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Fractalkine-induced smooth muscle cell proliferation in pulmonary hypertension

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ABSTRACT

Introduction: Pulmonary hypertension is characterized by a progressive increase in pulmonary arterial resistance due to endothelial and smooth muscle cell proliferation resulting in chronic obstruction of small pulmonary arteries. There is evidence that inflammatory mechanisms may contribute to the pathogenesis of human and experimental pulmonary hypertension.

Objective: The aim of this study was to address the role of fractalkine (CX3CL1) in the inflammatory responses and in the pulmonary vascular remodeling of a monocrotaline-induced pulmonary hypertension model.

Methods: We studied the expression of CX3CL1 and its receptor CX3CR1 in monocrotaline-induced pulmonary hypertension by means of immunohistochemistry and quantitative reverse-transcription polymerase chain reaction on laser-captured microdissected pulmonary arteries.

Results: We demonstrated that CX3CL1 was expressed by inflammatory cells surrounding pulmonary arterial lesions and that smooth muscle cells from these vessels had increased CX3CR1 expression. Then we showed that cultured rat pulmonary artery smooth muscle cells expressed CX3CR1 and that CX3CL1 induced proliferation but not migration of these cells.

Conclusions: We propose that fractalkine may act as a growth factor for pulmonary artery smooth muscle cells. Chemokines may thus play a role in pulmonary artery remodeling.

Pulmonary arterial hypertension (PAH) is characterized by a progressive increase in pulmonary vascular resistance leading to right ventricular failure[1]. The main pathological finding related to PAH is an abnormal pulmonary artery endothelial and smooth muscle cell (PASMC) proliferation resulting in obstruction of small pulmonary arteries[2, 3]. In addition, there is evidence that inflammatory events may also be involved in its pathogenesis. Indeed, it is established that PAH can develop as consequence of systemic inflammatory conditions, such as connective tissue diseases[4]. Furthermore, the presence of mononuclear cells and lymphocytes infiltration in plexiform lesions indicates that inflammatory mechanisms may have a role as inciting, modulating or resulting event in the development of PAH[5]. The relevance of inflammatory events in PAH patients has been further supported by significant clinical and hemodynamic improvements in patients receiving corticosteroids and/or cyclophosphamide in the setting of PAH complicating systemic diseases[6, 7].

We hypothesize that mediators involved both in inflammatory responses and pulmonary vascular remodeling may explain at least partially the link between inflammation and PAH. The relation of PAH with mutations of genes encoding receptor members of the transforming growth factor-beta family[8, 9] has clearly highlighted a central role for cytokines in pulmonary vascular homeostasis. Moreover, overexpression of platelet-derived growth factor (PDGF) in human PAH as well as in monocrotaline-exposed rats has led to novel hypotheses and therapeutic strategies based on PDGF pathway inhibition with imatinib[10-13]. Last, proinflammatory cytokines and chemokines are produced in excess in patients displaying severe PAH[14-16], suggesting a role in the chain of events promoting PAH occurrence and/or progression.

Chemokines belong to a family of soluble proteins known to induce leukocyte migration and activation. Fractalkine (CX3CL1) is a unique chemokine once it exists in a soluble form and in a membrane-anchored form on endothelial cells[17]. It is normally expressed in neurons

and other nonlymphoid lineages, like endothelial and epithelial cells[18-21] acting through a transmembrane receptor (CX3CR1) expressed in several cellular types including monocytes and T-lymphocytes[22, 23]. There is a growing interest in chemokines in vascular disorders once their significant effect has been shown after endothelial injury[20]. High levels of fractalkine have been measured in advanced atherosclerotic arteries[24, 25]. Importantly, fractalkine may act beyond inflammatory cell recruitment, as suggested by its proliferative effect on SMC demonstrated in atherosclerosis[26]. Due to the major role of PASMC proliferation in PAH, we thus hypothesized that fractalkine may have a role in pulmonary vascular remodeling. For that purpose, we first demonstrated increased fractalkine and its receptor expression in the lungs and pulmonary arteries of rats displaying monocrotaline-induced pulmonary hypertension. Based on these data and our previous demonstration of fractalkine overexpression in the lungs of PAH patients[15], we then showed that rat PASMC could express the fractalkine receptor and that CX3CL1 may act as a growth factor for PASMC.

METHODS

Animal model

Young male albinos Wistar rats received a single subcutaneous injection of 60mg/kg monocrotaline (Sigma-Aldrich, Saint Quentin Fallavier, France) or saline. Ten animals (5 saline and 5 monocrotaline exposed) were sacrificed on days 0, 0.5, 1, 5, 10, 15 and 21, after complete hemodynamic analysis, as previously described[27]; lungs were then explanted and stored at -80^{o}C. Right ventricular hypertrophy was analyzed through weight ratio of the right ventricle over the left ventricle plus septum [RV/(LV+S)].

