EDITORIAL
BASIC SCIENCE

Putting the spotlight on macrophage-derived cathepsin in the pathophysiology of obliterative bronchiolitis

Olivier Brugiere [1,2] and Stijn E. Verleden[3]

Affiliations: [1]Lung Transplant Dept, Foch Hospital, Suresnes, France. [2]Inserm UMR S 1152, Physiopathologie et Epidémiologie des Maladies Respiratoires, Paris, France. [3]BREATHE, Dept of CHROMETA, KU Leuven, Leuven, Belgium.

Correspondence: Olivier Brugière, Service de Transplantation Pulmonaire, Hôpital Foch, 40 rue Worth, 92150 Suresnes, France. E-mail: o.brugiere@hopital-foch.com

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New findings for the puzzle of bronchiolitis obliterans syndrome (BOS) are pointing to the role of CatB-procollagen-TGF-β signalling pathways, linked to fibrosis mechanisms and tissue damage control https://bit.ly/3qQPax9

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While the experience of lung transplantation (LTx) is growing worldwide, long-term outcomes are not improving accordingly. Next to oncological and infectious complications, chronic rejection, clinically defined as chronic lung allograft dysfunction (CLAD), remains the major bottleneck to improving long-term outcomes [1, 2]. Increased recognition of clinical phenotypes of CLAD assists in predicting patient prognosis; however, mechanistically, we are still far from unravelling the pathophysiological processes underlying CLAD. Indeed, the internationally endorsed recognition of an obstructive (bronchiolitis obliterans syndrome; BOS) and restrictive (restrictive allograft syndrome; RAS) phenotype of CLAD leads us to critically review historical mechanistic studies, as these are not focused on separate phenotypes [1, 3]. Whether both phenotypes share common pathophysiological mechanisms remains unknown; however, obliterative bronchiolitis (OB), pathological scarring of the small airways, is found in both phenotypes in varying degrees and is therefore a major target for further research because adequate therapy is lacking [4]. One of the major reasons for this poor knowledge of the mechanism of CLAD has been the lack of an adequate animal model of CLAD. Indeed, animal models are key for our further understanding of pathophysiological mechanisms. Although the initially proposed heterotopic trachea transplant model has its merits, the scientific community was especially intrigued by the murine orthotopic left LTx, as this was regarded as the ultimate model of CLAD. Key pathological findings include peribronchial inflammation, peribronchial thickening, vascular rejection and alveolar fibrosis.

The high prevalence of BOS in patients with allogenic mismatch of the lung and haematopoietic cells (*i.e.* lung and bone marrow transplant recipients) [5], indicates a crucial role for alloimmune injuries in the development of OB. Both alloimmune T- and B-cell reactivity have been demonstrated as the main triggers of the pathological cascades inducing airflow limitation observed in BOS [6–8], associated with autoimmunity after exposition of sequestered self-antigens resulting from immune or nonimmune tissue insults [9]. Given this pivotal role of allo- and autoimmunity in BOS development, most therapies developed in human BOS post-LTx to date have focused on anti-T- and anti-B-cell therapies, or therapy directed against the complement cascade resulting from antibody-mediated rejection [10–12].

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More recently, attention has been paid to mechanisms of tissue damage control involved in OB, linked to the dysregulated fibrotic repair observed after graft injuries (*i.e.* colonisation or infection with micro-organisms [12]), and presumed to drive mesenchymal cell infiltration and collagen deposition. This dysregulated fibrotic repair is well-evidenced by histological features of BO, with architectural remodelling and scarring of the airways [13], involving increased accumulation of myofibroblasts [14], increased extracellular matrix (ECM) synthesis [15, 16], epithelial–mesenchymal transition (EMT) [16–18], and increased protein levels of platelet derived growth factor, vascular endothelial growth factor, fibroblast growth factor and insulin-like growth factor [16, 19–22].

