et al. 2003), and osteopontin (Lorenzen et al. 2011). The RV capacity to adapt to increased afterload is highly heterogeneous among PAH patients: some are adaptive remodelers with RV hypertrophy and preserved RV function, whereas others are maladaptive remodelers and rapidly develop RV failure (Sztrymf et al. 2010). Some studies have suggested the implication of inflammation in RV remodeling and hypertrophy (Overbeek et al. 2008). However, very little is known about the molecular and cellular mechanisms involved in RV adaptation.

Part of the answer could be found through the analysis of SSc-PAH patients, which have a worse prognosis than iPAH patients (Condliffe et al. 2009; Kawut et al. 2003). This difference may be partly explained by the older age of SSc patients and their comorbidities. Nevertheless, recent data suggest that their RV adapts differently compared to iPAH patients (Chung et al. 2010), including higher N-terminal BNP levels and increased neurohormonal activation found in SSc-PAH compared to iPAH despite of less severe hemodynamic abnormalities (Mathai et al. 2009; Overbeek et al. 2008). Indirect evidence of increased RV inflammation in SSc-PAH has been suggested. For instance, an increased signal intensity in T2-weighted sequence (abnormalities commonly associated with inflammatory myocarditis in the absence of coronary artery disease) is documented on cardiac magnetic resonance imaging studies in SSc-PAH patients compared to SSc-patients without PAH (Hachulla et al. 2009). Recently, Overbeek et al. (2008) confirmed that RV from SSc-PAH ($n = 5$) had more neutrophilic granulocytes, macrophages, and lymphocytes than in iPAH ($n = 9$) or controls ($n = 4$), whereas RV interstitial fibrosis was similar in all groups. However, the participation of inflammation to RV failure is also suspected in all PAH types except, perhaps, for patients with left-to-right shunt and Eisenmenger physiology. In this setting, cardiomyocyte contractions may produce a trigger for autocrine, paracrine, and neuroendocrine signaling pathways leading to a vicious circle of RV inflammation and ischemia, leading to cardiomyocytes apoptosis and RV failure (Bogaard et al. 2009a). Nonetheless, observed RV recovery after lung transplantation supports the idea that RV failure is not irreversible and may be amenable to specific interventions in PAH. Interestingly, Bogaard et al. (2009a) recently demonstrated that a mechanical model of chronic progressive RV pressure overload using PA banding did not lead to fatal RV failure. In contrast, the Sugen model, an established model of angioproliferative PAH, showed myocardial apoptosis, fibrosis, decreased RV capillary density, and VEGF expression despite increased nuclear stabilization of

Hypoxia Inducible Factor-1 (HIF-1), ultimately leading to RV dilation and failure. A more recent animal study from this group assessed a potential RV failure molecular signature (Bogaard et al. 2009b). Their results suggest that RV hypertrophy and/or failure phenotypes in Sugen rats are characterized by distinct patterns of gene expression (mRNA and microRNA) related to cell growth, angiogenesis, and energy metabolism (Drake et al. 2011). Additionally, using PA banding and monocrotaline (MCT) rat models, Piao et al. (2010a, b) demonstrated that in RV hypertrophy, there was a mitochondrial metabolic switch from glucose oxidation to glycolysis. This increase in glucose uptake by the RV can be seen on PET scan, compared to a normal RV that is usually invisible (Bokhari et al. 2011; Can et al. 2011). The increase in glycolysis may temporarily preserve the energetic balance, but ultimately becomes maladaptive (Piao et al. 2010a, b). The reversibility of mitochondrial dysfunction in animals with RV hypertrophy, by a oxidative phosphorylation restoration using a prototypic pyruvate dehydrogenase kinase inhibitor (dichloroacetate), may offer selective strategies for improving RV function; this is in addition to known positive effects of dichloroacetate on PA remodeling in multiple experimental PAH models (Guignabert et al. 2009; McMurtry et al. 2004; Michelakis et al. 2002). These data suggest that complex molecular, cellular, and hemodynamic heart–lung interactions may be involved in the transition from compensated RV hypertrophy to failure in PAH. New therapeutic approaches based, among others, on RV inflammation and abnormal glycolytic metabolism may thus be of interest in PAH.

5 Available Immunosuppressive Agents: Mode of Action and Use in Human Diseases Predisposing to PAH

The mechanisms of action of common immunosuppressive agents are summarized in Table 1 and schematized in Fig. 2.

