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SERIES "ADVANCES IN PATHOBIOLOGY, DIAGNOSIS, AND TREATMENT OF PULMONARY HYPERTENSION"
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Inflammation in pulmonary arterial hypertension

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Inflammation in pulmonary arterial hypertension. P. Dorfmüller, F. Perros, K. Balabanian, M. Humbert. ©ERS Journals Ltd 2003.
ABSTRACT: Inflammatory mechanisms appear to play a significant role in some types of pulmonary hypertension (PH), including monocrotaline-induced PH in rats and pulmonary arterial hypertension of various origins in humans, such as connective tissue diseases (scleroderma, systemic lupus erythematosus, mixed connective disease), human immunodeficiency virus infection, or plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, monoclonal (M) protein and skin changes (POEMS) syndrome.

Interestingly, some patients with severe pulmonary arterial hypertension associated with systemic lupus erythematosus have experienced significant improvements with immunosuppressive therapy, emphasising the relevance of inflammation in a subset of patients presenting with PH. Patients with primary PH (PPH) also have some immunological disturbances, suggesting a possible role for inflammation in the pathophysiology of this disease. A subset of PPH patients have been shown to have circulating autoantibodies, including antinuclear antibodies, as well as elevated circulating levels of the pro-infammatory cytokines, interleukins -1 and -6. Lung histology has also revealed inflammatory infiltrates in the range of plexiform lesions in patients displaying severe PPH, as well as an increased expression of the chemokines regulated upon activation, normal T-cell expressed and secreted (RANTES) and fractalkine.

Further analysis of the role of inflammatory mechanisms is necessary to understand whether this component of the disease is relevant to its pathophysiology.
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Pulmonary arterial hypertension (PAH) is characterised by an elevated mean pulmonary artery pressure $\geqslant$25 mmHg at rest, with a normal pulmonary artery wedge pressure. This severe condition leads to progressive right heart failure and ultimately death [1]. The Evian Classification reflects recent advances in the understanding of pulmonary hypertensive diseases, and recognises the similarity between "unexplained" pulmonary hypertension (PH) (primary PH (PPH)) and PAH of certain known aetiologies, such as collagen vascular diseases, human immunodeficiency virus (HIV) infection, portal hypertension, congenital systemic-to-pulmonary shunts and anorexigen exposure [2].

PAH results from chronic obstruction of small pulmonary arteries, which is due, at least in part, to endothelial and vascular smooth muscle cell dysfunction and proliferation [3]. The recent discovery that a significant proportion of patients with familial, as well as sporadic, PPH have germline mutations of genes encoding receptor members of the transforming growth factor (TGF)-β family (bone morphogenetic protein receptor-II and activin receptor-like kinase-1), suggests that dysfunctional TGF-β signalling could lead to an abnormal proliferation of pulmonary vascular cells [4, 5]. Although these major advances have improved the understanding of PAH, more information is needed to evaluate the possible involvement of additional factors in its pathogenesis. The authors and others have recently proposed that inflammatory mechanisms could play a part in the genesis or progression of PAH. This review will analyse recent information supporting the relevance of inflammation in animal models [6, 7] and patients displaying PH.

The role of inflammation and autoimmunity in pulmonary arterial hypertension

Pulmonary arterial hypertension in connective tissue diseases

PAH is a common complication of systemic inflammatory conditions, such as scleroderma and systemic lupus erythematosus. Pulmonary arterial lesions in the lungs of

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patients suffering from connective tissue diseases (CTD) with isolated PH are often similar to those found in lungs displaying PPH, including plexogenic arteriopathy. Resemblance in pathological anatomy may suggest an identical pathophysiology. Besides medial hypertrophy, intimal "onion bulb" lesions and characteristic glomoid-like plexiform lesions, COOL *et al.* [8] have reported that in patients with scleroderma-related PH, mononuclear inflammatory cells surround vascular sites of plexiform growth, but not uninvolved vessels or extravascular lung structures. In addition, TUDER *et al.* [9] were the first to identify inflammatory infiltrates in the range of plexiform lesions in the lungs of patients displaying severe PPH. This common denominator of PPH and PAH associated with CTD underlines a possible role for vascular inflammation in PAH. In *in vitro* experiments, auto-antibodies from patients with CTD (anti-U1-ribonucleoprotein antibodies, anti-double-stranded deoxyribonucleic acid antibodies) have been shown to induce up-regulation of immuno-active molecules, such as intercellular adhesion molecule-1, endothelial leukocycte adhesion molecule-1 and major histocompatibility complex class II, on human pulmonary endothelial cells, suggesting that such immunitary/inflammatory processes could lead to a proliferative and inflammatory pulmonary vasculopathy [10]. Some studies have reported a significant improvement in PAH associated with CTD after immunosuppressive therapy [11]. However, this clinical observation still needs to be confirmed by large prospective studies (see below).