One molecule that has always drawn interest in the fibrotic cascade is transforming growth factor-β (TGF-β) [8, 16]. Its profibrotic role and importance in idiopathic pulmonary fibrosis (IPF) has also triggered interest in the LTx community. While a recent study has implicated TGF-β specifically in RAS as an inducer of mesothelial-to-mesenchymal transition [8], its role in BOS was previously suspected where positive TGF-β staining on transbronchial biopsies preceded the histological confirmation of OB by 6–18 months [23]. Linked to the TGF-β profibrotic pathway, cathepsine B (CatB), a cysteine protease negatively regulated by its endogenous inhibitor cystatin-C (CystC), was also previously demonstrated to implement TGF-β1 signalling and its level is significantly increased during lung [24–26] and liver fibrosis [27, 28].

In this issue of the *European Respiratory Journal*, Morrone *et al.* [29] investigates the CatB–procollagen–TGF-β axis in the field of BOS post-LTx. The authors used serial bronchoalveolar lavage (BAL) samples, explant tissues from human LTx recipients, a murine orthotopic LTx model and *in vitro* experiments with cell lines. They found a BOS stage-dependent increase in CatB and pro-collagen 1 levels in BAL in LTx-recipients, while the number of CystC-positive cells significantly decreased in explanted lung from end-stage BOS patients as compared to controls, indicating that CatB activity is crucial for collagen expression. This observation was further confirmed in the murine orthotopic LTx model, in which CatB and collagens were already upregulated 14 days after transplantation and where orthotopic transplantation in CatB-/- mice significantly reduced histopathological and physiological features of graft rejection. A suspected mode of action is shown in figure 1.

To date, the importance of cathepsins and CystC in organ fibrosis has been somewhat controversial. Because cathepsins can digest ECM proteins, they are expected to reduce the fibrotic burden. However, the fibrosis-promoting effects of CatB have been demonstrated in lung [24–26] and liver fibrosis [27, 28] by facilitating TGF-β-driven differentiation of fibroblasts [26, 30]. Accordingly, given that it potently inhibits cysteine proteases, such as cathepsins, CystC is expected to stimulate fibrosis by inhibiting the

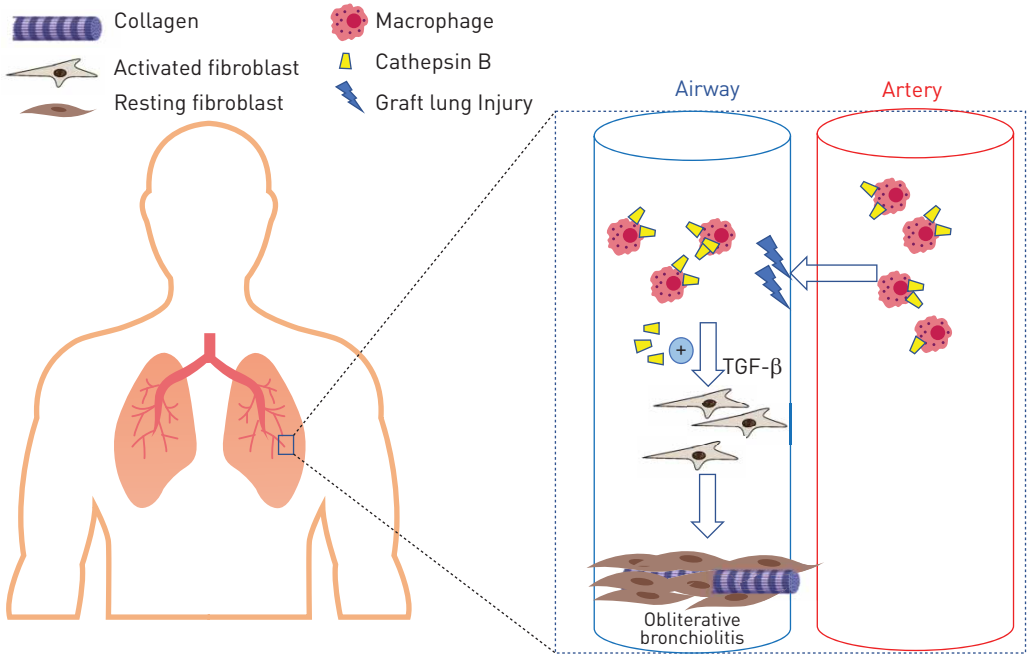

FIGURE 1 Proposed mechanism of action as investigated by Morrone *et al.* [29], where a series of insults lead to cathepsin-loaded macrophages infiltrating the airways. These recipient-derived macrophages will interact to release transforming growth factor (TGF)-β, attracting active (myo-)fibroblasts from different sources, which will produce collagen 1 and eventually obliterate the complete airway lumen.

