48. Immunobiology in the transplanted lung: experimental and clinical evaluations

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Late-breaking abstract: Non specific IgG replacement in lung transplantation recipients with low IgG plasma levels: Effects on survival and bronchiolitis obliterans syndrome occurrence

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After lung transplantation, IgG plasma levels < 6 gr/L are recorded in more than 50% of cases resulting in adverse events (JHLT 2001;71:242; Transplantation 2005;79:1723). We conducted an open study with non specific IgG, Tegelines® in all patients with IgG plasma < 6 g/L post transplantation since 1991 in 59 out of 119 consecutive recipients who survived more than 3 months in Grenoble. Both groups had similar donor and recipient characteristics and events during a mean follow-up of 4.14±3.4 years except for a shorter duration of ventilation in donors, a longer first hospitalization stay and more acute rejection/TBB performed 0.32±1.2 (*vs* 0.11±0.18) in non-substituted group. IgG substitution started 4.5 [0-76] and lasted 5 [0-115] months, mean cumulative doses were 53±48 gr. In multivariate Cox regression model, IgG substitution conferred a net benefit in terms of survival at 5 years HR: 0.40 [0.18-0.88] 95%CI, p:0.022 taking in account age of recipients and donors, annualized number of treated infection and rejection episodes, and BOS free survival at 5 years HR: 0.50 [0.25-1.03] 95%CI, p:0.06 taking in account age of recipients and donors, type IC *vs* EC preservation solution, use of cardiopulmonary by-pass, annualized number of treated infection and rejection episodes. We conclude that replacement therapy with non specific IgG in lung transplant recipients with low IgG plasma levels resulted in a better survival and BOS free survival at 5 years post-transplantation as compared to non-substituted group. Limitation: non randomized design. A prospective controlled multicentre study is warranted. Funds from LFB, France.

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Significance of anti-HLA immunization in lung transplantation

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Objectives: HLA immunization is triggered by pregnancy, blood transfusion or organ transplantation. It is recognized factor for graft dysfunction after kidney transplantation. This study explores influence of HLA antibodies on occurrence and severity of Bronchiolitis Obliterans Syndrome (BOS), and survival after lung transplantation (LT).

Methods: Retrospective study from January 2004 to June 2010 including 99 LT in 96 patients. We determined 3 groups: specifically immunized against their pulmonary graft (group 1, n=16), non-specifically immunized (group 2, n=19), non-immunized (group 3, n=64). We compared FEV1 evolution curves at 1, 3, 6 months and every 6 months, survival with and without BOS (Kaplan Meier and Log rank), incidences of acute cellular rejection (ACR), lower respiratory infections and bronchial stenosis (Chi-2 and ANOVA).

Results: Specific immunization (group 1) appeared 15 months (±14) after LT. Non-specific immunization (group 2) was detected 9 months (±32) before LT. A decrease of FEV1 was observed in group 1 at 24 and 30 months after LT (compared to group 3: $p < 0,05$). There was a linear relationship between antibodies rates and FEV1 decrease in group 1. Mean delays between LT and BOS diagnosis were 20 (±14), 14 (±13), and 25 (± 11) months for group 1, group 2, and group 3 respectively. Incidences of BOS 0p and ACR were increased in group 2 compared to group 3 ($p < 0,05$).

Conclusion: Anti-HLA immunization is related to early onset of BOS. Specific antibodies probably lead to humoral rejection. Non specific antibodies indicated sensitized status with increased incidence of ACR.

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Effect of cyclosporine A in *ex vivo* reperfused pig lungs

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Rationale: The *ex vivo* pulmonary reperfusion is a suitable method of evaluation of lung ischemia reperfusion injuries (IRI). The role of the Cyclosporine A (CsA) in the prevention of IRI has been shown in different organs of several animal species but not clearly evaluated in lungs. Our objective was to evaluate the effects of CsA in *ex vivo* reperfused pig lungs.

Methods: 10 lungs were perfused with an extracorporeal perfusion circuit, and mechanically ventilated. CsA was administered before and during reperfusion procedure (either at 1 or 30µM).

Results: Lungs treated by 30 µM of CsA had increased capillary pressure (Pcap), pulmonary vascular resistances (PVR), lung permeability to proteins, IL1 beta and TNF alpha concentrations in bronchoalveolar lavage (BAL). 1µM of CsA seemed to have no effect compared to control group.