Primary smooth muscle cell cultures

At baseline (control) and 21 days after being exposed to monocrotaline, five rats were killed by an overdose of pentobarbital. Lungs were immediately removed and proximal pulmonary arteries were isolated. Smooth muscle cells were obtained by enzymatic digestion as previously described[28]

Immunohistochemistry

Immunohistochemistry was performed on 8 µm-thick sections of frozen tissue or on PASMC grown on Lab-tek eight-well chamber slides (Dominique Dutscher, Brumath, France). After routine preparation, slides were processed with the following antibodies: anti-CD3 (T lymphocytes, 1F4, Serotec, Cergy Saint-Christophe, France), anti-CD68 (macrophages, ED1, Serotec, France), anti-CX3CL1 (TP203, CliniSciences, Montrouge, France) and anti-CX3CR1 (TP502, AbCys, Paris, France). Control used for these antibodies included omission of the primary anti-body and substitution of the primary antibody by rabbit IgG.

Laser capture microdissection and cDNA preparation of bronchus-associated pulmonary arteries

Pulmonary artery media of a cross-sectional diameter of 100 to 200 µm, were captured using the AS LMD laser microdissection microscope (Leica, Rueil-Malmaison, France). RNA was extracted from microdissected PA with a picopure RNA isolation kit (Alphelys, Plaisir, France) and then eluted from silicate columns and reverse-transcribed using Sensiscript Reverse Transcription kit (Qiagen, Courtaboeuf, France).

Gene quantification by real-time reverse transcription polymerase chain reaction (PCR)

Total lung PCR reactions were followed in an ABI Prism7000 Sequence Detection System with SYBR green PCR reagents (Applied Biosystems, Courtaboeuf, France). Oligonucleotide primers were designed using the Primer Express software, based on sequences from the GenBank database (18s:F.5'- AAGTCCCTGCCCTTTGTACACA-3',R.5'-GATCCGAGGGCCTCACTAAAC-3',GenBank Accession X01117; *cx3cl1*: F.5'-TGCACAGCCCAGATCATTCA -3',R.5'-CTGCGCTCTCAGATGTAGGAAA-3',GenBank Accession NM_134455; *cx3cr1*:F.5'-GGAGCAGGCAGGACAGCAT-3', R.5'-CCCTCTCCCTCGCTTGTGTA-3',GenBank Accession NM_133534). For nano-quantities of cDNA obtained by microdissection, we used Applied Biosystems TaqMan Gene Expression Assays with TaqMan Universal PCR Master Mix (Applied Biosystems, Courtaboeuf, France). Results were analysed with ABI Prism 7000 SDS Software using the second derivative maximum method to set CT with 18s as an internal housekeeping gene control.

Western blot analysis

Western blot analysis of CX3CR1 in PASMC was performed, as previously described[29].

Measurement of PA-SMC proliferation.

PA-SMC were serum-starved for 48 hours, then incubated with recombinant rat fractalkine (catalog number: 537-FT) or recombinant rat PDGF-BB (catalog number: 520-BB) (R&Dsystem, Lille, France) and [^{3}H]thymidine for 24 hours. Cell proliferation was detected by the measurement of thymidine incorporation[30].

Migration assay

Trypsinized PA-SMC were transferred into the upper chambers of 8-µm-pore transwell plates (vWR, Fontenay-sous-Bois, France). Recombinant rat fractalkine or recombinant rat PDGF-BB were added to the lower chamber. After 24 hours at 37°C, migration was quantified by counting cells in the bottom of the membrane stained with Diff Quick (Dade Behring, Paris, France). The number of cells on lower surface of filter was counted in eight field by light microscopy under high power field (x200). Actin polymerization assay was performed as previously described[31].

Statistical analysis

Results are presented as mean and standard error of mean (SE) unless otherwise stated. Analysis of variance for repeated measures was used for multiple groups comparison with Fisher projected least significant difference (PLSD) test for the post-hoc analysis.

RESULTS

Hemodynamics and right ventricular hypertrophy in monocrotaline-exposed rats

Control rats had a mean pulmonary artery pressure (mPAP) of 14±1 mmHg and a total pulmonary vascular resistance (TPR) of 23±1 units/kg, while all monocrotaline-exposed rats had pulmonary hypertension at day 21 (mPAP= 28±2 mmHg, TPR= 56±5 units/kg). A compensatory right ventricle hypertrophy developed, as demonstrated by the ratio RV/(LV+S) (0.62±0.03 in the monocrotaline group versus 0.29±0.02 in the control group, $p<0.0001$) which correlated with mPAP ($r^2 = 0.65$, $p<0.0001$) and TPR ($r^2 = 0.64$, $p<0.0001$).

