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Small airway obstruction in severe pulmonary arterial hypertension correlates with increased airway CD8+ T-cells and fractalkine expression

To the Editors:

Pulmonary arterial hypertension (PAH) is primarily a disease of lung small arteries. However, several studies have documented mild-to-moderate peripheral airflow obstruction in most adults [1–3] and children [4] with PAH, which could be significantly reversed with β_2 -agonist administration [5]. Although peribronchial infiltrates have been scarcely reported in PAH [1], it is still unclear whether airway dysfunction occurs as a result of lung vascular disease or from a direct impairment of the airways.

In lung specimens from six patients with severe group I PAH [6] undergoing lung transplantation (and who did not display signs of cardiopulmonary decompensation at that time), we assessed airway inflammatory cell infiltration and PAH-related chemokine expression in comparison with specimens from nine nonsmoking controls (sampled as far as possible from the tumour) and correlated findings to lung function data. Patient's characteristics and lung function tests are summarised in table 1.

No impairment of routine lung function tests was observed in PAH patients, except a trend for lower forced expiratory volume in 1 s and increased residual volume. However, among indices of small airway function, corrected mean maximal expiratory flow (MEF50) [2] was significantly decreased in severe PAH patients compared with controls (table 1; $p<0.05$). Highly significant increases in CD3+ T-cells were observed within the epithelium of large and small airways of PAH patients ($p<0.01$), as well as in submucosal glands and in lamina of bronchioles. CD4/CD8 immunohistochemistry indicated that these T lymphocytes consisted mainly of CD8+ T-cells (fig. 1). In contrast, no significant changes were observed for neutrophils and macrophages, except a modest increase of macrophages in small airways ($p=0.03$). In addition, intraepithelial infiltration of CD8+ T-cells correlated with corrected MEF50 ($r_s = -0.81$, $p=0.05$) in small airways; a functional parameter of small airway obstruction in these patients (fig. 2). Moreover, immunoreactive cells for fractalkine were observed in small pulmonary arteries of lung sections from PAH patients, as well as in large ($p=0.003$) and small airways ($p=0.002$) that consisted of

structural cells such as epithelial cells and subepithelial inflammatory cells, in contrast to RANTES which did not significantly differ between groups (fig. 3).

In three out of 10 PAH patients for whom lung specimens were available, FERNANDEZ-BONETTI and LUPI [1] described the existence of airway narrowing, bronchial wall thickening and mononuclear infiltrates dominated by lymphocytes with some plasma cells, as well as a few granulocytes. Accordingly, we showed that this inflammatory infiltrate mainly consists of CD3+ T-cells and, to a marginal extent, macrophages, and that large airways are also consistently infiltrated by T-cells. Although we can not formally exclude a difference in sensitivity of CD8 *versus* CD4 tissue immunodetection, T-cell infiltrates seem to predominantly consist of CD8+ lymphocytes. It is tempting to speculate that fractalkine, which

TABLE 1 Patients' characteristics with hemodynamic and lung function tests

	PAH	Controls
Subjects n	6	9
Age yrs	45±14	62±7
Male:female n	4:2	4:5
$\bar{P}_{pa}$ mmHg	73±9	ND
FEV₁ % pred	81±10	107±3
FEV₁/VC %	70±6	76±5
TLC % pred	98±5	111±4
RV % pred	136±31	80±7
MEF₅₀ % pred	82	86
Corr MEF₅₀ s⁻¹#	1.5±0.5*	1.9±0.2
DL_{CO} % pred	80±24	86±10

Data are presented as mean±SD, unless otherwise stated. PAH: pulmonary arterial hypertension; $\bar{P}_{pa}$: mean pulmonary artery pressure; FEV₁: forced expiratory volume in 1 s; % pred: % predicted; VC: vital capacity; TLC: total lung capacity; RV: residual volume; MEF₅₀: mean maximal expiratory flow; DL_{CO}: diffusing capacity of the lung for carbon monoxide; ND: not determined. #: MEF₅₀ corrected for remaining VC fraction, corr MEF₅₀=MEF₅₀ × (0.50 × VC)⁻¹. *: $p<0.05$.

FIGURE 1. Photomicrographs of lung sections from a pulmonary arterial hypertension (PAH) patient immunostained for a) CD3, b) CD4 and c) CD8. d) CD3 expression in a control patient. Scale bars=160 μ m. Arrows indicate examples of stained T-cells. e) CD3+ T-cells in airway tissue from severe PAH patients versus controls (C). Mean optical density of CD3 staining is shown in the different microcompartments of large (□) and small (■) airways, in PAH versus control patients. Horizontal bars indicate medians. Intergroup comparison performed by the Mann-Whitney U-test. AU: arbitrary units; Epith.: epithelium. #: $p=0.002$; *: $p=0.004$; +: $p=0.009$; §: $p=0.03$.

FIGURE 2. CD8+ T-cells plotted against corrected mean maximal expiratory flow (MEF₅₀) in severe pulmonary arterial hypertension patients, and assessed by the Spearman's correlation test. MOD: mean optical density; AU: arbitrary units. $r_s = -0.81$; $p = 0.05$.

FIGURE 3. Photomicrographs from a, b) pulmonary arterial hypertension (PAH) versus c, d) control patients immunostained for fractalkine (a, c) or RANTES (b, d). The arrows indicate positive cells. Scale bars=80 μ m. e) Fractalkine and f) RANTES immunodetection in airway tissue from severe PAH versus control (C) patients. Intergroup comparison was performed by the Mann-Whitney U-test. ○: large airways; ●: small airways. #: $p=0.003$; *: $p=0.002$; NS: nonsignificant.

appears to be increased within epithelial and subepithelial areas of the airways in our severe PAH patients, could trigger smooth muscle hypertrophy [7] around the airways that could account for peripheral airway obstruction and hyperresponsiveness. Airway infiltration by CD8+ T-cells has been observed in chronic obstructive pulmonary disease (COPD)

[8], as well as in severe asthma [9] and has been related to lung function decline.

The mechanisms of airway inflammation in PAH are unknown. T-cell influx to the airways could potentially result in PAH from a spill over of chemokines, such as fractalkine or RANTES [10], in surrounding peripheral airways. However, this seems unlikely, notably because T-cells infiltrate both large and small airways. An alternative explanation is airway epithelial damage (by viruses, toxins, autoimmunity or drugs), which could occur concomitantly to the endothelial injury that primarily underlies PAH. However, we did not observe airway epithelial changes in secretory component or Clara cell protein content in these PAH patients, used as controls in our previous study of COPD patients [11].

Our data suggest that CD8+ T-cell infiltration of the airways is not only observed in typical obstructive airway diseases, such as COPD and severe asthma, but also in severe PAH. The clinical relevance of airway inflammation in PAH could include asthma-like symptoms, while PAH masquerading asthma has also been related to dilation of proximal bronchial vessels [12]. Future studies should confirm these findings in a larger series of PAH patients of varying phenotype and severity, as well as explore the molecular determinants and clinical relevance of CD8-mediated inflammatory responses in the airways.

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