Toll-like Receptor 4 Signaling Affects Myofibroblasts Expression in Mice Tracheal Allograft
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Background
Development of chronic lung allograft dysfunction involves various alloimmune-independetn insults including those mediated by toll-like receptor (TLR) signaling. The purpose of this study is to investigate a role of toll-like receptor 4 in allograft airway fibrosis.

Methods
Orthotopic tracheal transplantation was conducted between MHC-mismatched BALB/c and wild-type C3H (C3H (WT)) or C3H-derived TLR4 mutant mice (C3H (Tlr4<sup>ΔPS</sup>)) with cyclosporine treatment until postoperative day 10 (25 mg/kg/day). Syngenic transplantation was done using C3H (WT) as a control. To evaluate a role of TLR4 function in allograft, combinations of donors and recipients in allotransplantation were divided into four groups as follows; Allograft (i): BALB/c > C3H (WT), Allograft (ii): BALB/c > C3H (Tlr4<sup>ΔPS</sup>), Allograft (iii): C3H (WT) > BALB/c, Allograft (iv): C3H (Tlr4<sup>ΔPS</sup>) > BALB/c. Grafts were evaluated on postoperative day 21 by means of hematoxylin and eosin (HE) staining and immunohistochemistry for α-smooth muscle actin (SMA).

Results
In Allograft (i) group, tracheal specimens of TLR4 wild-type recipients showed higher density of subepithelial layer in HE and larger α-SMA positive area than the others (Figure 1). The α-SMA positive area of allograft (i) was significantly different compared with Allograft (ii) group (Figure 2).

In Allograft (iii) and (iv) group, on the other hand, allografts of both TLR4 wild-type and non-functional donors showed similar appearance in HE and no statistical difference in αSMA-positive area (Figure 1 and 2).

Discussion
TLR is one of innate immune receptors which recognize bacterial components to provoke host defense system. TLR is reported to affect post-transplant outcome (Kastelijin EA et al. J Heart Lung Transplant 2010, 29(6) 665-71). In our experimental model, allograft of TLR4 wild type recipients showed significant myofibroblast infiltration compared with that of TLR4 non-functional recipients (Allograft (i) vs Allograft (ii)). However, TLR4 function of donors did not cause remarkable morphological differences (Allograft (iii) vs Allograft (iv)). These results mean that recipients’ TLR4 function may have an important role for allograft airway fibrosis in this model.

Conclusion
Recipients’ TLR4 signaling contributed to airway allograft fibrosis in the orthotopic tracheal transplant model. This result suggests that TLR signaling pathway of recipient cells can be a potential therapeutic target for CLAD management. Further investigation in the mechanism is required.

Conflicts of Interest: Nothing to declare