Lungs parameters

	Pcap (mmHg)	PVR total (mmHg/l/min)	K (min^{-1})	[IL1β] in BAL (pg/mL)	[TNFα] in BAL (pg/mL)
Control (n=4)	7.62±1.20	9.54±0.62	0.006±0.004	66.1±70.1	17.7±25.8
CsA 1µM (n=3)	8.80±1.19	13.08±4.99	0.012±0.010	78.3±92.7	10.0±14.1
CsA 30µM (n=3)	13.37±3.39*	16.02±1.53*	0.024±0.009*	198.2±2.1*	46.3±10.7*

*p<0.05 between control and CsA 30µM ; K = coefficient of permeability to Dextran of the capillary-alveolar membrane.

Discussion: CsA, at the concentration of 30 µM, seems to be deleterious in lung IRI. The hemodynamic effects of CsA may explain these results.

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Comparison between SCOT-15® and Perfadex® as lung preservation solutions

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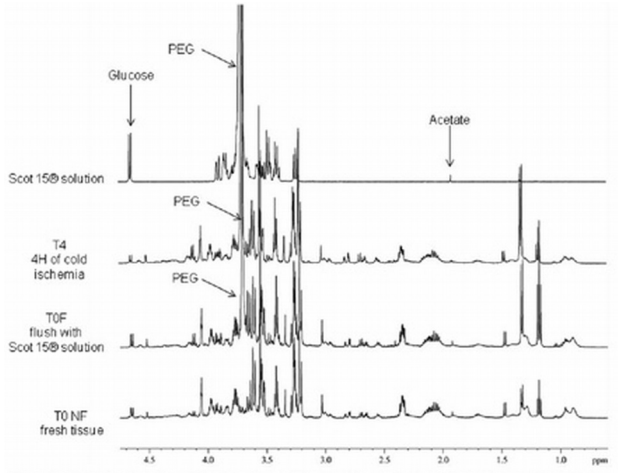
Objectives: SCOT-15® is a low K+ solution including polyethyleneglycol (PEG) as a colloid for protection of endothelium. PEG was demonstrated to have immunocamouflage properties and has been tested for kidney, pancreas and liver preservation. This study compares the properties of SCOT-15® for lung preservation with Perfadex® as a golden standard.

Methods: Two groups of 6 pigs each were compared. After 2L cold pulmoplegia with either Perfadex® [P] or SCOT-15® [S], lungs were stored cold for 4 hours. Peripheral lung biopsies were taken for High Resolution Magic Angle Spinning (HRMAS) detection of colloids. Lung function was assessed in an ex vivo lung perfusion and ventilation model. Pulmonary artery flow and pressure were recorded for pulmonary vascular resistance (PVR), mean airway pressure (mAwP) for lung compliance and blood gases on the perfusion outflow line for partial oxygen pressure (PO_2). Wet-to-dry weight ratio (W/D) was recorded for edema.

Results: PVR was significantly lower in [S] compared to [P] (p=0.04). There were no differences in PO_2 (p=0.13), mAwP (p=0.24) and W/D (p=0.06). HRMAS spectra showed presence of PEG in peripheral lung tissue in [S].



1D 1H HRMAS CPMG spectra of lung biopsies collected before and after flushing with Scot15® solution T0 NF (fresh tissue) and T0F respectively, and after 4 hours of cold ischemia (T4). The upper spectrum represents the metabolic profile of Scot15® solution.
Those spectra clearly show the distribution of the solution containing polyethylene glycol (PEG) in the tissue perfused with Scot15®, indeed, the PEG signal identified at 3,71ppm appeared after flush with Scot15® and not in fresh tissue. In addition, either glucose or acetate concentrations does not increase after flush with Scot15® solution, this data suggest that glucose or acetate present in tissue are not taken from the solution.

Conclusion: Lungs preserved with SCOT-15® had lower vascular resistance with comparable oxygenation capacity reflecting well preserved endothelial function. Experiments with longer cold ischemia are needed to assess the relevance of this solution.

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MSK1 kinase in obliterative bronchiolitis after heterotopic tracheal transplantation