Fractalkine and fractalkine receptor expression in rat lungs and microdissected pulmonary arteries

Whole lung fractalkine and fractalkine receptor expression was markedly overexpressed after monocrotaline exposure (Figure 1). There was an early gene overexpression immediately after monocrotaline-induced pulmonary injury with a 29- and 26-fold increase in fractalkine and fractalkine receptor expression at 12 hours, as compared to baseline values. After this initial peak, fractalkine and fractalkine receptor gene expression remained elevated as compared to baseline values. In order to investigate whether pulmonary artery fractalkine and fractalkine receptor gene expression was also raised in the pulmonary arterial wall after monocrotaline exposure, we studied tissue obtained by laser capture microdissection of pulmonary artery from pulmonary hypertensive rats analyzed 21 days after monocrotaline exposure. For that purpose we microdissected the pulmonary artery media of a cross-sectional diameter of 100 to 200 μm as shown in Figure 2A. In the collected material, we found that CX3CR1 but not CX3CL1 was overexpressed (Figure 2B).

CD3, CD68, CX3CL1 and CX3CR1 staining by immunohistochemistry in pulmonary arteries from monocrotaline-exposed rats

Immunohistochemistry demonstrated that perivascular inflammatory cells from pulmonary hypertensive rats corresponded to CD68-positive macrophages and CD3-positive lymphocytes. In addition, CX3CL1 stained weakly PASMC and strongly perivascular inflammatory cells whilst CX3CR1 positive cells were both PASMC and perivascular inflammatory cells. Stained Western-Blot analysis of culture PASMC indicated a production of CX3CR1 with expected bands at 27 and 30 KDa[29] (figure 3).

Fractalkine-induced proliferation of rat pulmonary artery smooth muscle cells

[^{3H}]thymidine incorporation in PASMC in response to fractalkine and PDGF-BB indicated that both fractalkine and PDGF BB could promote PASMC proliferation (Figure 4). There was no difference in terms of fractalkine-induced proliferation between PASMC obtained from control and pulmonary hypertensive rats (data not shown).

Lack of fractalkine-induced migration of rat pulmonary artery smooth muscle cells

The process of chemotaxis can be measured at its initiation by evaluating actin polymerization within cells, a physiological requirement for cell movement. No actin polymerization could be demonstrated with fractalkine stimulation in PASMC, whereas a significant polymerization occurred in response to PDGF-BB stimulation (figure 5). These results were confirmed by transwell assay showing that fractalkine could not promote PASMC migration whereas a dose-response cell migration was demonstrated with PDGF-BB ($p<0.0001$, Figure 6).

DISCUSSION

Experimental pulmonary hypertension in rats is associated with fractalkine and fractalkine receptor overexpression, raising a possible role for this chemokine in the pathogenesis of pulmonary hypertension, as previously suggested by our data obtained in human PAH. In addition, we demonstrated that rat PASMC express the fractalkine receptor CX3CR1 and that PASMC proliferated but did not migrate in response to fractalkine. We thus hypothesize that CX3CL1 overexpression may not only promote cell recruitment, but that it may also play a direct role on pulmonary artery remodeling thanks to its PASMC proliferative effect.

A feature common to all forms of pulmonary hypertension remodeling is the appearance of a layer of smooth muscle cells in small peripheral, normally nonmuscular, pulmonary arteries within the respiratory acinus[2]. The cellular processes underlying muscularization of this distal part of the pulmonary arterial tree are incompletely understood. This cellular proliferation results in chronic obstruction of small pulmonary arteries, a major characteristic of PAH explaining raised pulmonary vascular resistance. In human PAH, several mediators may promote PASMC proliferation including serotonin (5-HT), endothelin-1, transforming growth factor beta and PDGF[3]. In addition *BMPR2* germline mutations detected in a significant proportion of familial and idiopathic PAH has clearly underlined that abnormal cellular growth is certainly a key feature of all types of pulmonary hypertension[8, 9]. Our present data add fractalkine to the list of agents able to promote PASMC proliferation and by inference remodeling and obstruction of small pulmonary arteries although one of the limitations of our study lies in the fact that no specific fractalkine antagonist was used to counterbalance its effects. The effect of fractalkine on CX3CR1-bearing PASMC is in keeping with recent data obtained in smooth muscle cells from atherosclerotic plaques[32].