Disturbances of the immune system can be complicated by pulmonary arterial hypertension

The course of other immunological disturbances, such as HIV infection [2] or plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes (POEMS syndrome) [12], can be complicated with significant PAH. Inflammatory action in the range of affected vessels has been observed in HIV patients with PAH, although development of severe PAH seems to be unrelated to the degree of immune deficiency [8]. Nevertheless, in a previous study it was demonstrated that a group of HIV patients displaying PAH had significantly higher auto-antibody levels than a matched HIV non-PAH control group [13]. This could indicate a role for a complicating auto-immunity status in the evolution of some seropositive patients, triggering the development of PAH and thereby worsening prognosis [14]. Excessive production of immune mediators in the rare POEMS syndrome with PH has been reported by FEINBERG *et al.* [15]. Increased baseline levels of tumour necrosing factor (TNF)-α, soluble TNF-receptor type I (sTNF-RI), interleukin (IL)-6, interferon gamma, IL-2, soluble IL-2 receptor (sIL-2R) and abnormally low levels of sIL-6R normalised with steroid application and plasmapheresis, with an improvement of disease status. As the interplay of IL-6 and its receptor sIL-6R appears to be relevant to the pathogenic manifestations of POEMS syndrome with PH, it is remarkable that excessive IL-1 and -6 serum levels have been described in pure PPH, as compared with PH secondary to chronic obstructive pulmonary disease [16]. The authors have recently reported an exaggerated production of CX3C-chemokine fractalkine (FKN) and an increased interaction with its receptor, CX3C-R1, in the lungs of patients suffering from severe PPH [17].

Primary pulmonary hypertension patients show a pattern of autoimmunity and inflammation

A large proportion of "pure" PPH patients without immunodeficiency or other associated systemic diseases have evidence of autoimmunity and/or active inflammation, including detectable levels of circulating antinuclear antibodies [18], elevated serum levels of the pro-inflammatory cytokines IL-1 and -6 [16], and increased pulmonary expression of platelet-derived growth factor [19] or macrophage inflammatory protein-1α [20]. For example, anti-fibrillin-auto-antibodies (anti-Fbn-1) are commonly found in systemic sclerosis, calcinosis, Raynaud′s phenomenon, oesophageal involvement, sclerodactyly, telangiectasia syndrome (CREST) and mixed connective tissue disease. MORSE *et al.* [21] have reported elevated frequency of anti-Fbn-1 in patient groups of adults with PPH (93%, n=75), children with PPH (84%, n=33) and patients with appetite suppressant-associated PPH (67%, n=18), as compared with healthy individuals. In addition, recent data providing evidence for a close association of PPH and autoimmune thyroid disease, such as Grave′s disease or Hashimoto-thyreoiditis, has revealed the possibility of an autoimmunitary pathomechanism in PPH [22]. Further evidence supporting the concept of a systemic inflammatory component in PPH was recently given by BALABANIAN *et al.* [17], who demonstrated significantly increased plasma levels of various inflammatory markers in patients with severe PPH, as compared with normal controls (table 1).

