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Title: Vascularization of heterotopic tracheal transplant in mice

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Body: Heterotopic tracheal transplantation (HTT) is a commonly used model to study obliterative bronchiolitis (OB) in mice. Vascularization of the graft in this model has not been reported. We here describe that vascularization occurs early after HTT. Methods. HTT (iso- and allograft) was performed (D0-D21). The graft was collected, paraffin-embedded, and cut into sections for anti-CD31 (Santa Cruz) and anti-LYVE1 (Abcam) immunostaining. Groups of 4 grafts were pooled for RNA extraction, and VEGF, angiopoietin-2 and podoplanin mRNAs quantification in grafts versus control non transplanted tracheas. In another series of experiments, biotinylated dextran was injected I.V. 10min before graft harvest. Results. CD31 immunostaining increased from D0 to D7 in the tracheal tissue of iso- and allograft (72±12 and 43±8 vessels/mm2), indicating neovascularization of the tissue. VEGF mRNA expression increased with a peak at D1 (8±3-fold) both in iso- and allografts, whereas angiopoietin-2 mRNA increased from D7 (42±10-fold) to D21 (40±17-fold) in allograft, significantly higher than in isografts. Immunostaining of the lymphatic vessels with LYVE1 occurs at D21 in allografts, and expression of podoplanin mRNA increased at D3 and D7 to return to baseline levels at D21. Functional microvessels labeled with biotinylated dextran were observed in the subepithelium both in iso- and allografts (35±20 and 48±16: D3; 131±42 and 56±11: D7). At D21, labeled microvessels vascularized the trachea (44±03) and the fibroproliferative tissue (OB) (43±17). Conclusion. The grafts in HTT are vascularized with functional blood and lymphatic vessels. Our data are in strong support of the use of the HTT model for proof of concept studies in OB.