Our initial hypothesis was that chemokines such as fractalkine (CX3CL1) and RANTES (CCL5) may induce pulmonary vascular inflammatory cell recruitment, and therefore promote inflammatory damage leading to an abnormal scarring and remodeling of pulmonary arteries. Although this predominant inflammatory component is presumably of significant importance in active inflammatory conditions such as systemic lupus erythematosus, a condition which may be reversible when treated with anti-inflammatory agents, it is more debatable to play a key role in other forms of symptomatic PAH including idiopathic and familial PAH. We and other have however demonstrated that inflammatory cells may indeed infiltrate remodeled small pulmonary arteries in the setting of established idiopathic PAH[5, 15, 16]. Nevertheless, anti-inflammatory agents are usually ineffective in idiopathic PAH, and the exact role of inflammatory cells and their mediators remains uncertain in this setting. We hypothesize that these cells and mediators may play a role either early in the course of the disease prior to the development of end-stage fixed pulmonary vascular obstruction or that they may also contribute to disease progression. In order to support this latter hypothesis we have proposed that inflammatory mediators in general and more precisely chemokines such as fractalkine may act beyond inflammatory cell recruitment. This is in keeping with our present data produced in experimental pulmonary hypertension showing a proliferative effect of fractalkine on PASMC.

Endothelial cell dysfunction is another hallmark of PAH, and reduced production of endothelium-derived prostacyclin and nitric oxide, as well as increased production of endothelin-1 are characteristic of the disease[3]. This endothelial dysfunction will in turn promote PASMC constriction and growth. Novel therapeutic strategies targeting these dysfunctional pathways have been shown to improve clinical and hemodynamic parameters in patients displaying severe PAH[1]. We previously published that pulmonary endothelial cells are the main pulmonary artery source of fractalkine in human PAH[15] and we proposed that

PAH dysfunctional pulmonary endothelial cells may be also characterized by elevated production of CX3CL1 which in turn may promote not only inflammatory cell recruitment but also PASMC growth. Monocrotaline-induced pulmonary hypertension is a useful model of pulmonary hypertension. However, one must bear in mind that this model certainly highlights some components of pulmonary hypertension pathogenesis, such as exaggerated pulmonary vascular inflammation. Indeed, the monocrotaline animal model of PH presents striking differences with human PAH. The development of PAH in humans usually takes years and the role of inflammatory processes is not clinically predominant in idiopathic PAH. By contrast monocrotaline exposure in rats induces an acute "toxic" pulmonary artery endothelial cell damage rapidly followed by a significant inflammatory reaction and subsequent obstruction of small pulmonary arteries by proliferating PASMC. These events lead to significant pulmonary hypertension 14 to 21 days after exposure[33]. Interestingly our present data show that fractalkine and its receptor were markedly overexpressed during the early inflammatory burst with a peak detected as early as 12 hours after monocrotaline exposure. However CX3CL1/CX3CR1 gene expression remained elevated during the whole course of the disease after exposure when PASMC proliferation is a key event. In spite of the obvious weaknesses of the monocrotaline model, it has been successfully used to validate important concepts which were later confirmed in human PAH (for instance major pharmaceutical agents widely used in PAH such as prostacyclin derivatives, phosphodiesterase type 5 inhibitors and endothelin receptor antagonists have been tested in this model and later in large placebo-controlled trials in human PAH). Moreover, several inflammatory conditions, such as connective tissue diseases, Hashimoto-thyroiditis and HIV infection can lead to PAH. Besides, auto-immunity is a common denominator in several forms of PAH.

Chemokine antagonists such as anti MCP-1 antibodies have been shown to successfully prevent monocrotaline-induced pulmonary hypertension in rats[34] and we propose that chemokine

antagonists may act at least in part as anti-remodeling agents. Whether this potential therapeutic approach aiming at reducing PASMC chemokine-induced growth will be successful in human PAH remains, however, to be demonstrated. Recent data indicate that imatinib is able to inhibit PDGF-induced PASMC proliferation and can prevent monocrotaline-induced pulmonary hypertension in rats[11]. Of note, preliminary case reports plead in favor of imatinib as an antiremodeling agent in severe human PAH[12, 13]. These preliminary data need to be precisely reproduced in the setting of well-designed placebo-controlled studies. Similarly treatment strategies based on chemokine antagonism also have to be tested properly.

CX3CR1 has two common coding polymorphisms, namely V249I and T280M, that are in strong linkage disequilibrium (almost always occurring on the same allele) and have been associated with inter individual differences in susceptibility to both HIV infection and atherosclerosis[35]. It has been reported a significant association between coronary vascular endothelial dysfunction in humans and a polymorphism in the CX3CR1 gene that affects receptor expression and ligand binding affinity. Most important is the strong association found between this polymorphism and both the extent and complications of coronary artery disease, independent of established risk factors, suggesting a link between reduced CX3CR1 expression/function and the prevalence and severity of atherosclerosis.

Raised expression of CX3CL1/CX3CR1 may be a mere consequence of the abnormal hemodynamic status in the setting of experimental pulmonary hypertension; however, the monocrotaline rat model clearly indicates that CX3CL1 overexpression occurred several days before the development of abnormal pulmonary hemodynamics, and that locally produced fractalkine may indeed act as a growth factor for CX3CR1 expressing PASMC. Our data strongly support the concept that inflammatory cell-derived fractalkine may interact with CX3CR1 at the cell surface of PASMC and subsequently promote cell proliferation. We thus conclude that the present data add fractalkine/CX3CL1 to the list of proliferative agents which

may actively contribute to PASMC proliferation in pulmonary hypertension. Further studies are needed to confirm and extend these findings, with the aim of identifying novel therapeutic pathways in PAH.