Table 1. – Plasma concentrations of soluble markers in patients with pulmonary arterial hypertension (PAH) and controls

Molecules	PAH	Controls	p-values
Subjects n	29	26	
sCD25 $\mu g \cdot mL^{-1}$	2.8±0.3	1.9±0.2	0.025
sP-sel $\mu g \cdot mL^{-1}$	66.6±5.4	52.2±4.3	0.04
sE-sel $ng \cdot mL^{-1}$	79.5±6.9	37.3±3.6	0.0001
sICAM-1 $ng \cdot mL^{-1}$	370.8±27.3	212.6±11.9	0.0001
sVCAM-1 $\mu g \cdot mL^{-1}$	2.5±0.1	1.3±0.1	0.0001
sIL-6 $pg \cdot mL^{-1}$	13.8±6.0	3.7±1.3	0.0001
vWF $U \cdot dL^{-1}$	165.6±14.5	100.2±7.4	0.0005

Data are presented as mean±SE unless otherwise stated. sCD25: soluble CD25; sP-sel: soluble P-selectin; sE-sel: soluble E-selectin; sICAM-1: soluble ICAM-1; sVCAM-1: soluble VCAM-1; sIL-6: soluble interleukin-6; vWF: von Willebrand Factor. Table modified from [17].

The monocrotaline model

Pathological changes in lungs of patients displaying PAH do not concern the whole pulmonary arterial tree, but remain restricted to certain levels of the vessel. The classical hypertensive pulmonary arteriopathy concerns overall muscular arteries of $\leqslant 500$ μm in diameter, corresponding to the subsegmentary arteries and their down-stream colaterals. Different and characteristic lesions, such as isolated medial hypertrophy, concentric intimal fibrosis, *in situ* thrombosis, pulmonary arteritis and typical plexiform lesions with their glomoid-like exhuberant endothelial cell proliferation, are found [23]. Perivascular inflammatory infiltrates with macrophages and lymphocytes in the range of occlusive lesions can be observed in PAH [9]. Endothelial cell dysfunction with deregulated expression of vasoactive, mitogenic and pro-inflammatory mediators may be the cause of these changes [24, 25]. Monocrotaline, a plant-derived toxine, causes endothelial cell injury and subsequently a massive mononuclear infiltration into the perivascular regions of arterioles and musculary arteries when injected into rats. These animals develop severe PH after monocrotaline exposure [26]. Although typical plexiform lesions are not normally found in monocrotaline-induced PH, it is used as a standard model for PAH and PPH. The important role of inflammation in this model has led to several studies focusing on immunosuppressive and

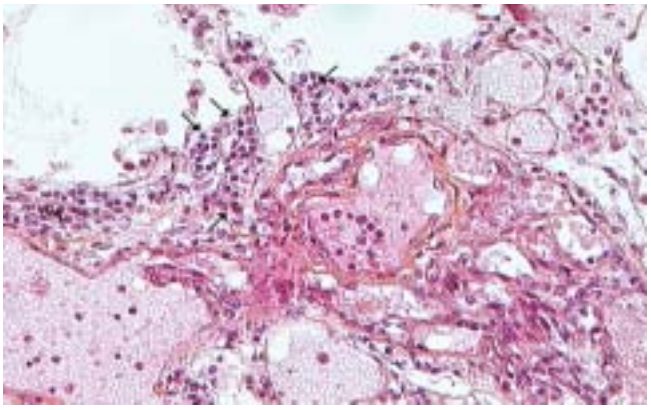

Fig. 1. – Plexiform lesion with strong, mainly lymphocytic (arrows) inflammatory infiltrate in a lung sample of a patient with severe primary pulmonary hypertension.

anti-cytokine treatment (see below), and, therefore, raises the question of how involved inflammatory cascades are in the installation and evolution of PAH lesions in humans.

The role of chemokines in pulmonary arterial hypertension

Identification of perivascular inflammatory cell infiltrates, comprised of T- and B-lymphocytes and macrophages, has supported the concept that inflammatory cells may play a role in PAH. The involvement of leukocytes, such as macrophages and lymphocytes, in complex lesions of PPH was initially described by TUDER *et al.* [9]. Recent studies by DORFMÜLLER *et al.* [27] have confirmed this observation, stressing the possible role of perivascular lymphocytic infiltrates. This inflammatory pattern has been demonstrated in plexiform lesions, as well as in other vascular lesions of PAH-affected lungs (fig. 1).

