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# The role of recipient derived interleukin-17A in a murine orthotopic lung transplant model of restrictive chronic lung allograft dysfunction

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ABSTRACT

The single most important cause of late mortality after lung transplantation is chronic lung allograft dysfunction (CLAD). However, the pathological development of CLAD was not as simple as previously presumed and subclassification phenotypes, bronchiolitis obliterans syndrome (BOS) and restrictive CLAD (rCLAD), have been introduced. We want to re-investigate how CLAD manifests in the murine orthotopic lung transplant model and investigate the role of interleukin 17A (IL-17A) within this model.

Orthotopic LTx was performed in CB57BL/6, IL-17 WT and IL-17 KO mice. In a first experiment, CB57BL/6 mice receiving an isograft (CB57BL/6) or allograft (BALB/C) were compared. In a second experiment IL-17 WT and IL-17 KO mice (both CB57BL/6 background) received an allograft (BALB/C). Mice received daily immunosuppression with steroids and cyclosporine and were sacrificed 10 weeks after transplantation for histopathological analysis by an experienced lung pathologist.

After murine orthotopic lung transplantation, the allograft histopathologically presented features of human rCLAD (i.e. overt inflammation, pleural/parenchymal fibrosis and obliterative bronchiolitis). In the IL-17A KO group, less inflammation in the bronchovascular axis ($p = 0.03$) was observed and a non-significant trend towards less bronchovascular fibrosis, pleural/septal inflammation and fibrosis, and parenchymal inflammation and fibrosis when compared to WT mice.

The major mismatch orthotopic lung transplant model resembles features of human rCLAD. IL-17A mediated immunity is involved in the inflammatory component, but had little influence on the degree of fibrosis. Further mechanistic and therapeutic studies in this mouse model are needed to fully understand the mechanisms in rCLAD.



## 1. Introduction

Lung transplantation (LTx) is the ultimate treatment option for patients with end-stage pulmonary diseases including cystic fibrosis, chronic obstructive lung disease, pulmonary arterial hypertension, and interstitial pulmonary fibrosis. However, long-term survival remains poor, with a 5 year survival of only 50%, predominantly due to the development of chronic rejection [1].

Chronic rejection (CR) was up to several years ago clinically recognized as bronchiolitis obliterans syndrome (BOS), which is diagnosed by a persistent decline in forced expiratory volume in 1 s ($FEV_1$) in the absence of other identifiable causes [2]. Histologically, BOS was documented as an obliteration of the small airways by scar tissue [2], of which the pathogenesis remains largely elusive. The working hypothesis was that chronic rejection resulted from insults to the airway epithelium by pathogens, cigarette smoke, primary graft dysfunction (PGD) etc., which triggered an innate and adaptive inflammatory response with a prominent role for IL-17A [3]. The cytokine IL-17A is the most important member of the IL-17 superfamily of cytokines and is produced by several subsets of lymphocytes including T helper cells (Th17), cytotoxic T cells (Tc17), invariant natural killer T cells (iNKT-17), gamma delta T cells (γδT-17) and innate lymphocyte cells (ILC-17) [3]. The most important feature of this cytokine is the induction of neutrophilic

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inflammation, by which it is involved in host defense, allergic diseases, autoimmune disorders, malignancies and graft versus host diseases [4]. It appears to be a key cytokine in several chronic inflammatory disorders and also in driving chronic rejection after lung transplantation [5,6].

Recurrent exposure to donor HLA and other antigens will result in ongoing mucosal injury that is likely to tip the balance from graft tolerance to immune activation which can induce severe (mostly lymphocytic) inflammation [3]. This overt inflammation can further damage the airway epithelium by releasing reactive oxygen species, chemokines, alarmins and metalloproteinases, which results in fibroproliferation by recruitment of fibrocytes and proliferation of resident fibroblasts and fibrocytes, and the activation of epithelial to mesenchymal cell transition [7]. The end result is a fibrous scarring of small airways leading to bronchiolar obstruction (obliterative bronchiolitis) and respiratory insufficiency.

However, in recent years it became increasingly clear that this concept of chronic rejection did not fit all manifestations and that different clinical phenotypes could be distinguished. As a consequence, the overarching term chronic lung allograft dysfunction (CLAD) was introduced, to describe every possible cause of chronic pulmonary function decline [8]. In addition to BOS, a restrictive phenotype of CLAD (rCLAD or restrictive allograft syndrome, RAS) was recently described and accepted as another manifestation of CR. rCLAD occurs in approximately 30% of CLAD patients and is characterized by a restrictive pulmonary function defect (persistent decline in $FEV_1$, FVC and TLC), persistent parenchymal infiltrates and (sub-) pleural thickening on chest CT scan, as well as pleuroparenchymal fibroelastosis and concurrent obliterative bronchiolitis on histopathologic examination. Once diagnosed, the rCLAD median survival is only 6 to 18 months compared with 3 to 5 years in patients with BOS [8].