5.1 Glucocorticoids

Glucocorticoids are small lipophilic compounds affecting B cell and T cell development, differentiation, and function. Their major mechanism for immune suppression is through NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) inhibition. NF-κB is involved in many cytokines and/or chemokines synthesis. Inhibition of this transcription factor, therefore, blunts the immune system capacity to mount a response (Rhen and Cidlowski 2005). Glucocorticoids also suppress cell-mediated immunity by inhibiting genes coding for different cytokines such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, and interferon-γ. They can also stimulate T cell apoptosis by triggering the transcription of different genes (Leung and Bloom 2003). Finally, glucocorticoids not only suppress immune response but

Table 1 Immunosuppressive agents in pulmonary arterial hypertension

	Glucocorticoids (prednisone, dexamethasone...)	Cyclophosphamide (CYC)	Mycophenolate mofetil (MMF)	Methotrexate (MTX)	Ciclosporin A (CsA)
Mechanisms of action	Steroid hormones regulate function and numbers of lymphocytes. They also inhibit phospholipase A2 and cyclooxygenase, two important inflammation pathways (Goppelt-Struebe et al. 1989; Leung and Bloom 2003; Rhen and Cidlowski 2005)	Alkylating agent Direct cytotoxicity on bone marrow and mature lymphocytes (Brode and Cooke 2008; Manno and Boin 2010; Marder and McCune 2007; McCune et al. 1988)	Inhibitor of purine synthesis, thus inhibiting cells proliferation (Suzuki et al. 2006; Zheng et al. 2010)	Antimetabolite (dihyrdofolate reductase inhibitor), leading to antiproliferative and cytotoxic effects (Pope et al. 2001)	Calcineurin inhibitor interfering with T-cell activation and IL-2 production (Grinyo et al. 2004; Kobayashi et al. 2007; Tsuda et al. 2012)
Effects in experimental PAH	Dexamethasone (5 mg/kg) and prednisone show beneficial effects in MCT-induced PAH (Price et al. 2011; Wang et al. 2011)	Not reported	Reverses PAH in MCT-induced rats (20 and 40 mg/kg). Does not affect T cells (Suzuki et al. 2006; Zheng et al. 2010)	Not reported	Shows beneficial effect in MCT-induced and hypoxia-induced PAH (Bonnet et al. 2007; Guignabert et al. 2006)
Common dosage in inflammatory disorders	Prednisone: 0.5–1.5 mg/kg/day initially, then slowly tapered afterward Lowest effective dose for long-term use (Jais et al. 2008)	1–2 mg/kg p.o. daily or 500–750 mg/m² iv. monthly (Jais et al. 2008)	500–1,500 mg p.o. twice daily (Contreras et al. 2004; Derk et al. 2009; Gerbino et al. 2008; Zamora et al. 2008)	10–25 mg p.o. or im. weekly (Pope et al. 2001; van den Hoogen et al. 1996; Yildirim-Toruner and Diamond 2011)	2.5–5 mg/kg p.o. daily (Clements et al. 1993; Filaci et al. 1999)

Indications, use and clinical reports	Inflammatory manifestations of CTD and other inflammatory disorders (e.g., POEMS, sarcoidosis) (Bertsias et al. 2008; Coker 2007; Dispenzieri 2011; Kowal-Bielecka et al. 2009)	ILD related to SSc Neuropsychiatric involvement of SLE (Hoyles et al. 2006; Marder and McCune 2007; Nadashkevich et al. 2006; Nannini et al. 2008; Tashkin et al. 2006) Lupus nephritis (Barile-Fabris et al. 2005; Stojanovich et al. 2003)	ILD related to SSc (Gerbino et al. 2008; Zamora et al. 2008) SSc-skin manifestations (Derk et al. 2009) Induction therapy in lupus nephritis (Bertsias et al. 2008; Contreras et al. 2004)	Inflammatory arthritis/myositis in many CTD Unsignificant efficacy on SSc-skin disease and lung function (Pope et al. 2001; van denHoogen et al. 1996) Skin manifestations of SLE (Yildirim-Toruner and Diamond 2011)	SSc-skin involvement (Clements et al. 1993; Filaci et al. 1999) Lupus nephritis : may be effective (Moroni, G, Nephrol Dial Transplant 2009) but not recommended (Bertsias G, Ann Rheum Dis 2008)
Effects in human PAH	Observational studies suggest that 40–50 % of SLE and MCTD-PAH may respond to a combination of CYC and glucocorticoids. (Jais et al. 2008; Ribeiro et al. 2001) Rare cases of POEMS syndrome have responded to steroids (Lesprit et al. 1998)	Observational studies suggest that 40–50 % of SLE and MCTD-PAH may respond to a combination of CYC and glucocorticoids. (Jais et al. 2008; Ribeiro et al. 2001)	Not reported	One case of presumed iPAH reported clinical response to MTX coadministered with prednisone (Bellotto et al. 1999)	Not reported
Main side effects	Muscle weakness[a] (may limit physical exertion), hyperglycemia, anxiety, agitation, insomnia, hypokalemia[a] (may be aggravated by	Anemia[a] (may be poorly tolerated in patients with low cardiac output), risk of bleeding[a] in case of thrombocytopenia or hemorrhagic cystitis (may be	Gastrointestinal disturbance (may be relevant for patients treated with prostanoids whose frequently complaint of diarrhea)[a], risk of	Rare cases of pneumonitis and pulmonary fibrosis Hepatotoxicity and elevated liver function test (may be confounding in	Renal toxicity Systemic hypertension Hirsutism, tremor Gum hyperplasia Increased risk of infection[b] (Bertsias et al. 2008; Denton