The role of T-lymphocyte recruitment by chemotactic cytokines in PAH has also been previously evaluated. Leukocyte trafficking involves successive events, including rolling, firm adhesion and extravasation, in response to a chemoattractant gradient that may involve chemokines [28]. Chemokines are soluble, secreted basic proteins that direct the migration of specific subsets of leukocytes [28, 29]. They play a major role in the different steps of leukocyte recruitment, including rolling, activation, adherence and extravasation into the inflammed tissue. The above-mentioned studies by DORFMÜLLER *et al.* [27] and BALABANIAN *et al.* [17] have attempted to analyse those chemokine-dependent mechanisms leading to inflammatory cell recruitment in the lungs of patients displaying PAH. FKN/CX3CL1 is a unique chemokine, since it exists in both a soluble form as a chemotactic protein and in a membrane-anchored form as a cell-adhesion molecule on endothelial cells [30, 31]. Its actions are mediated by CX3CR1, a seven transmembrane receptor that is expressed by monocytes, microglial cells, neurons, natural killer cells, mast cells and subpopulations of T-lymphocytes [32–37]. FKN promotes CX3CR1-expressing leukocyte recruitment, but, unlike other chemokines, it can mediate the rapid-capture, integrin-independent adhesion and activation of circulating CX3CR1+ leukocytes under high blood flow [38–40]. Several recent studies have reported a polymorphism in CX3CR1 associated with a reduced risk of acute coronary artery disease, suggesting that FKN plays a critical role in monocyte/T-cell recruitment to the vessel wall [41, 42]. The authors were able to demonstrate the following: 1) CX3CR1 was upregulated in circulating CD4+ and CD8+ T-lymphocytes from PAH patients, as compared with controls; 2) this deregulation of CX3CR1 expression accounted for the increased sensitivity of these cells to soluble FKN (sFKN) (fig. 2); 3) the abnormal response of T-lymphocytes to FKN was not the mere consequence of PH, as it was not present in patients with PH secondary to chronic thrombembolic PH (CTEPH); 4) elevated sFKN plasma concentrations were measured in PAH patients, as compared with CTEPH patients and normal controls; 5) lung samples from PAH patients showed an increased FKN messenger ribonucleic acid (mRNA) expression, as compared with controls, and pulmonary artery endothelial cells from PAH patients expressed FKN (fig. 3).

Regulated upon activation, normal T-cell expressed and secreted (RANTES) is an important chemoattractant for monocytes and T-cells [43, 44]. RANTES presumably plays a key role in a number of arterial inflammatory processes, such as glomerulonephritis [45], Kawasaki disease [46] and Takayasu arthritis [47]. In addition, successful antagonisation of RANTES has been reported in animal models of inflammatory disease [48–50]. RANTES may also play an indirect role in PAH through the induction of endothelin-converting enzyme-1 and endothelin-1, a potent endothelium-derived factor with strong vasoconstrictive and mitogenic action [51]. In their recent work, DORFMÜLLER *et al.* [27] have found new evidence for a possible involvement of this potent mediator in the evolution of PAH. Experiments on patients and healthy controls showed the following: 1) RANTES mRNA was detected by competitive reverse transcriptase-polmerase chain reaction in lung samples from all PAH patients and controls; 2) the number of RANTES mRNA copies was significantly elevated in the lungs of PAH patients as compared with controls (fig. 4); and 3) endothelial cells were the major source of RANTES, identified by *in situ* hybridisation and immunohistochemistry in PAH lung samples (fig. 5) [27].

Inflammation in pulmonary arterial hypertension: therapy

Immunosupressants in the therapy of pulmonary arterial hypertension

The effectiveness of corticosteroids and immunosuppressants in the treatement of PAH associated with connective tissue disorders has been discussed previously. In the absence of a larger placebo-controlled study, evaluations about efficacy of such treatment rely on case reports and observations in smaller groups. Nevertheless, many of these publications have reported an improvement after treatment, usually with a combination of immunosuppressants and corticoids [11, 52]. Interestingly, convincing results with decreasing mean pulmonary arterial pressures of <28 mmHg have been frequently reported in patients suffering from systemic lupus erythematosus [53, 54]. Conversely, treatment in patients with systemic sclerosis seems to be less effective on PH, suggesting that different mechanisms may be involved in the pathogenesis of PH secondary to scleroderma. Usually, corticosteroids associated with immunosuppressants, such as cyclophosphamide in bolus infusion, seem to be the most effective treatment. However, immunosuppressive protocols vary from one study to another and comparison is difficult. Lastly, there can be difficulties in evaluating the effects of immunosuppressive therapy alone because of the frequent use of vasodilator therapy [11]. In conclusion, immunosuppressants should be considered for patients with CTD and PAH, with the important exception of scleroderma. Here, strict clinical and haemodynamical criteria are necessary to evaluate the efficacy of such a treatment. If there is no clinical and haemodynamical improvement after 3–6 months of therapy

