was 38. On investigation of the HLA Class II DSA development it was identified that the majority of DSAs were directed against HLA-DQA/B eplets.
Conclusion: This observation supports the concept of RAS as a manifestation of humoral rejection, mediated predominantly by alloantibodies directed against HLA Class II.

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A Single Donor TARC/CCL17 Promotor Polymorphism Correlates with Serum TARC/CCL17 Levels and Is Associated with Impaired Clinical Outcome after Lung Transplantation

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Purpose: Lung transplantation (LTx) outcome is hampered by development of chronic rejection, presented as the bronchiolitis obliterans syndrome (BOS). TARC/CCL17 is a chemo-attractant of which serum levels measured during the first month post-LTx are predictive for BOS development. Since TARC/CCL17 promotor polymorphisms correlate with serum TARC/CCL17 levels, we hypothesized that single nucleotide polymorphisms (SNPs) present in this region could be associated with the clinical outcome after LTx.
Methods: We analyzed seven selected donor and patient SNP configurations and haplotypes in the promotor region of TARC/CCL17 and correlated both individual SNPs and identified haplotypes to serum concentrations of TARC/CCL17, the incidence of BOS, and overall survival after LTx.
Results: We identified a single promotor polymorphism in the donor correlating with patient TARC/CCL17 serum levels post-transplantation (p=0.066). Interestingly, this SNP configuration in patients did not show any correlation with pre-LTx TARC/CCL17 serum levels (p=0.776). Survival analysis showed that receiving a graft from a donor heterozygous for this specific position has a disadvantageous impact on transplantation outcome. When stratified per donor SNP genotype, patients receiving a transplant from a heterozygous donor showed a significant lower BOS-free survival (50% vs. 75%, p=0.023) and lower survival rate (50% vs 80%, p=0.0079). Since the identified SNP is located within a potential NFκB binding site, heterozygosity at this position could result in a reduced expression of TARC/CCL17.
Conclusion: Our data indicate that a single SNP in the promotor region of TARC/CCL17 in the donor correlates with lower serum TARC/CCL17 levels measured in the recipient one month after LTx. Furthermore, receiving a graft heterozygous for this specific promotor SNP affects clinical outcome after transplantation.

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Bile Acid Aspiration Associates with CLAD and Affects the Bronchial District Lipid Profile: Targeted Bile Acid Metabolomics and Lipidomics in Bronchial Washing

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Purpose: Lung transplant is the ultimate option for end stage lung disease pts. Overall survival is halted by rejection and chronic lung allograft dysfunction (CLAD). Gastro-esophageal reflux and aspiration has been considered a risk factor for CLAD. Bile acid aspiration was investigated by targeted metabolomics in the bronchial washings from lung transplant patients. Given the detergent properties of bile acids, their effect of the bronchial district lipids was studied using a lipidomics approach.
Methods: Bronchial washings (BW) (56 samples) were prospectively collected from 51 patients at routine surveillance post-transplant bronchoscopies. Liquid chromatography-mass spectrometry retrospectively assayed BW for 13 bile acids and 25 lipid families inclusive of 250 lipids. Patients were monitored for CLAD and transbronchial biopsies for rejection and inflammation.
Results: Bile acids were detected in all BW. BW from pts with CLAD (22/51) had overall greater levels of bile acids (Mann Whitney p=0.03). Different levels (Kruscal Wallis p<0.05) were noted in BW from pts with early (onset <24 mo) CLAD (19.4 nM/L, IQR 15.2 - 35.8), late CLAD (26, 19.3-55.9), and no-CLAD (17.1, 13.5-26.4). Late CLAD vrs no-CLAD group (p=0.02); late CLAD vrs early CLAD (p=0.05); no difference for early CLAD vrs. no CLAD. Total Bile acids correlated (p<0.0005) with: cholesteryl-esters (CE) (Spearman r=0.6); Sphingomyelin (SM) (r=0.5); dihydrosphingomyelin (dhSM) (r=0.55); lactosyl-ceramide (Lac-Cer) (r=0.6); Lysophosphatidylcholine ether (LPCe) (r=0.5). Bile acids levels, high (upper quartile >27.5 nM/L) vrs. low, showed greater levels (p<0.05) of: CE, SM, dhSM, hexosyl-Cer, Lac-Cer, ganglioside GM3, phosphatidic acid, dipalmitoylphosphatidylcholine phosphatidylcholin ether, lysophosphatidylcholine, LPCe, lysophosphatidylinositol, phosphatidylethanolamine-p, lysophosphatidylethanolamine, lysophosphatidylethanolamine-p, phosphatidylinositol, and acylphosphatidylglycerol.
Conclusion: Bile acids assayed by mass spec are detected in all lung transplant bronchial washings. High level of bile acids associate with late CLAD onset and have a significant dose related effect on the bronchial district lipidomic profile.