(continued)

Table 1 (continued)

	Glucorticoids (prednisone, dexamethasone...)	Cyclophosphamide (CYC)	Mycophenolate mofetil (MMF)	Methotrexate (MTX)	Ciclosporin A (CsA)
Significant interactions with specific PAH therapy and with warfarin	Not reported with PAH therapy Moderate interactions with *warfarin* (may increase or decrease the anticoagulant activity)[a] (Drugs.com 2010)	Not reported with PAH therapy Moderate interactions with *warfarin* (may increase or decrease the anticoagulant activity)[a] (Drugs.com 2010)	Not reported with PAH therapy No interactions with warfarin	The use of MTX in combination with potentially hepatotoxic agents (like *ERA*) is generally not recommended unless the potential benefit outweighs the risk[a] (Drugs.com 2010) No interactions with warfarin	*ERA*: Contraindication with bosentan[a] (Pharmaceuticals 2011; Venitz et al. 2012) (competitive inhibition of bosentan metabolism via CYP450 3A4 and/or inhibition of intestinal P-glycoprotein secretion by CsA). Ambrisentan dose should be limited to 5 mg[a] (Spence et al. 2010) (CsA inhibition of P-glycoprotein efflux

	Glucorticoids (prednisone, dexamethasone...)	Cyclophosphamide (CYC)	Mycophenolate mofetil (MMF)	Methotrexate (MTX)	Ciclosporin A (CsA)
	diuretics), fluid retention[a] (may simulate RV failure signs), weight gain[a] (may exacerbate dyspnea), may increase the risk of renal crisis in SSc patients, increased risk of infection[b] (Drugs.com 2010)	aggravated by warfarin), Infertility, nausea and vomiting, alopecia, increased risk of infection[b] (Drugs.com 2010)	bleeding in case of thrombocytopenia (may be aggravated by warfarin)[a], leukopenia, increased risk of infection[b] (Derk et al. 2009; Drugs.com 2010)	patients treated with *ERA*)[a] Stomatitis Nausea and abdominal distress Pericardial effusion[a] Thrombocytopenia Increased risk of infection[b] (Drugs.com 2010)	et al. 1994; Kowal-Bielecka et al. 2009)

transporter and organic anion transporting polypeptide, of which ambrisentan is a substrate)

PDE5i: Coadministration with CsA (inhibitors of CYP450 3A4) may increase the plasma concentrations of PDE5i (Schwartz and Kloner 2010) (Sildenafil or Tadalafil), dosage adjustments may be appropriate[a]

Moderate interactions with *warfarin* (may increase or decrease the anticoagulant activity)[a] (Drugs.com 2010)

PAH pulmonary arterial hypertension; *MCT* monocrotaline; *CTD* connective tissue disease; *POEMS* polyneuropathy, monoclonal gammapathy, skin changes; *ILD* interstitial lung disease; *SSc* systemic scleroderma; *SLE* systemic lupus erythematosus; *MCTD* mixed CTD; *iPAH* idiopathic PAH; *RV* right ventricular; *ERA* endothelin receptor antagonist; *CYP* cytochrome; *PDE5i* phosphodiesterase type 5 inhibitors

[a]Of particular interest in PAH because of disease pathophysiology or drug interaction

[b]Particularly of concern for patients with a central venous line for epoprostenol infusion
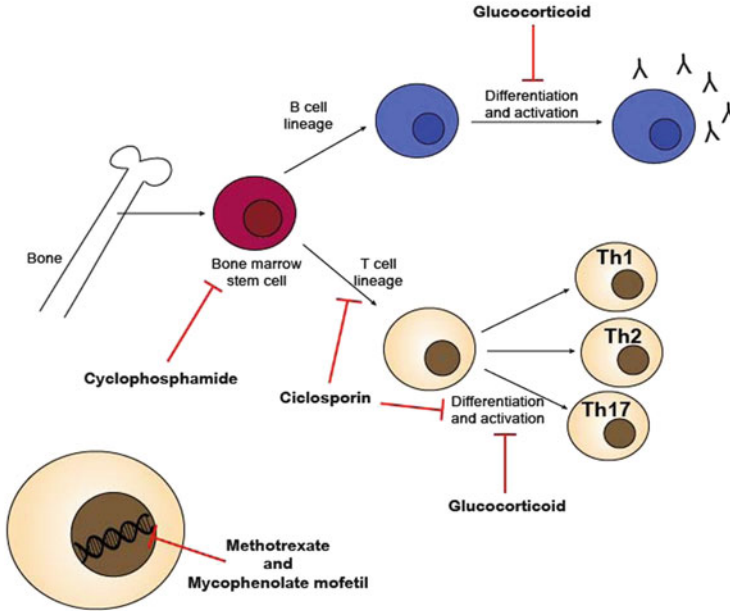
Fig. 2 Mode of action of different immunosuppressive agents as described in text. Cyclophosphamide targets bone marrow stem cells and inhibits their differentiation into B and T cells. Glucocorticoids target B and T cell differentiation and activation. Ciclosporin has a more T cell-specific mode of action as it inhibits differentiation of T cells into Th1, Th2, and Th17. Other molecules such as methotrexate and mycophenolate mofetil target DNA and RNA synthesis, thus blocking proliferation