protease-mediated digestion of ECM [27, 28]. In contrast, and in accordance with findings of Morrone *et al.* [29] in this issue, CysC expression was found markedly reduced in mouse and human lungs with interstitial fibrosis [31], and was suggested as a potential therapy in lung fibrosis.

Additionally, Morrone *et al.* [29] found CatB most often in macrophages, again putting the macrophage at the centre stage of BOS development and progression. Further *in vitro* experiments specifically indicated the M1 macrophages to be involved in the cathepsin–TGF-β axis, resulting in the activation of fibroblasts, in line with previous *in vitro* evidence that tumour necrosis factor-α from activated macrophages accentuates EMT [17]. Indeed, while the macrophage is often neglected as a key player in the pathophysiology of BOS, other recent evidence indicates that the recipient-derived mononuclear phagocyte system gives rise to the majority of myofibroblasts found in occluded airways of OB [32]. The origin of these myofibroblasts in OB remains highly controversial [33, 34], and previous studies suggested that these can arise from the donor [33, 35–37], for example *via* EMT [17, 18]. However, an increasing body of evidence has now demonstrated their predominant recipient origin [32, 38, 39], as confirmed by the finding of an increased proportion of myofibroblasts expressing the macrophage marker CD68 in BOS explants, as compared to controls [32]. In the most likely scenario, the combination of recipient- and donor-derived factors account for the onset and progression of BOS [40].

One particular interesting finding from the Morrone *et al.* [29] study also included that the clear association between decreased levels of CystC and increased CatB activity was specifically valid in patients with a history of pulmonary fibrosis. This was further validated in different existing single-cell RNA-sequencing datasets, which demonstrated that CatB expression in macrophages was increased in lung fibrosis patients, indicative of a higher bio-availability of CatB in pulmonary fibrosis patients. This finding might be specifically relevant given that the outcome post-LTx in pulmonary fibrosis patients is inferior to that for other common indications for LTx [41]. The reason remains unknown but higher bio-availability of CatB could be important to answer this question, especially since this might be therapeutically modulated. This finding, if validated, could be also be relevant for future biomarker research within the pulmonary fibrosis field as well.

Other important areas of further validation include the importance of the CatB–procollagen–TGF-β axis in RAS. In BOS, only the airways are affected by fibrosis formation, whereas in RAS, the fibrotic response is not only limited to the airways; the mesothelium and alveoli are also involved. Therefore, CatB could be of even more importance in RAS, especially because TGF-β concentrations in BAL and tissue are high in RAS and are associated with outcome for these patients [8]. Additionally, the murine orthotopic LTx model is thought to show features that are more reminiscent of RAS, with increased collagen accumulation, septal inflammation and fibrosis, decreased elastance, and increased compliance [42].

Hence, most current therapies in BO are directed towards its known immune and non-immune triggers, but the Morrone *et al.* [29] study adds new findings for the puzzle of BOS/OB development and potential target therapies, pointing to the CatB signalling pathways linked to fibrosis mechanisms and tissue damage control. Anti-fibrotic therapies approved in slowing down IPF and non-IPF interstitial lung diseases [43–45] are already under evaluation in BOS post-LTx in placebo-controlled trials (pirfenidone in the EPOS study: ClinicalTrials.gov identifier NCT02262299; nintedanib in the infinitixBOS study: ClinicalTrials.gov identifier NCT03283007). However, specific CatB-triggered therapy in BOS patients may open new avenues in the near future. Morrone *et al.* [29] bring a strong basis to further investigate this specific pathway in LTx recipients affected by BOS and eventually RAS, and a glimpse of hope to turn the balance in the everlasting battle with CLAD post-LTx.

Conflict of interest: None declared.

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