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Introduction: Obliterative bronchiolitis (OB) occurs during chronic allograft rejection of lung transplantation. OB is characterized by airway epithelium degradation, and obstruction of the small airways with inflammatory infiltrate and fibrosis. MSK1 is a nuclear kinase that activates NFκB in inflammation. Our hypothesis proposes MSK1 as an actor in OB *via* NFκB-induced activation of pro-inflammatory genes like IL-6.
Methods: In the mouse model of heterotopic tracheal transplantation, tracheal MSK1 and IL-6 mRNA was quantified by qPCR. Mice were treated I.P. with compound H89, a MSK1 inhibitor (10mg/kg/day) *vs* solvent (DMSO 5%). Tracheal sections were stained with hematoxylin-eosin, and epithelium degradation measured. CD3+ cells (SP7 anti-CD3 mAb, Abcam) were counted and dendritic cells (DC, H2-I-A/I-E mAb, M5/114) labeled on fresh tracheas.
Results: MSK1 and IL-6 mRNA levels were increased in allografts by 68±8% and 88±2%, respectively, at D7 as compared to D0 and unmodified in isografts. Progressive epithelium degradation reached 77±4% of the total epithelium at D7 and was inhibited upon H89 treatment of 43±10% ($p<0.05$). In isografts, epithelium degradation also occurred (18±10%). CD3+ cell recruitment reached 208±82 cells/mm^2 at D7 and was inhibited to 16±8 cells/mm^2 ($p<0.05$), compared to isografts (17±9 cells/mm^2, NS). DC recruitment also occurred in allografts (525±73 cells/mm^2 at D3 and 1065±368 at D7) as compared to D0 (290±17). No effect of H89 was observed on DC recruitment at D3. Inflammatory luminal infiltrate was inhibited at D7 by 44±15%.
Conclusion: Inhibiting MSK1 is therefore a potential strategy to help combat obstruction after lung transplantation in this OB model.

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Development of a novel model of obliterative bronchiolitis following orthotopic lung transplantation in the rat

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Background. Transplantation (Tx) is the only effective treatment for several end-stage lung diseases, but long-term survival after Tx is precluded by the development of obliterative bronchiolitis (OB). The specific etiology and pathogenesis of OB are not fully understood. Animal models could be crucial to elucidate the immunological and non-immunological pathogenetic mechanisms leading to OB, to identify early markers and to test effectiveness of new treatments.
Methods: Left lung allografts from Lewis rats were orthotopically transplanted into Fisher 344 rats [n=19]. Animals were sacrificed at day 30 [group A, n=6] or day 90 [group B, n=6; C, n=4; D, n=3] post-transplantation. In animals from group C and D, cyclosporine A (CyA) was administered from day 1 to 7 or from day 7 to 14, respectively. Following HE-staining, lung rejection was graded according to the working formulation of ISHLT. The presence of circulating donor-specific (DSA) antibodies was determined by flow cytometry.
Results: Acute rejection (AR) occurred in 25 to 33% of the animals in the different groups. OB occurred in 17% of animals in group A, in 33% of animals in group B and D, and in 75% of animals in group C. High levels of DSA IgG were observed in all cases of AR and, to a lower extent, in all animals with OB. Furthermore, early administration of CyA reduced the levels of DSA but did not prevent OB occurrence.
Conclusions: A novel model of pulmonary OB was developed in the rat. To obtain a reproducible onset of OB, short-term and sub therapeutic CyA administration appears indispensable, at least in our species combination. Furthermore, a 90-day postoperative period is required for OB to take place.

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A new mouse model of bronchiolitis obliterans syndrome

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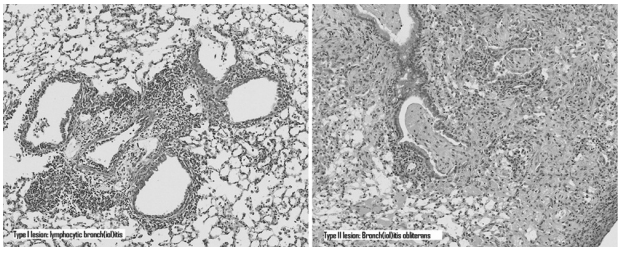
Background: Long-term survival after lung transplantation (LTx) is hampered by Bronchiolitis Obliterans Syndrome (BOS), morphologically presented by OB. Since the pathogenesis is still not fully understood and the prognosis remains poor a good animal model is indispensable.
Aim: The development of a new model of BOS after LTx.
Methods: C57Bl6 mice underwent LTx with BALB/C donor lungs and were sacrificed at 2, 4, 6, 10 and 12 weeks after LTx. Staining with H&E and Sirius Red (not shown) were performed.
Results: Histology showed two types of lesions. Type I lesions are characterized by lymphocytic bronch(iol)itis and functional lung parenchyma. These lesions seem to resolve over time. Type II lesions are demonstrated by fibrotic plugs growing into the airway lumen, resembling true BO lesions in humans. The surrounding parenchyma however is not functional.



Conclusions: Allograft LTx in mice mimics human histology of BO, optimalisation of this model will open new perspectives to study pathogenesis of chronic rejection after LTx.