also inhibit the two main products of inflammation: prostaglandins synthesis at the phospholipase A2 level as well as leukotrienes synthesis at the cyclooxygenase/PGE isomerase level (Goppelt-Struebe et al. 1989). Glucocorticoids are thus potent anti-inflammatory agents, regardless of the inflammation's cause. Therefore, glucocorticoids are often recommended by experts for the treatment of inflammatory manifestations of CTD (Bertsias et al. 2008; Kowal-Bielecka et al. 2009) as well as other inflammatory disorders like POEMS syndrome (Dispenzieri 2011) and sarcoidosis (Coker 2007). Some experimental data also support that glucocorticoids (prednisolone) have an antiproliferative effect on cultured PASMC from patients with iPAH (Ogawa et al. 2005).

5.2 Cyclophosphamide

Cyclophosphamide (CYC) is an alkylating agent used in chemotherapy to slow or stop cell growth. It alkylates or binds to DNA, cross-linking DNA, and RNA strands, thus inhibiting protein synthesis. These actions are neither cell cycle specific nor cell type specific. CYC also exerts its anti-inflammatory function

through direct cytoxicity of bone marrow precursors and mature lymphocytes (Fig. 2), leading to a reduction of T and B cells as well as the CD4:CD8 T cell ratio (Manno and Boin 2010; Marder and McCune 2007; McCune et al. 1988), but depending on the dose used, it can also act on Treg and in this case stimulate the immune system (Brode and Cooke 2008). CYC is currently used, most commonly with concomitant corticosteroids, to treat some severe manifestations of CTD, including in interstitial lung disease related to SSc (Hoyles et al. 2006; Marder and McCune 2007; Nadashkevich et al. 2006; Nannini et al. 2008; Tashkin et al. 2006) and neuropsychiatric involvement related to lupus (Barile-Fabris et al. 2005; Stojanovich et al. 2003). Because of many side effects of CYC, however, other immunosuppressive agents are generally preferred for less severe complications of CTD or for maintaining remission.

5.3 Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is an immunosuppressive drug with antiproliferative effects on inflammatory cells through the inhibition of the $5'$-monophosphate dehydrogenase. This enzyme is involved in purines synthesis, an essential step for DNA synthesis in lymphocytes (Fig. 2). This agent is mainly used following organ transplantation. MMF has also been shown to be as effective and better tolerated than CYC for the treatment of lupus nephritis (Bertsias et al. 2008; Contreras et al. 2004). In addition, small series have suggested moderate benefit for interstitial lung disease (Gerbino et al. 2008; Zamora et al. 2008) and skin score (Derk et al. 2009) in SSc.

5.4 Methotrexate

Methotrexate (MTX) is a folic acid analogue and a potent competitive inhibitor of dihydrofolate reductase. It inhibits both DNA and RNA synthesis (Fig. 2). MTX is frequently used in SSc to treat inflammatory arthritis and myositis. Its efficiency for skin disease and interstitial lung disease is however limited (Pope et al. 2001; van den Hoogen et al. 1996). Similarly, MTX has an uncontested role in the management of arthritis and skin manifestations of SLE (Yildirim-Toruner and Diamond 2011), although it has no role for major organ involvement related to SLE (Carneiro and Sato 1999).

5.5 Ciclosporin

Ciclosporin A (CsA) is frequently used for Th1-, Th2-, and Th17-related disorders. It exerts its immunosuppressive function by interfering with T-cell production as

well as Th differentiation and function (Grinyo et al. 2004; Kobayashi et al. 2007) (Fig. 2). CsA inhibits calcium synergic action and suppresses the calcineurin pathway, although the precise pharmacological mechanisms of CsA have not yet been fully elucidated (Tsuda et al. 2012). Calcineurin is a key molecule for the nuclear factor for activated T cells (NFAT) activation, which is the main transcription factor for IL-2. Additional anti-fibrotic effects as well as efficiency in chronic graft-versus-host disease have prompted consideration for SSc treatment. In a small open-label study, CsA was associated with a 36 % decrease in skin scores (Clements et al. 1993). Similar improvements were demonstrated in a small randomized trial testing CsA in combination with iloprost (Filaci et al. 1999). However, side effects like systemic hypertension and renal toxicity (Denton et al. 1994) have limited the clinical development of this calcineurin inhibitor in the spectrum of SSc, and this molecule is not currently recommended as first line immunosuppressive agent in SSc (Kowal-Bielecka et al. 2009). Similarly, CsA is not recommended for the management of SLE whatever its manifestations (Bertsias et al. 2008).

