ELSEVIER

Early Administration of FTY720 Prevents Chronic Airway as Well as Vascular Destruction in Experimental Rat Lung Transplantation

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ABSTRACT

Background. Chronic rejection (CR) in terms of bronchiolitis obliterans (BO) and vascular sclerosis (VS) still represents the major obstacle for pulmonary graft survival in the medium and long term course after lung transplantation (LTX). Aside from nonspecific stimuli, early acute rejection (AR) seems to be causative especially in cases of a late diagnosis or inadequate treatment. This study investigated the effects of FTY720, a new immunosuppressant that promotes lymphocyte sequestration into lymph nodes and Peyer's patches, on the development of CR after experimental LTX.

Methods. A total of 50 rats underwent allogenic (F344-to-WKY) and syngenic (WKY-to-WKY) left LTX. Group 1 animals had no treatment. Group 2 animals were administered FTY720 (3 mg/kg body weight per day) at the maximum time of AR (day 14) and continued up to day 100 after LTX. Group 3 animals were treated with the same dosage of FTY720 from day 0 to 100. The grades of AR and CR were classified according to the criteria of the International Society for Heart and Lung Transplantation.

Results. Within 14 days after allogenic LTX, all nontreated rats developed early AR followed by severe CR with VS and BO. Similar data were observed for FTY720 treatment of existing AR (group 2). Only early administration of FTY720 (at the time of LTX) significantly reduced the proportion of animals with severe acute vascular rejection ($P < .001$). However, all of these allografts showed high-grade acute airway inflammation. After long-term application, the chronic inflammatory response was absent; none of the allografts developed BO and VS.

Conclusion. Only application of FTY720 immediately after LTX prevented lymphocyte recirculation and lung injury.

LONG-TERM SUCCESS after lung transplantation (LTX) is limited by chronic rejection (CR), in terms of vascular sclerosis (VS) and bronchiolitis obliterans (BO), which are characterized by progressive inflammatory/fibrotic processes that affect the small vessels and airways respectively.[1] Various animal models have been utilized to analyze the mechanisms of VS and BO. FTY720, a sphingosine-1-phosphate receptor agonist, has shown beneficial effects to preserve the respiratory epithelium in allografted tracheae.[2] However, the heterotopic tracheal transplantation model has major drawbacks compared with clinical reality. A relevant orthotopic rat LTX model (F344-to-WKY)[3] was used herein to evaluate the efficacy of FTY720 to treat CR.

MATERIALS AND METHODS

Inbred male Fischer F344 (RT1lvl) and Wistar Kyoto WKY (RT1l) rats (Charles River, Sulzfeld, Germany) with a mean body weight of 295 ± 62 g were used as donors and recipients, respectively, for allogeneic left LTX.[3,4] Procedures were approved by the local authorities according to regional laws. FTY720 (Selleck Chemicals, Houston, Tex, USA) dissolved in distilled water was administered daily (3 mg/kg weight, intraperitoneally) without additional immunosuppression. Rats were treated as group 1 ($n = 14$), no treatment; group 2 ($n = 10$), FTY720 from postoperative day (POD) 14 to 100 and group 3 ($n = 11$), FTY720 from POD 0 to 100. In each group, five syngeneic

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Table 1. Histological Classification of Acute Vascular (ISHLT-A) and Airway (ISHLT-B) Rejection After Rat Lung Transplantation (According to Stewart et al 2007[1]) (POD 20)

Group #	ISHLT-A	*P* Value	ISHLT-B	*P* Value
1	9x A4		9x B2R	
2	A3.5; 4x A4	n.s.	5x B2R	n.s.
3	A 2.5; 2.5; 3; 3; 3; 3.5	*	B 1R; 1R-2R; 2R; 2R; 2R; 2R	n.s.

ISHLT-grade, classification of acute vascular (ISHLT-A) and airway rejection (ISHLT-B) according to the actual working formulation of the International Society of Heart and Lung Transplantation (ISHLT); POD, postoperative day. Statistics included the difference in the proportion of animals with high-grade rejecting allografts (ISHLT-A 3.5-4.0; ISHLT-B2R) (vs group 1). n.s., non-significant.
*$P < .05$.

transplants were sacrificed at POD 100. Rat lungs were harvested on POD 20 (early phase) or 100 (late phase).[3] Lung sections were stained with hematoxylin-eosin and Masson Goldner Trichrome to grade acute rejection (AR) and CR according to the classification of the International Society for Heart and Lung Transplantation (ISHLT-A 0–4: degree of acute vascular rejection; ISHLT-B 0, 1R, 2R, X: degree of acute airway inflammation; ISHLT-C: 0 (absence) or 1 (presence) of chronic airway rejection (BO); ISHLT-D: 0 (absence) or 1 (presence) of chronic vascular rejection).[1] The definition of BO included both histological variants "constructive bronchiolitis" and "organizing pneumonia."[5]