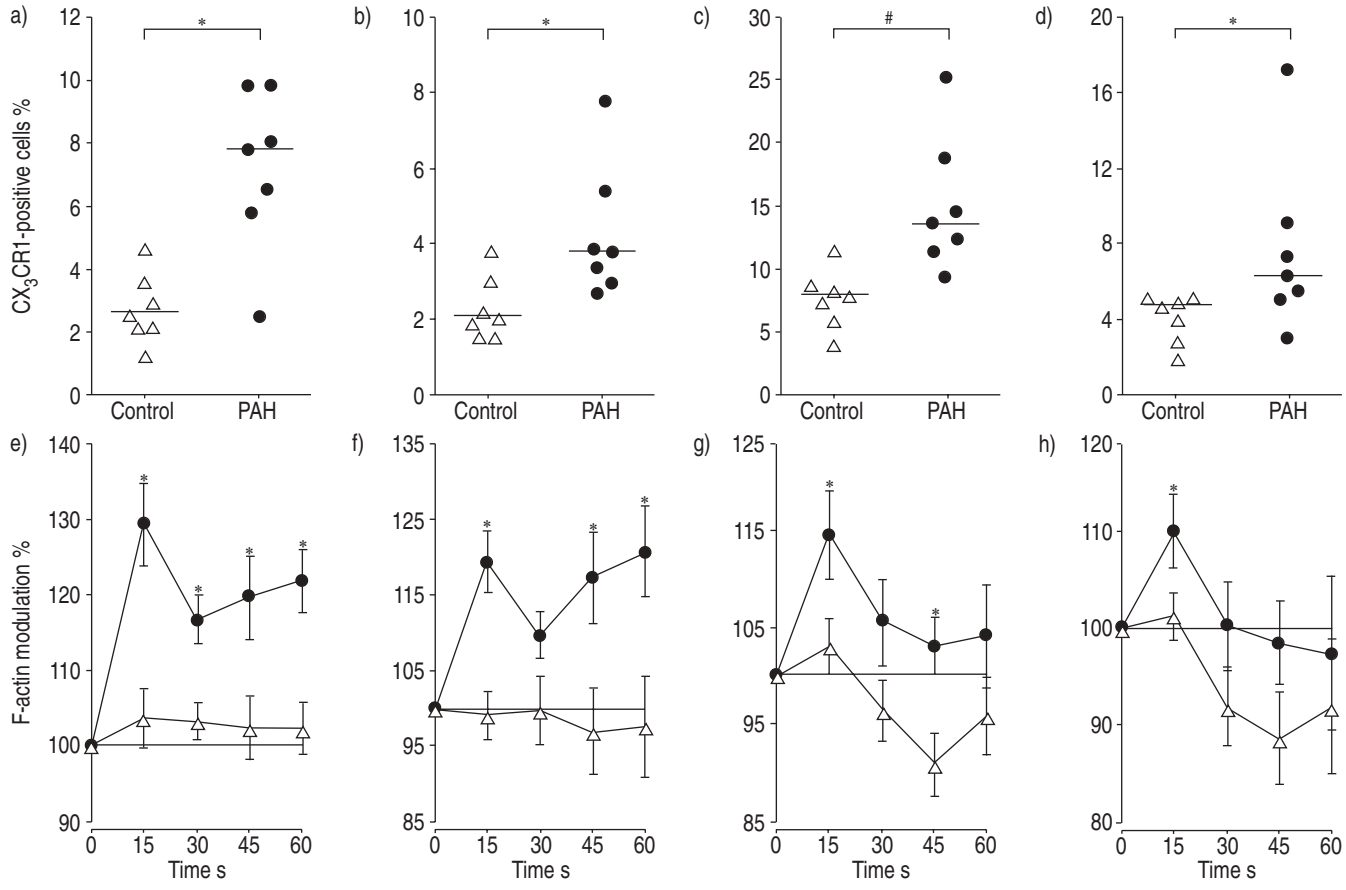

Fig. 2. – a–d) Expression and function of CX_3CR1 by T-lymphocytes from pulmonary arterial hypertension (PAH) patients. Expression of CX_3CR1 was analysed by flow cytometry in memory (CD45RO+) (a and c) and naive (CD45RO-) (b and d) CD4+ and CD8+ T-lymphocytes. Results are expressed as the proportion of labelled cells for each healthy control (n=7, △) and PAH patient (n=7, ●). *: $p<0.05$; #: $p<0.005$. e–h) In the same individuals the function of CX_3CR1 was tested by monitoring actin polymerisation (●: PAH patients, n=7; △: healthy controls, n=7). Results show the kinetics of actin polymerisation following fractalkine addition, with time. Baseline level, before fractalkine addition, are represented as 100%. *: $p<0.05$. Error bars show SEM. Horizontal lines show median values. Figure modified from [17].

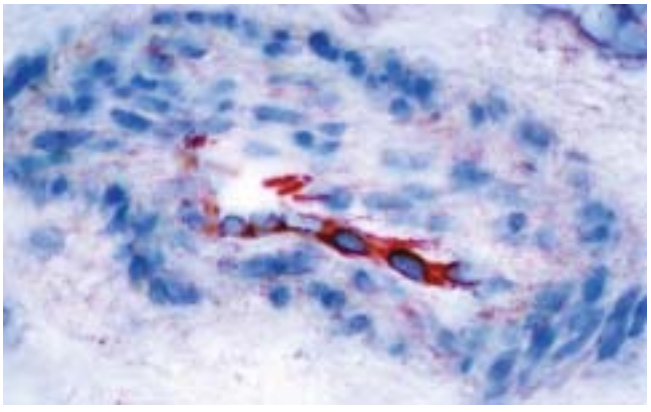