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Delayed Non-Myeloablative Irradiation after Lung Transplantation to Induce Allograft Acceptance in a Large Animal Model

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Purpose: Previously, we induced long term tolerance in animals that underwent perioperative irradiation and a donor-specific alloantigen infusion in our miniature swine lung transplantation model. Here, we delayed induction to improve its clinical applicability.
Methods: Left sided single lung transplantation from MHC mismatched male donors was performed in 11 female outbred minipigs. Group 1 (n=5) received non-myeloablative irradiation (7 Gy thymus and 1,5 Gy whole body, IRR) 12 hours before transplantation and perioperative donor-specific splenocyte infusion (SpTx). Group 2 (n=3) also received a perioperative SpTx, but underwent delayed irradiation 3 days after transplantation. Group 3 (n=3) was also exposed to delayed IRR but did not receive a donor-specific SpTx. Immunosuppression was maintained for 28 days with tacrolimus and methylprednisolone. Peripheral blood chimerism was monitored by flow cytometry and real-time polymerase chain reaction.
Results: In group 1, three animals never rejected their grafts until elective sacrifice at postoperative days (POD) 800+, the other two showed rejection on POD 239 and 360. In group 2, median allograft survival was 92 days. Out of the third group, one animal showed rejection on POD 226, the other two are currently alive at POD 216 and 393. In all groups, chimerism was detectable 1 hour after reperfusion of the lung, and peaked 1 hour after SpTx in groups 1 and 2. Group 1 showed early leukocyte chimerism levels of up to 30%. Analysis of putative regulatory CD4+CD25 high T cells revealed a rising tendency in group 1, whereas groups 2 and 3 had decreasing levels of this T cell subpopulation after withdrawal of immunosuppression.
Conclusion: Preoperative irradiation and perioperative SpTx had the potential to upregulate the frequency of putative regulatory CD4+CD25 high T cells. SpTx without preceding IRR seemed to rather upregulate the effector arm, than inducing Treg proliferation. Delayed IRR only may lead to prolonged allograft survival in all three animals. This unresponsiveness might be due to induced anergy.

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The Presence of Complement (C4d) Broncho-Alveolar Lavage in Phenotypes of Chronic Lung Allograft Dysfunction

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Purpose: Chronic lung allograft dysfunction (CLAD) is one of the major complications after lung transplantation (LTx). The two major phenotypes of CLAD are restrictive allograft syndrome (RAS) and bronchiolitis obliterans syndrome (BOS), of which the exact pathophysiology remains elusive. Complement activation is suggestive for antibody-mediated rejection (AMR), which might be an important trigger of CLAD. We investigated complement (C4d and C1q) concentration in broncho-alveolar lavage (BAL) samples selected at time of diagnosis of CLAD with clear BOS or RAS phenotypes.
Methods: BOS and RAS were separated using a combination of pulmonary function (obstructive vs. restrictive), pathology (airtrapping vs. persistent