5.6 Anti-infectious Agents

5.6.1 Highly Active Antiretroviral Therapy in HIV-Related PAH

Considering on one hand the absence of apparent correlation between the stage of HIV infection, the degree of immunodeficiency, the $CD4^+$ T lymphocytes counts, and the occurrence or severity of HIV-PAH (Mehta et al. 2000; Nunes et al. 2003) and, on the other hand, the heterogeneous evolution of HIV-PAH following antiretroviral therapy initiation, experts do not recommend systematic HAART initiation in case of HIV-PAH (Galie et al. 2009; Hammer et al. 2008). In fact, a large majority of patients are diagnosed for HIV-PAH while already on HAART. Nevertheless, a stable prevalence of HIV-PAH overtime despite HIV patients live much longer than in the 1980s suggest HAART may be associated with a lower incidence of PAH among HIV-infected patients (Opravil and Sereni 2008; Sitbon et al. 2008).

5.6.2 Antihelmintic Treatment in Schistosomiasis-Related PAH

Prazicantel is the most widely used antihelmintic drug to treat schistosomiasis in humans. It is a pyrazinoisoquinoline derivative only active against the adult worms (Ross et al. 2002). In mice, hepatic fibrosis and lung granuloma formation has been shown to be partly reversed by prazicantel and other antihelmintic treatment (de Almeida and Andrade 1983). In a murine model, prazicantel also reversed pulmonary vascular remodeling and prevented the development of schistosomiasis-related PAH in association with a reduction of lung mRNA expression of cytokines IL-13, IL-8, and IL-4 (Crosby et al. 2011). In humans, prazicantel is usually given

to prevent further progression of the disease, even if pulmonary embolism composed of dead worms may follow this specific treatment and lead to a superimposed acute cor pulmonale (Lambertucci et al. 2000). However, antischistosomal therapy has not demonstrated any effect on pulmonary hemodynamics in schistosomiasis-related PAH, despite one case report documenting remission of PAH after prazicantel treatment (Bouree et al. 1990). New therapeutic strategies potentially targeting the inflammatory component of the disease (e.g., IL-13 antagonists) are thus needed for schistosomiasis-related PAH (Fernandes et al. 2011).

6 Immunosuppressive Agents in Experimental PH

6.1 Presence of Inflammation in Different PH Experimental Models

To study PH pathobiology, three different models are commonly used: hypoxia model, monocrotaline-induced model (MCT) and the newly developed Sugen-induced model (SU5416, a VEGF receptor inhibitor associated to chronic hypoxia) (Ryan et al. 2011). Other models [e.g., transgenic mice overexpressing 5-HTT specifically in PASMC and fawn-hooded rat are also used but at lesser extent (Guignabert et al. 2006)]. The hypoxia model develops mild to moderate PH with modest inflammation. Conversely, the MCT model develops significant inflammation with higher cytokine levels and marked inflammatory cells infiltrates in remodeled vascular wall, with inflammatory cells displaying a strong IL-6 expression (Bhargava et al. 1999; Price et al. 2011). In addition to these changes, the Sugen model shows plexiform-like lesions and, as in humans, many inflammatory cells are present in these vascular lesions. All these models show an increase in T and B cells, macrophages, mast cells, monocytes, and dendritic cells.

In these experimental models, inflammatory cells are not only innocent bystanders of the pulmonary vascular remodeling but also influence its development. For instance, absence of T cells increases vascular remodeling and worsens PH in the Sugen model (Taraseviciene-Stewart et al. 2007). Similarly, euthymic rat develop severe PH when submitted to both SU5416 injection and chronic hypoxia, whereas athymic rat develop severe vascular remodeling even in normoxia. In athymic rats, pulmonary arterioles become occluded by proliferating EC and are surrounded by mast cells, B cells, and macrophages. IL-4, proliferating cell nuclear antigen, and collagen type I levels are also markedly increased. These studies demonstrate the complex interactions between the immune system, the VEGF receptor signaling pathway, and the pulmonary vascular remodeling process. As a result, different immunosuppressive treatments have been tested in experimental PH.

6.2   Glucocorticoids

Some experimental data support that glucocorticoid treatments (with prednisolone) have an antiproliferative effect on PASMC isolated from iPAH patients (Ogawa et al. 2005). While PDGF induces iPAH-PASMC proliferation and migration, prednisolone reverses this effect in a dose-dependent manner (Ogawa et al. 2005). Very high dose of prednisolone thus caused cell cycle arrest of PASMCs with subsequent suppression of proliferation, whereas prednisolone has no effect on PASMC from controls.