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LEGENDS FOR FIGURES

Figure 1: Fractalkine and fractalkine receptor expression in rat lungs

Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) expression was measured by means of real time PCR prior and after monocrotaline exposure. There was a marked overexpression of CX3CL1 and CX3CR1 after monocrotaline (MCT) exposure.

* : p<0.05 versus control

** : p<0.01 versus control

: p<0.0001 versus control

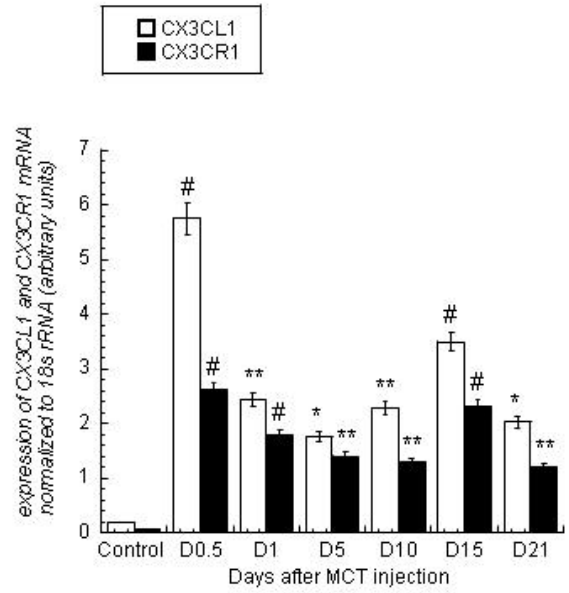

Figure 1

Figure 2: Microdissection of small and medium bronchus-associated pulmonary arteries (Panel A)

Analysis of microdissected arteries showed that CX3CL1 was not overexpressed, whilst there was a marked overexpression of CX3CR1 (Panel B).

- : $p<0.01$ versus control

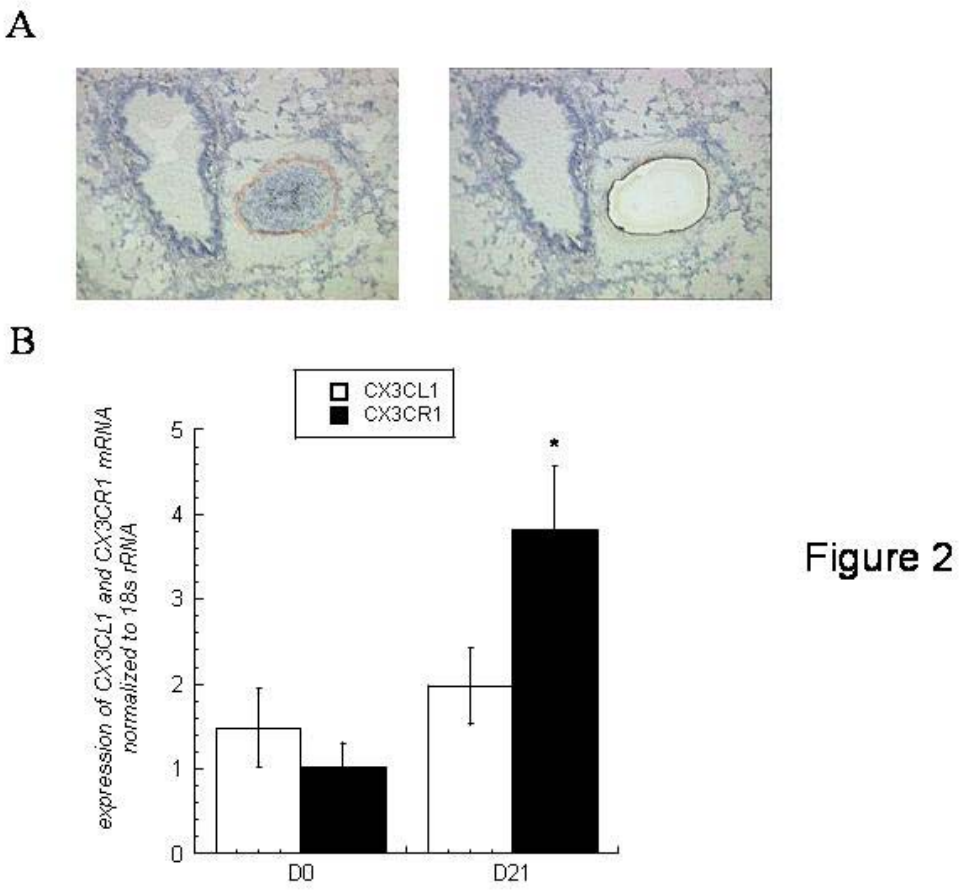

Figure 2

Figure 3. Immunhistochemical and western-blotting analysis of fractalkine and its receptor in pulmonary arteries from monocrotaline-exposed rats

Immunohistochemistry analysis of lungs from pulmonary hypertensive rats showed that CX3CL1 stained weakly PASMC and strongly perivascular inflammatory cells (A) whilst CX3CR1 positive cells were both PASMC and perivascular inflammatory cells (B). Original magnification x 400. Western-Blot analysis of PASMC confirmed a production of CX3CR1 with expected bands at 27 et 30 Kda (C).