Histological scoring was performed three times in blinded fashion. We tested the hypothesis of a difference among populations as the proportion of animals with high-grade rejected allografts

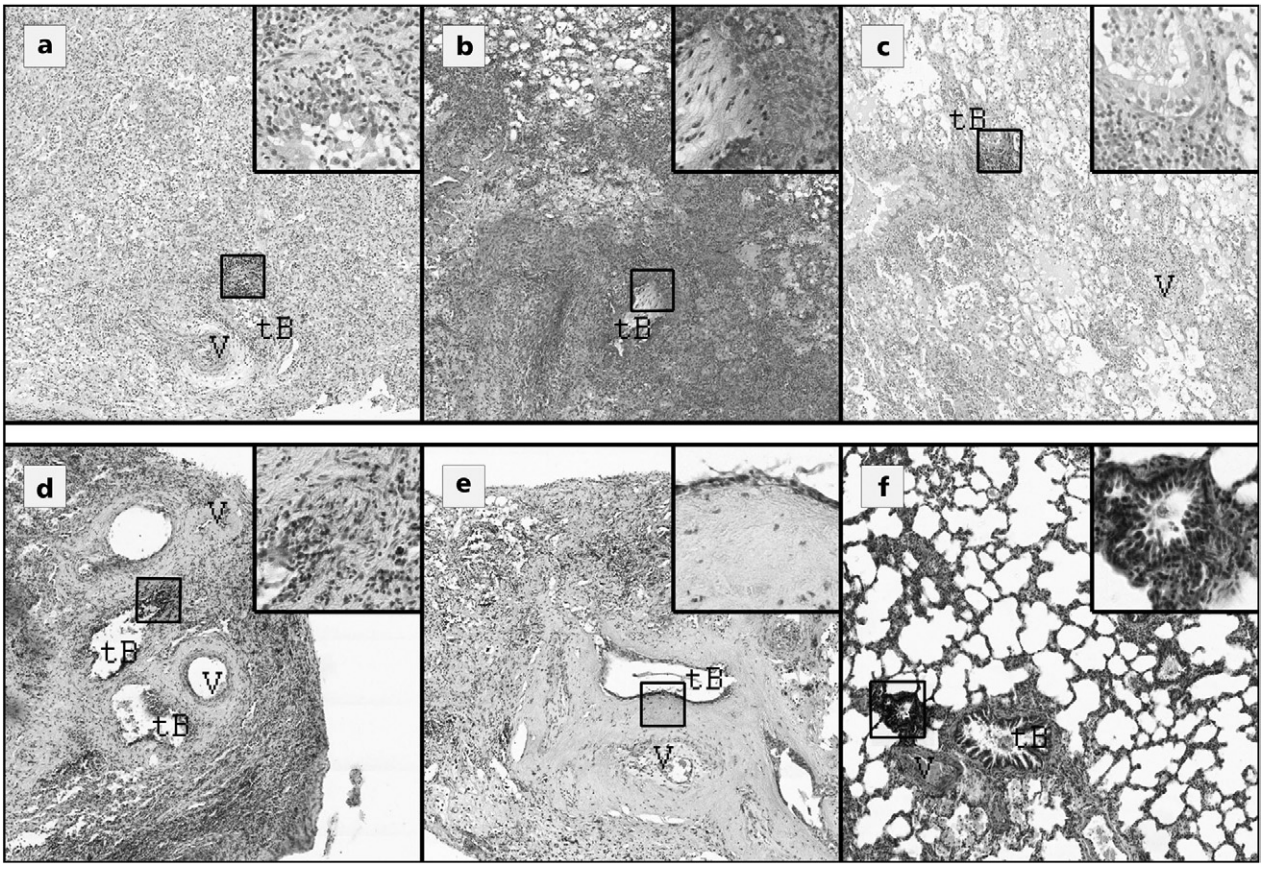

Fig 1. Representative histopathology of allografts from postoperative day (POD) 20 **(a–c)** and POD 100 **(d–f)** (magnification, 50x). Inserts (magnification, 400x) presented details from a representative terminal bronchiolus wall. Allografts from group 1 **(a)** and group 2 **(b)** were diagnosed with International Society of Heart and Lung Transplantation (ISHLT)-A4/B2R and presented first signs of perivascular and fibrointimal fibrosis. **(a)** Destruction of the smooth muscle cell layer and a subepithelial infiltration of mononuclear cells. **(b)** Infiltrated fibrocytes in the airway lumen. Allografts from group 3 **(c)** show ISHLT-A3/B1R-B2R without chronic alterations. The insert demonstrates peribronchiolar mononuclear cell infiltration. On POD 100, group 1 **(d)** and group 2 **(e)** commonly demonstrate bronchiolitis obliterans (BO)-like lesions in form of "constrictive bronchiolitis" (as described by Jonigk et al[5]). In addition, vasculopathy is also common in these allografts. Allografts from group 3 **(f)** were free of BO and chronic vascular rejection. tB, terminal bronchiolus; V, vessel.