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Lymphangiogenesis in COPD: Another link in the pathogenesis of the disease

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Summary
Background: New lymphatic vessels are associated with tissue injury and repair. Recent studies have shown increased lymphatic follicles formation in the lungs of COPD patients. We hypothesized that lymphatic vascular remodeling could be part of COPD pathogenesis.
Aim: To investigate the lymphangiogenetic process in COPD we measured the lymphatic microvessel density (LMVD), the lymphatic invasion (L.I), and their correlation with clinical and laboratory parameters.
Methods: Lung tissue from 20 COPD patients and 20 non-COPD smokers was immunohistochemically stained for D2-40 (lymphatic endothelial cell marker), and LYVE-1 (lymphatic endothelial hyaluronan receptor 1). Both groups had similar age and smoking history.
Results: D2-40 and LYVE-1 were expressed in all specimens. Lymphatic invasion was presented only in COPD specimens. Lymphatic microvessel density (LMVD) as revealed by D2-40 and LYVE-1 markers was statistically significantly higher in COPD patients when compared with non-COPD smokers. Both markers (D2-40, LYVE-1) were correlated with FEV1 (% pred) ($R^2 = 0.415$, $R^2 = 0.605$, respectively).
Conclusions: We report for the first time high lymphatic microvessel density and lymphatic invasion in COPD patients, related to the degree of airway obstruction. Our findings could provide novel insights in the pathogenesis of the disease.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a major public health and economical problem worldwide.[1] It affects about 10% of the general population but its prevalence among smokers may reach as much as 50%.[1,2] Smoking, occupational exposures, environmental pollution as well as certain genetic variations are some of the risk factors for the disease.[1–3] COPD is associated with epithelial mucous metaplasia, inflammation, parenchymal destruction and extensive lung remodeling in a way that is difficult to maintain normal lung function.[1,2,4]

In a recent study Hogg *et al*[2] demonstrated that the disease progression correlates with the accumulation of mucous exudates within the lumen and infiltration of the walls by inflammatory cells that form lymphoid follicles. These changes participate in the process of "remodeling" that increases the thickness of the airways' walls. The lymphoid structures are generally the driver point of chronic inflammation.[2] Inflammatory cells and various molecules (*e.g.* IL-1β, TNF-α, VEGF-C) have been recognized to play important roles in lymphangiogenesis (formation of lymphatic vessels) in experimental models[5] and human diseases.[6–11] In addition, soluble molecules, such as hyaluronic acid, involved in lung injury and repair can also induce lymphangiogenesis.[12–14]

Recently, El-Chemaly *et al*[6] in order to test the hypothesis whether lymphangiogenesis is part of the "remodeling" in idiopathic pulmonary fibrosis (IPF) demonstrated that the alveolar microenvironment in IPF contains soluble factors (*e.g.* hyaluronic acid) and cells (*e.g.* CD11b + macrophages) that enhance lymphangiogenesis. Although inflammatory cells, such as macrophages, and lung mediators such as hyaluronic acid are common features in COPD,[2,4,5,8,9] no study thus far, investigated lymphangiogenesis in COPD.

At present, the identification of lymphatic vessels in normal tissue and in vascular malformations has been considerably improved by the newly discovered lymphatic endothelial markers, D2-40 and LYVE-1.[13,14] We have chosen the LYVE-1 (lymphatic vessel endothelial hyaluronan receptor 1) and the D2-40 (a marker of lymphatic endothelial cells) in order to evaluate the lymphatic microvessel density (LMVD) and the presence of lymphatic invasion (L.I) in COPD. LYVE-1 binds to hyaluronan on the luminal surface of lymphatic vessels, while D2-40 specifically reacts with lymphatic vessels, allowing identification of lymphatic vessel invasion. Our findings demonstrated an intense lymphangiogenetic process in COPD, significantly correlated with the degree of airway obstruction.

Methods

We have studied 40 smokers who underwent open lung surgery for the excision of solitary pulmonary nodule. Twenty subjects were diagnosed with COPD and 20 were smokers without COPD. Table 1 presents the anthropometric and spirometric data of the patients studied. The surgical specimens were collected from the subpleural parenchyma at least 5 cm away from the solitary nodule, from an uninvolved segment. Formalin-fixed paraffin-embedded blocks of lung tissue were selected from each case on the basis of containing viable COPD tissue and surrounding normal lung parenchyma. The COPD viable tissue was comprised of either enlarged air spaces (emphysema) or infiltration by mononuclear inflammatory cells, with scattered lumen occlusion by mucus plugging, thickened walls by inflammation and fibrosis and prominence of smooth muscles (chronic bronchitis). Areas with necrosis were avoided and wedges were removed in case of over-staining. The former pathology was absent from normal controls. None of the specimens contained neoplastic tissue.

Table 1 Anthropometric and spirometric characteristics of non-COPD and COPD subjects.

	Non COPD median (IR)	COPD median (IR)	*p* value
No of subjects	20	20	
Age (years)	61 (16)	67 (9)	0.09
Sex (M/F)	18/2	19/1	
Smoking (packs/year)	50 (23)	65 (49)	0.17
FEV1 (%pred)	93 (29)	53 (22)	<0.001[a]
FVC (%pred)	93 (22)	68 (24)	<0.001[a]
FEV1/FVC	79 (10)	65 (7)	<0.001[a]

[a] Statistically significant *p* value.

Medical records (sex, age, spirometry values, and disease stage) were thoroughly reviewed. Diagnosis and staging of COPD patients were based upon GOLD classification (Global Initiative for Chronic Obstructive Lung Disease).[2] All the procedures followed were in accordance with international recommendations concerning human research and ethical standards and were approved by the Ethics Committee of the University Hospital of Heraklion, Crete, Greece. Informed consent was obtained from all patients participating in the study.

LYVE-1 protocol

Immunostaining of the formalin-fixed, paraffin embedded tissue sections was performed (EnVision System, DAKO). Consecutive 4 μm thick sections were sliced from each tissue block and used for the immunohistochemical study. Briefly, sections were deparaffinized and dehydrated to distilled water. Microwave treatment was performed twice in citrate buffer (pH = 6), at 300 W, for 15 min. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide in absolute methanol for 7 min in room temperature. Then, the slides were washed with distilled water, and Tris Buffer Saline (TBS) (DAKO). The LYVE-1 (Lymphatic Vessel Endothelial Receptor 1, R&D Systems), primary antibody was used, with an incubation time of 60 min at room temperature. The optimal dilution (1/50) was defined on the basis of a titration experiment. Samples were washed for 5 min. The incubation of the secondary monoclonal antibody against LYVE-1 followed, for 15 min. Slides were washed with TBS for 5 min, incubated with peroxidase streptavidin for 15 min, and then washed again for 5 min.