Dexamethasone treatment (5 mg/kg/day) also significantly improved survival in a rat model of in MCT-induced PH (Price et al. 2011; Wang et al. 2011). Furthermore, Price et al. (2011) demonstrated that dexamethasone not only prevented but also normalized hemodynamics and right ventricular hypertrophy in a dose-dependent manner in established PH. At the vascular level, dexamethasone reversed medial and adventitial thickening, reduced MCT-induced adventitial infiltration of IL-6-expressing inflammatory cells (Price et al. 2011), inhibited pulmonary IL-6 overexpression, reduced CX3CR1 expression (Wang et al. 2011), suppressed perivascular CD8$^+$ T cells, and restored the EC integrity (Wang et al. 2011).

6.3   Cyclophosphamide

No animal data was found.

6.4   Mycophenolate Mofetil

In the past decade, different groups demonstrated the beneficial effects of MMF (20–40 mg/kg/day), a prodrug of mycophenolic acid, on MCT-induced PH (Suzuki et al. 2006; Zheng et al. 2010). MMF decreased PASMC proliferation in both MCT-induced PH and in vitro (with fibroblasts to stimulate proliferation) in a dose-dependent manner (Zheng et al. 2010). MMF was also associated with a decrease in macrophages and IL-6 production in the pulmonary vasculature (Suzuki et al. 2006), whereas T cells or mast cells were unchanged (Zheng et al. 2010). From a hemodynamic point of view, MMF decreased RV systolic pressure and hypertrophy. These beneficial effects were seen with MMF concentrations within clinical applicable range, suggesting potentiality of MMF in the treatment of human PAH (Zheng et al. 2010).

6.5 Methotrexate

No animal data was found.

6.6 Ciclosporin

Koulmann et al. (2006) suggested that CsA treatment inhibits PH and RV hypertrophy either by inhibiting HIF-1 transcriptional activity in lung, by decreasing calcineurin activity in lung and heart, by direct effects of CsA, or by a combination of these factors. The CsA dose used in this study (25 mg/kg/day) is much higher than in more recent studies. The exact mechanism by which CsA inhibits PH has been determined more recently. Indeed, Bonnet et al. (2007) demonstrated that NFAT was overexpressed both in human and experimental PH and was largely involved in the PH pathobiology. NFATc2 was in fact mostly activated in iPAH PASMC (Bonnet et al. 2007). By different techniques, they provided evidence that the inhibition of NFAT using a specific [VIVIT (Aramburu et al. 1999)] or an indirect inhibitor (CsA) had beneficial effect on experimental (in vitro and in vivo) PH (Bonnet et al. 2007). Both CsA and VIVIT decreased PASMC proliferation and restored apoptosis levels in vitro. In established MCT-induced PH, CsA (1 mg/kg, similar to doses used clinically) conferred beneficial effects on hemodynamics, RV hypertrophy, and vascular remodeling (Bonnet et al. 2007). In a PH model of mice overexpressing 5-HTT (Guignabert et al. 2006), CsA decreased the pulmonary expression of NFAT and increased Kv1.5 protein levels but did not affect PH itself (mean PA pressure for example) (Guignabert et al. 2006). These results may be explained by the fact that inflammation is not an important component of SM22-5-HTT[+] PH mice model (Macian 2005). Whether NFAT inhibition using indirect (e.g., CsA) or specific (e.g., VIVIT) inhibitors is effective in human PH or RV hypertrophy and failure remains unknown (McKinsey and Olson 2005).

6.7 Dehydroepiandrosterone

Our team demonstrated that dehydroepiandrosterone (DHEA) confers beneficial effects on PH not only due to its vasodilator effects but also by disrupting the IL-6/STAT3/NFAT axis known to potentiate PH (Paulin et al. 2011). In vitro data demonstrated that DHEA treatment inhibits IL-6-induced PASMC proliferation. By doing so, it confers anti-inflammatory effects. In MCT-induced PH, DHEA therapy also reverses PH, as assessed by mean pulmonary arterial pressure, right ventricular hypertrophy, and vascular wall thickness.