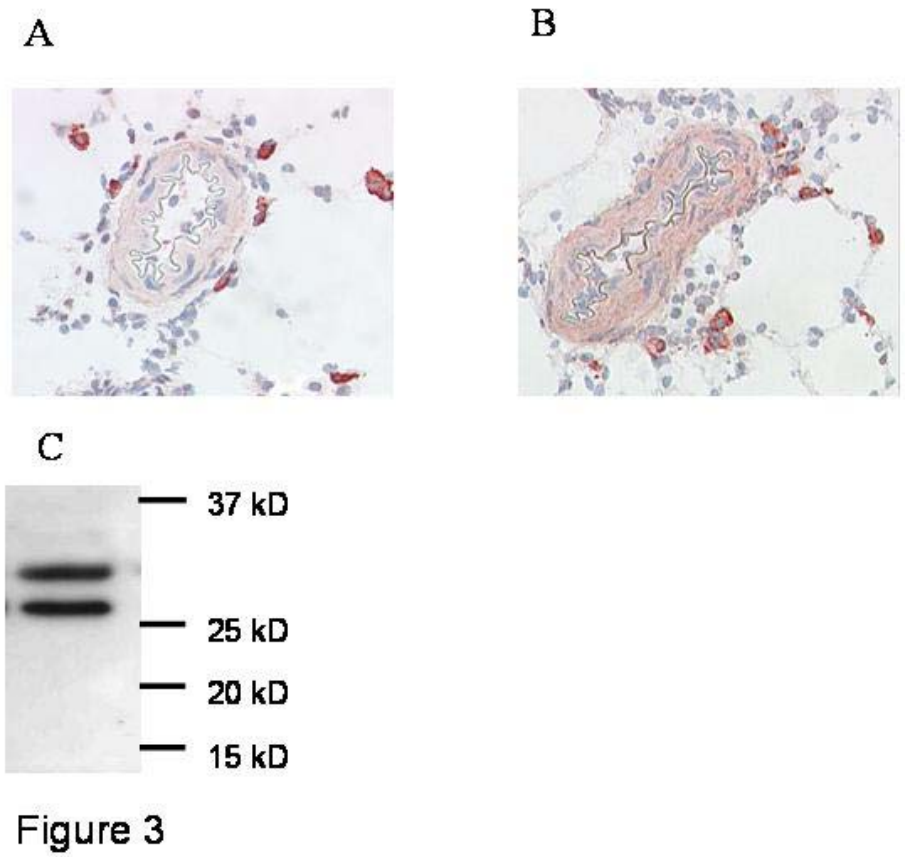

Figure 3

Figure 4. Fractalkine-induced proliferation of rat pulmonary artery smooth muscle cells

PASMC proliferation in response to fractalkine and PDGF-BB stimulation were measured by means of tritiated thymidine incorporation. We found that both fractalkine and PDGF BB could promote PASMC proliferation.

* : $p<0.05$ versus control

** : $p<0.01$ versus control

: $p<0.0001$ versus control

† : $p<0.05$ versus other conditions

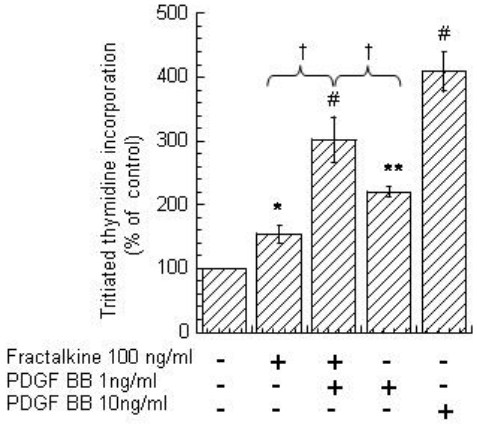

Figure 4

Figure 5. Lack of fractalkine-induced actin polymerization in rat pulmonary artery smooth muscle cells.

No actin polymerization could be demonstrated with fractalkine stimulation in PASMC, whereas a significant polymerization occurred in response to PDGF-BB stimulation.