It remains unclear if these are 2 totally different manifestations of chronic rejection, or a heterogenic presentation of the same disease. Therefore, the murine orthotopic single left lung transplant model could be used to improve the understanding of chronic rejection and to study the underlying pathophysiological mechanisms [9].

## 2. Objective

Our goal was to investigate the relationship of the major mismatched murine orthotopic lung transplant model and the different manifestations of CR. We wanted to determine if CR in this mouse model reflects the BOS or rCLAD phenotype of chronic rejection. Additionally, we aimed to investigate the role of IL-17A induced pulmonary inflammation in this model, given the prominent role of IL-17A in early (primary graft dysfunction) and late (chronic rejection) complications after lung transplantation [3,10].

## 3. Material & methods

### 3.1. Animal preparation and experimental groups

All animals received human care in compliance with the European Convention in Animal Care and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 86-23, 1996). The experimental procedure was approved by the Ethical Committee for Animal Research at the KU Leuven (P156/2011). For the experiments male C57BL/6J and BALB/C mice, 10–12 weeks old (20 to 25 g), were purchased from Harlan Laboratories (Huntingdon, UK). IL-17A $KO^{-/-}$ C57BL/6 mice were a kind gift from Y. Iwakura (Institute of Medical Science, University of Tokyo, Japan) and were further bred at the KU Leuven animal research facility. IL-17A $WT^{+/+}$ C57BL/6 were generated via multiple cross-breeding with IL-17A $KO^{-/-}$ and C57BL/6J mice until the desired genotype was achieved. Genotyping was performed after weaning and before transplantation surgery (Fig. 1). C57BL/6J mice (H-2Kb) were used as transplant recipients, with C57BL/6J mice as isograft donors and BALB/C mice (H-2Kd) as allograft donors. The study design with final n-values and groupings is illustrated in Fig. 2. N-values were dependent on the availability of the mice from the breeding and technical failures including mortality, surgical problems, early infection ($n = 4$ in the IL-17A KO allograft group) and lung necrosis observed macroscopically ($n = 2$ in the IL-17 WT allograft group and $n = 1$ in the IL-17A KO allograft group). The breeding of the IL-17A KO mice and its wild types was organized in our specific pathogen free (SPF) facility. To avoid bias due to strain specific genetic differences, only mice from the transgenic breeding were used as wild type mice (checked via PCR) for the IL17A knock-out experiments. After surgery all animals were housed in a conventional facility with IVC cages. Therefore all mice were housed and treated in exactly the same matter.

### 3.2. Orthotopic lung transplantation model

Orthotopic LTx was adapted from the technique described by Jungraithmayr et al. [9]. Donor mice were anesthetized with a mixture of medetomidine (1 mg/kg) and ketamine (75 mg/kg, Ketalar, Pfizer, Puurs, Belgium) intraperitoneally. A tracheostomy was performed and the mouse was connected to a ventilator (UNO microventilator UMV-03, UNO Roestvaststaal, Zevenaar, The Netherlands). Abdomen and thorax were opened and the lungs were flushed with 5 ml PerfadexH (Vitrolife, Göteborg, Sweden) through the arteria pulmonalis.

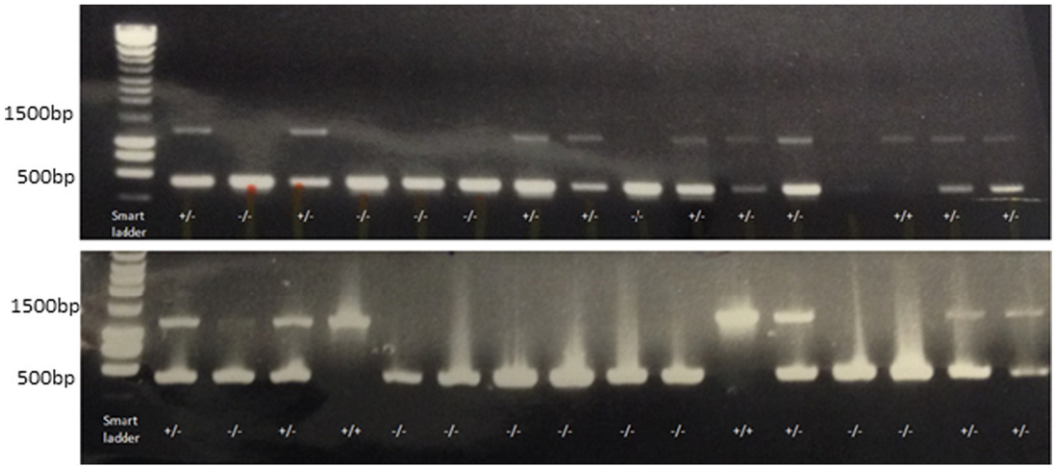


**Fig. 1.** Gel electrophoresis of IL-17A KO breeding. Heterozygous mice have 2 bands (±1300 bp and 500 bp) compared to KO (−/−, 500 bp) and WT (+/+, 1300 bp) animals.