6.8   Etanercept

Tumor necrosis factor alpha (TNF-α) is a key proinflammatory cytokine that is increased in human and experimental PH (Stojanovich et al. 2003). Furthermore, TNF transgenic rats overexpressing TNF-α develop PH demonstrating its implication in PH pathogenesis (Fujita et al. 2001). Etanercept is a recombinant TNF-α receptor which binds to circulating TNF-α and functionally inhibits its action by blocking binding to cell membrane receptors, thus weakening its pro-inflammatory action (Zhou 2005). In MCT-induced PH, etanercept administered at clinically relevant doses (Lovell et al. 2000) at the same time as MCT injection (in prevention) or 3 weeks later of when PH was established (as a reversal therapy) decreased PH severity and RV hypertrophy (Sutendra et al. 2011). Since TNF-α expression is driven by NFAT in T cells, they confirmed that NFAT activation led to increased levels of TNF-α in an autocrine manner in PASMC. Furthermore, NFAT activation (and its downstream target Kv1.5) was similarly decreased by etanercept and VIVIT, a specific NFAT inhibitor. MCT-PH rats treated with etanercept also demonstrated lower TNF-α and IL-6 levels (another NFAT target) and decreased CD8^{+} T cells (Sutendra et al. 2011). Etanercept also reduced PH in a late endotoxin-induced model in pigs (Mutschler et al. 2006). Conversely, etanercept at a significantly lower dose had a nonsignificant effect on RV systolic pressure in MCT-induced PH (Henriques-Coelho et al. 2008).

6.9   Sirolimus

Sirolimus (or rapamycin) is an immunosuppressive drug preventing T cells and B cells activation by blocking their response to IL-2. It also blocks the VEGF pathway. Unlike tacrolimus and CsA, sirolimus is not a calcineurin inhibitor but has similar effects on the immune system. While sirolimus may prevent the development of MCT-induced PH, it is ineffective in reversing established PH (McMurtry et al. 2007; Nishimura et al. 2001).

7   Immunosuppressive Agents in Human PAH

Even if preclinical data show some beneficial effects, therapies targeting inflammation have not been formally tested in human PAH. Nevertheless, anti-inflammatory/ immunosuppressive therapies are sometimes cautionary recommended by experts in exceptional circumstances. These pharmacological agents, their reported effects on inflammatory disorders and PAH, as well as their potential adverse effects and drug interactions with current PAH therapies are summarized Table 1.

7.1 Updated Experiment of Immunosuppressive Agents in Human PAH

7.1.1 Idiopathic PAH

Rare case reports suggested immunosuppressive therapy could be effective in iPAH. In 1999, Bellotto et al. described the case of an iPAH patient with sustained clinical and hemodynamic improvement following the initiation of prednisone and MTX (Bellotto et al. 1999). Recently, Ogawa et al. (2011) reported the case of a 34-year-old female with iPAH treated with epoprostenol for 3 years, who significantly improved after prednisolone was prescribed for concomitant idiopathic thrombocytopenia. Importantly, both cases of presumed iPAH were characterized by concomitant inflammatory disorders. Apart from these rare cases, no data support the use of immunosuppressive therapy in iPAH.

7.1.2 PAH Related to CTD

According to the EULAR Scleroderma Trials and Research database, PAH is a leading cause of SSc-related deaths even in the era of modern PAH therapies (Mathai and Hassoun 2011; Tyndall et al. 2010). Current guidelines recommend SSc-PAH should follow the same treatment algorithm as in iPAH (Galie et al. 2009). To date, the efficacy of immunosuppressive therapy in SSc-PAH is not supported by observational studies. Indeed, Sanchez et al. (2006) reported that, among a retrospective cohort of 28 CTD-PAH patients, none of the six SSc-PAH responded (defined as patients remaining in New York Heart Association functional class I or II with sustained hemodynamic improvement after at least 1 year without the addition of PAH specific therapy) to monthly cyclophosphamide and glucocorticoids alone. More pronounced fibrotic vascular disease and presence of major comorbidities are proposed reasons to explain the absence of response to immunotherapy in SSc-PAH (Le Pavec et al. 2011). Conversely, cases reported before the advent of specific therapies suggested a positive impact of immunosuppressive therapy on the SLE-PAH course (Groen et al. 1993; Kawaguchi et al. 1998; Morelli et al. 1993; Pines et al. 1982). In the same retrospective study from Sanchez et al. (2006), 5 of the 12 (42 %) SLE-PAH were considered as responder under immunosuppressive therapy alone. Later and from the same group, Jais et al. (2008) reported similar findings, with half of the SLE-PAH patients responding clinically and hemodynamically to immunosuppressive therapy. Patients with less severe PAH at the time of diagnosis were more likely to benefit from immunosuppressive therapy. Normalization of hemodynamic parameters was even observed in some patients with SLE-PAH who received immunosuppressive therapy either alone or in combination with PAH-specific treatment (Heresi and Minai 2007; Jais et al. 2008; Ribeiro et al. 2001). Clinical and hemodynamic improvements have also been observed, although less frequently, in patients with PAH associated with

mixed CTD (Sanchez et al. 2006). More recently, immunosuppression added to specific PAH therapy has been reported effective in a heterogeneous cohort of 13 CTD-PAH as compared to a historical group of 8 CTD-PAH receiving specific PAH therapy alone (Minai 2009). Unfortunately, repartition of CTD subtypes was different between the two groups (more SLE and less SSc in the "combination" group) limiting the interpretation of these results. Despite the limited data supporting the efficacy of immunosuppressive therapy, current guidelines suggest immunosuppression, most commonly in combination with PAH-specific agents, may be considered in SLE-PAH or mixed CTD-PAH (Jais et al. 2008; Ribeiro et al. 2001).

