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Title: A longitudinal characterization of lymphangiogenesis in bleomycin-induced pulmonary fibrosis mouse model

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Body: [Background] The roles of lymphangiogenesis in the pathogenesis of pulmonary fibrosis has been remained to be elucidated, although it has been associated with this condition in human. [Objective] To clarify longitudinal characteristics of lymphangiogenesis in bleomycin-induced pulmonary fibrosis mouse model. [Animal and Methods] Pulmonary fibrosis was induced in C57BL/6J female mice by intratracheal injection of 3.0 U/kg of bleomycin. Three mice were sacrificed in 6 individual days, including before the injection and at 7, 14, 21, 28 and 35 days after. Formalin fixed and paraffin embedded lung tissues were used for immunohistopathological and morphometric analyses. Antibodies specific for Vegfr-3, Cd31 and type I collagen were used to detect lymphatics, blood vessels and collagen, respectively. [Results] The dilatation of the existing lymphatics around bronchus and large blood vessels was observed on Day 7, and numerous lymphocytes were organized around them. The lymphatics were newly formed in fibrotic lesions on Day 14, although capillaries were barely detected in the lesions. In Masson Trichrom staining, connective tissue was most prominent on Day 21, and looser after that. The area densities of interstitium and hydroxyproline concentration in lung homogenates were maximally increased on Day 21. The existing and the newly formed lymphatic densities were significantly increased on Day 21, respectively ($p < 0.05$). [Conclusion] In the early stage, the existing lymphatics may play a role in the organization of lymphoid structure, which possibly facilitate the fibrogenesis. In the later stage, the newly formed lymphatics may be associated with the tissue resolution.