Fig. 3. – Fractalkine protein detected by immunohistochemistry in the endothelium of small muscular pulmonary arteries (lung sample taken at the time of lung transplantation in a patient suffering from severe pulmonary arterial hypertension), endothelial proliferation with obstruction of the vessel and strong endothelial staining. Figure modified from [27].

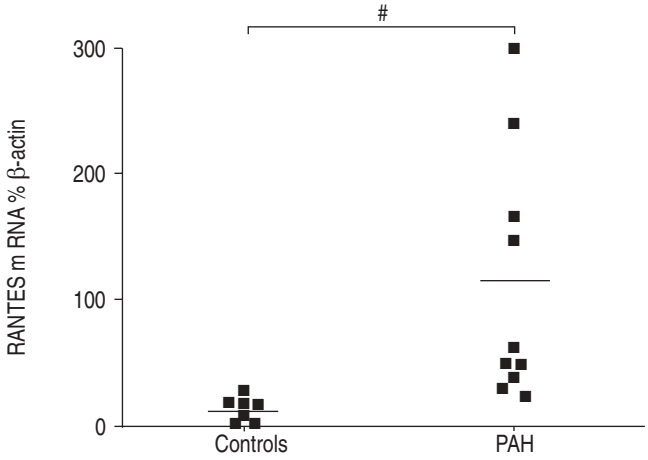

Fig. 4. – Regulated upon activation, normal T-cell expressed and secreted (RANTES) messenger ribonucleic acid (mRNA) expression detected by competitive reverse transcriptase-polymerase chain reaction in lung samples from patients suffering from severe pulmonary arterial hypertension (PAH) and controls. #: $p=0.017$. Horizontal lines show mean values. Figure modified from [27].

then treatment should be stopped because of possible complications, including infections and neoplasms.