7.1.3   POEMS Syndrome-Related PAH

POEMS syndrome has been occasionally associated with PAH. Recently, Jouve et al. (2007) described two cases of PAH related to POEMS syndrome, including one case ascribed to PAH and one case of post-capillary/high cardiac output PAH. In any case, clinical symptoms disappear and hemodynamic study showed a significant improvement in both patients (Jouve et al. 2007). Mukerjee et al. (2003a) also reported a single case of reversible POEMS-PAH after sequential therapy including initial iloprost therapy followed by immunosuppressive therapy. Finally, among 20 cases of POEMS syndrome, 5 cases of PAH were reported during the follow-up (Lesprit et al. 1998). Of note, however, PAH was confirmed by catheterization in only two of these patients. Overproduction IL-1β, IL6, TNF-α, and VEGF was found in all cases, suggesting that cytokines may mediate the development of PAH in the POEMS syndrome. However, only two of these five patients presented a sustained improvement under immunosuppressive therapy (Lesprit et al. 1998). Results about the benefit of anti-VEGF were also conflicting (Dispenzieri 2011).

7.2   Side Effects and Drug–Drug Interactions of Immunosuppressive Agents with PAH-Targeted Therapies

Potential side effects and drug–drug interactions were detected using the side effect and drug interactions checker within http://www.drugs.com database (2010). Few data are currently available about the safety profile of immunosuppressive drugs in the setting of PAH. Nevertheless, among the usual side effects of immunosuppressive therapies (Table 1), some are particularly of concern in the setting of PAH. For example, glucocorticoids promote hypokalemia, which may be aggravated by diuretics and may be associated with an increased risk of arrhythmias. CYC and MMF may reduce red blood cells and platelet production. Anemia may be poorly tolerated, especially in PAH patients with low cardiac output. Patients treated with

warfarin may also be at increased risk of bleeding in case of thrombocytopenia or CYC-related hemorrhagic cystitis. MMF may also worsen the gastrointestinal disturbance associated with prostanoid therapy. More widely, it is important to keep in mind that all immunosuppressive therapy increases risk of infection. This additional issue should be taken into consideration in the management of PAH patients, especially in those with a central venous line for epoprostenol infusion.

The main concern about drug–drug interaction relates to the cytochrome P450 metabolite pathway. Indeed, both bosentan and CsA are metabolized through the CYP450 3A4 isoenzyme. Coadministration of CsA and bosentan is contraindicated due to significant increased bosentan exposure and decreased CsA exposure (Pharmaceuticals 2011; Venitz et al. 2012). Clinicians should also be cautious if tacrolimus (another calcineurin inhibitor) and bosentan are used together. Conversely, coadministration of ambrisentan and CsA or tacrolimus is not contraindicated, although it is recommended to limit the ambrisentan dose to 5 mg once daily (Spence et al. 2010). Limited data are available for phosphodiesterase 5 inhibitors, which are also primarily metabolized by the CYP450 3A4 pathway (Schwartz and Kloner 2010). Therefore, coadministration with inhibitors of CYP450 3A4 may increase plasma concentrations of sildenafil or tadalafil, and possibility of prolonged and/or increased pharmacologic effects of these drugs should be considered. A Spanish renal transplant study was reassuring as sildenafil therapy was safe for the treatment of erectile dysfunction and did not modify CsA and tacrolimus blood levels (Cofan et al. 2002). MMF is not expected to compete with ambrisentan metabolism (Mandagere et al. 2010). Similarly, CYC is not expected to interact with specific PAH treatments because of its multiple metabolic pathways. Finally, no specific data are available concerning immunosuppressive therapy interactions with prostanoids agents.

8 Conclusion

Inflammation is undoubtedly part of the PAH pathobiology. Giving the poor outcomes in PAH despite recently developed treatments, there is an urgent need for new therapies targeting novel pathophysiological pathways. In many animal models of PAH, targeting the inflammation by current or new immunosuppressive agents improves PAH and pulmonary vascular remodeling. Treating the underlying inflammatory condition in human PAH associated with inflammatory disorders such as SLE, mixed CTD, and POEMS syndrome also led, in some cases, to marked clinical and hemodynamic improvements. Nevertheless, further studies are needed to characterize the elusive mechanisms by which inflammation triggers and sustains vascular remodeling and likely RV failure. More importantly, the poor safety profile of existing immunosuppressive drugs and the potential for drug–drug interactions with current PAH therapies mandate that appropriate large-scaled randomized trials are performed before immunosuppression becomes standard of care in any PAH type.

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