* : $p<0.05$ versus control

** : $p<0.01$ versus control

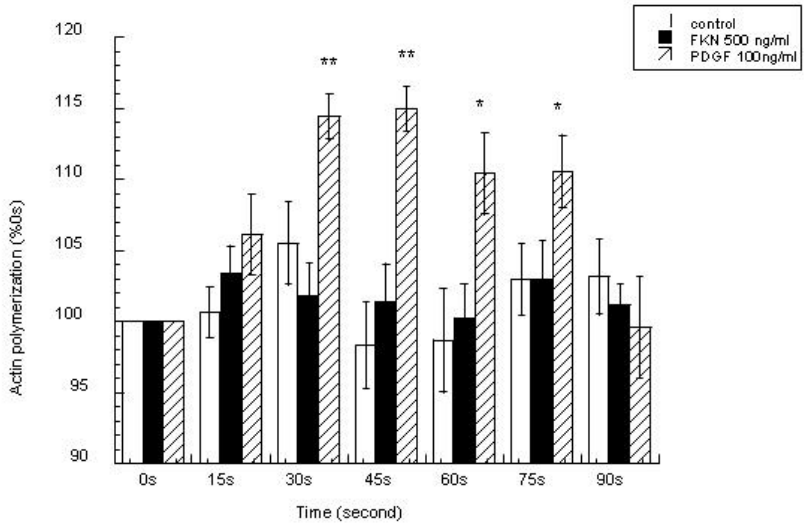

Figure 5

Figure 6. Lack of fractalkine-induced migration of rat pulmonary artery smooth muscle cells Transwell assay demonstrated that fractalkine could not promote PASMC migration whereas a dose response cell migration was demonstrated with PDGF-BB.

* : $p<0.01$ versus control

** : $p<0.0001$ versus control

: $p<0.0001$ versus PDGF BB 1ng/ml

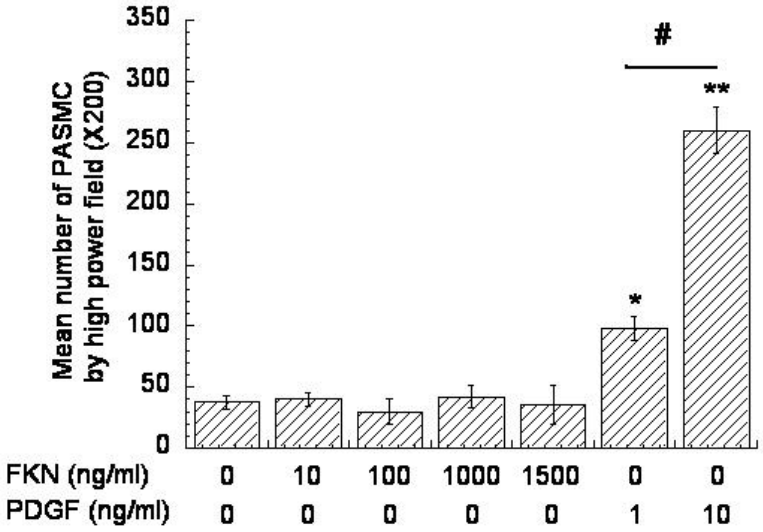
Mean number of PASMC by high power field (X200)
350
300
250
200
150
100
50
0
#
**
*
FKN (ng/ml) 0 10 100 1000 1500 0 0
PDGF (ng/ml) 0 0 0 0 0 1 10

Figure 6

REFERENCES

1. Humbert M, Sitbon O, and Simonneau G. Treatment of pulmonary arterial hypertension. *N Engl J Med* 2004;**351**:1425-36.
2. Pietra GG, Capron F, Stewart S, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. *J Am Coll Cardiol* 2004;**43**:25S-32S.
3. Humbert M, Morrell NW, Archer SL, et al. Cellular and molecular pathobiology of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004;**43**:13S-24S.
4. Barst RJ, McGoon M, Torbicki A, et al. Diagnosis and differential assessment of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004;**43**:40S-47S.
5. Tuder RM and Voelkel NF. Pulmonary hypertension and inflammation. *J Lab Clin Med* 1998;**132**:16-24.
6. Montani D, Achouh L, Marcelin AG, et al. Reversibility of pulmonary arterial hypertension in HIV/HHV8-associated Castleman's disease. *Eur Respir J* 2005;**26**:969-72.
7. Sanchez O, Sitbon O, Jais X, et al. Immunosuppressive therapy in connective tissue diseases associated pulmonary arterial hypertension. *Chest* 2006;in press.
8. Humbert M and Trembath RC. Genetics of pulmonary hypertension: from bench to bedside. *Eur Respir J* 2002;**20**:741-9.
9. Trembath RC, Thomson JR, Machado RD, et al. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med* 2001;**345**:325-34.
10. Humbert M, Monti G, Fartoukh M, et al. Platelet-derived growth factor expression in primary pulmonary hypertension: comparison of HIV seropositive and HIV seronegative patients. *Eur Respir J* 1998;**11**:554-9.