Recent studies on animal models with induced PH have highlightened two other substances with immunosuppressive effects in the treatment of PH. Rapamycin, a macrolide immunosuppressant currently being used in therapy for chronic allograft rejection, and triptolide, a herb used in

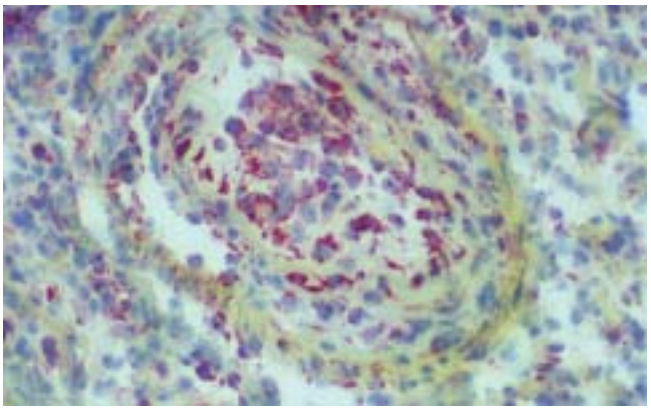

Fig. 5. – Regulated upon activation, normal T-cell expressed and secreted (RANTES) messenger ribonucleic acid expression detected by *in situ* hybridisation in the endothelium of small muscular pulmonary arteries, and to a lesser extent in perivasular cells (lung sample taken at the time of lung transplantation in a patient suffering from severe pulmonary arterial hypertension), plexiform lesion with endothelial and perivascular staining. Figure modified from [27].

traditional chinese medicine to treat rheumatoid arthritis and other autoimmune diseases, have been tested on rats being pneumectomised and subsequently treated with monocrotaline [26, 55]. Both rapamycin and tryptolide showed significant effects with lower mean pulmonary arterial pressures, as compared with vehicle-treated controls. As a morphological correlate to these findings, a significantly less right ventricular hypertrophy and pulmonary arterial neointimal formation were demonstrated. It is noteworthy that tryptolide shows anti-inflammatory properties by inhibiting T-cell activation at the level of cytokine gene transcription [56].

Anti-cytokine treatment in pulmonary arterial hypertension

Cytokine antagonists have been mainly tested on the monocrotaline rat model. VOELKEL *et al.* [6] have studied the role of IL-1, a strong pro-infammatory cytokine, in monocrotaline-induced PH as compared with chronic hypoxia PH, showing that IL-1 is excessively produced in the lungs of rats treated with monocrotaline. Repeated injections of IL-1 receptor antagonist reduced PH and right heart hypertrophy in the monocrotaline model but not in the chronic hypoxia model. If studies showing elevated circulating levels of IL-1 and -6 in patients displaying PPH, but not in patients with PH secondary to COPD are considered, a possible role for mediators of inflammation in some forms of PH can be assumed [16]. However, the authors are not aware of any studies attempting to evaluate anti-cytokine therapies in PH patients.

Numerous studies on inflammatory diseases and the effects of anti-cytokine treatment have stressed the importance of such therapeutic alternatives. Animal model experiments with RANTES-receptor antagonists (Met-RANTES), show relevant anti-inflammatory properties in the treatment of chronic allograft nephropathy. In their latest study, SONG *et al.* [57] showed that Met-RANTES diminishes the early infiltration and activation of mononuclear cells in grafts of transplanted rat kidneys accompanied by a local decrease of IL-1, -2 and TNF-α, as well as RANTES, and thereby reduces the pace of chronic allograft nephropathy. Moreover, recent studies demonstrated the prevention of crescentic glomerulonephritis in animal models by immunoneutralisation of the FKN-receptor CX3CR1 [40]. Considering these studies on FKN and RANTES, and the possible role for these two chemokines in PAH, further studies on animal models should evaluate a possible role for anti-cytokine treatment in this condition.

When assessing these different observations, inflammation and autoimmunity seem to have at least some influence on the evolution of the disease. The frequent association of pulmonary arterial hypertension and well-defined inflammatory conditions, as well as the presence of complicating pulmonary arterial hypertension in autoimmunitary disturbances, indicate a possible role for inflammatory cascades, leading to inflammatory infiltrates and remodeling of the vessel. Latest results from the authors suggest that increased activation of circulating inflammatory cells in affected individuals is a primary event, rather than a pure response to the altered physiological condition of the patient.

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