11. Schermuly RT, Dony E, Ghofrani HA, et al. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest* 2005;**115**:2811-21.

12. Souza R, Sitbon O, Parent F, et al. Long-term imatinib therapy in pulmonary arterial hypertension. *Thorax* 2006; **61**:736.

13. Ghofrani HA, Seeger W, and Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. *N Engl J Med* 2005;**353**:1412-3.

14. Humbert M, Monti G, Brenot F, et al. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. *Am J Respir Crit Care Med* 1995;**151**:1628-31.

15. Balabanian K, Foussat A, Dorfmuller P, et al. CX(3)C chemokine fractalkine in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2002;**165**:1419-25.

16. Dorfmuller P, Zarka V, Durand-Gasselin I, et al. Chemokine RANTES in severe pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2002;**165**:534-9.

17. Bazan JF, Bacon KB, Hardiman G, et al. A new class of membrane-bound chemokine with a CX3C motif. *Nature* 1997;**385**:640-4.

18. Harrison JK, Jiang Y, Chen S, et al. Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. *Proc Natl Acad Sci U S A* 1998;**95**:10896-901.

19. Harrison JK, Jiang Y, Wees EA, et al. Inflammatory agents regulate in vivo expression of fractalkine in endothelial cells of the rat heart. *J Leukoc Biol* 1999;**66**:937-44.

20. Imaizumi T, Yoshida H, and Satoh K. Regulation of CX3CL1/fractalkine expression in endothelial cells. *J Atheroscler Thromb* 2004;**11**:15-21.

21. Lucas AD, Chadwick N, Warren BF, et al. The transmembrane form of the CX3CL1 chemokine fractalkine is expressed predominantly by epithelial cells in vivo. *Am J Pathol* 2001;**158**:855-66.

22. Imai T, Hieshima K, Haskell C, et al. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 1997;**91**:521-30.

23. Umehara H, Bloom ET, Okazaki T, et al. Fractalkine in vascular biology: from basic research to clinical disease. *Arterioscler Thromb Vasc Biol* 2004;**24**:34-40.

24. Wong BW, Wong D, and McManus BM. Characterization of fractalkine (CX3CL1) and CX3CR1 in human coronary arteries with native atherosclerosis, diabetes mellitus, and transplant vascular disease. *Cardiovasc Pathol* 2002;**11**:332-8.

25. Lesnik P, Haskell CA, and Charo IF. Decreased atherosclerosis in CX3CR1-/- mice reveals a role for fractalkine in atherogenesis. *J Clin Invest* 2003;**111**:333-40.

26. Chandrasekar B, Mummidi S, Perla RP, et al. Fractalkine (CX3CL1) stimulated by nuclear factor kappaB (NF-kappaB)-dependent inflammatory signals induces aortic smooth muscle cell proliferation through an autocrine pathway. *Biochem J* 2003;**373**:547-58.

27. Stinger RB, Iacopino VJ, Alter I, et al. Catheterization of the pulmonary artery in the closed-chest rat. *J Appl Physiol* 1981;**51**:1047-50.

28. Eddahibi S, Fabre V, Boni C, et al. Induction of serotonin transporter by hypoxia in pulmonary vascular smooth muscle cells. Relationship with the mitogenic action of serotonin. *Circ Res* 1999;**84**:329-36.

29. Garin A, Tarantino N, Faure S, et al. Two novel fully functional isoforms of CX3CR1 are potent HIV coreceptors. *J Immunol* 2003;**171**:5305-12.

30. Eddahibi S, Humbert M, Fadel E, et al. Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. *J Clin Invest* 2001;**108**:1141-50.

31. Foussat A, Bouchet-Delbos L, Berrebi D, et al. Deregulation of the expression of the fractalkine/fractalkine receptor complex in HIV-1-infected patients. *Blood* 2001;**98**:1678-86.

32. Lucas AD, Bursill C, Guzik TJ, et al. Smooth muscle cells in human atherosclerotic plaques express the fractalkine receptor CX3CR1 and undergo chemotaxis to the CX3C chemokine fractalkine (CX3CL1). *Circulation* 2003;**108**:2498-504.

33. Meyrick B, Gamble W, and Reid L. Development of Crotalaria pulmonary hypertension: hemodynamic and structural study. *Am J Physiol* 1980;**239**:H692-702.

34. Kimura H, Kasahara Y, Kurosu K, et al. Alleviation of monocrotaline-induced pulmonary hypertension by antibodies to monocyte chemotactic and activating factor/monocyte chemoattractant protein-1. *Lab Invest* 1998;**78**:571-81.

35. Moatti D, Faure S, Fumeron F, et al. Polymorphism in the fractalkine receptor CX3CR1 as a genetic risk factor for coronary artery disease. *Blood* 2001;**97**:1925-8.