Presidential Session

Best Abstracts awards: Clinical Transplantation and Basic Science in Transplantation

O-337 THE IMPACT STUDY: PROPHYLAXIS WITH VALGANCICLOVIR FOR UP TO 200 DAYS POST-TRANSPLANT IN HIGH RISK KIDNEY RECIPIENTS SIGNIFICANTLY REDUCES THE INCIDENCE OF CMV DISEASE COMPARED TO 100 DAYS USE FOLLOWED BY PLACEBO

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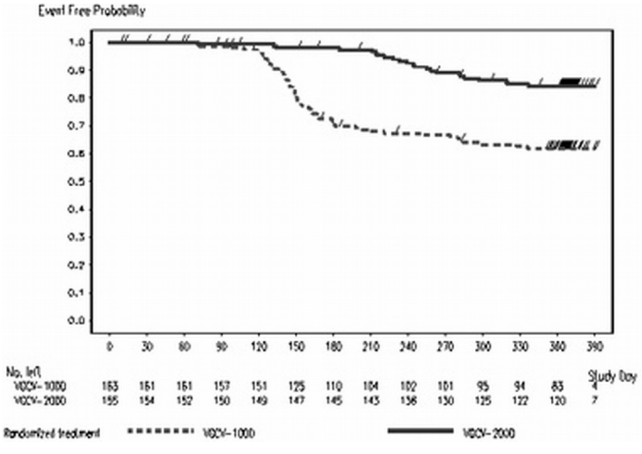
Rationale: Current immunosuppressive regimens significantly prolong the risk of CMV disease in high-risk transplant recipients beyond 100 days use of antiviral prophylaxis.
Methods: In this international, randomized, prospective, double-blind study, 318 CMV D+R- kidney transplant recipients received prophylaxis with valganciclovir 900mg once daily for up to 100 days, then placebo until day 200 post transplant compared to valganciclovir 900mg once daily for up to 200 days post transplant, with dose adjusted according to renal function.
Results: Confirmed CMV disease, defined as CMV syndrome or tissue invasive disease, developed in 36.8% vs. 16.1% of patients in the 100-day prophylaxis group vs the 200-day prophylaxis group respectively ($p<0.0001$).



Rates of acute rejection (17.2% vs 11%; p=0.11) and graft loss (1.8% vs 1.9%; p=0.9) were low in the 100 and 200-day prophylaxis groups, respectively.
There was no significant difference in overall tolerability and the incidence of marked hematological changes (neutrophils, hemoglobin and platelets) were comparable.
Conclusion: 200 days of once-daily oral valganciclovir prophylaxis significantly reduced the incidence of CMV disease compared to 100 days use in high risk kidney transplant recipients, as assessed 12 months post transplant, without adversely impacting the safety profile.

O-338 OPERATIONALLY TOLERANT KIDNEY GRAFT RECIPIENTS DISPLAY INCREASED NUMBERS OF PERIPHERAL B CELLS WITH AN INHIBITORY PHENOTYPE

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Background: We have previously reported on the clinical characteristics of kidney transplant recipients with operational tolerance, i.e. long-term graft acceptance in the absence of immunosuppression. We showed that these patients were characterized by a high number of peripheral B cells. We analyzed these peripheral blood B cells according to the Bm1-Bm5 classification and associated markers compared with different cohorts of kidney recipients. We observed a significant increase in both absolute cell number and frequency of activated, memory and early memory B cells (Bm2, EBm5 and Bm5) in patients with operational tolerance. We additionally found costimulatory-migratory molecules (B7-1/CD86, B7-2/CD80, CD40 and CD62L) to be up-regulated in B cells and particularly in memory B cell populations in these patients. This profile was associated with a significant decrease in the FcRIIA/FcRIIB index of activation/inhibition in B cells as well as an increase in CD5 and CD1d protein expression. Moreover, transcripts for BANK1, a negative modulator of CD40-mediated AKT activation and a hypo responsive cytokine profile were also increased, suggesting an inhibitory blood B cell profile in patients with operational tolerance. Finally, B cells from these tolerant recipients over-expressed BAFF-R transcripts, the B cell survivor and specific receptor of BAFF, as well as numerous genes involved in cell cycle that could explain their higher peripheral B cell count. Altogether our data show that, in contrast to T cells, the blood B cell phenotype and key molecules of B cell regulation are profoundly regulated in operationally tolerant recipients.

O-339 CAN MOLECULAR CHANGES IN PROTOCOL BIOPSIES PREDICT THE FUTURE? CORRELATING MICROARRAY DATA TO FUTURE SCARRING, DYSFUNCTION, AND REJECTION IN RENAL ALLOGRAFTS

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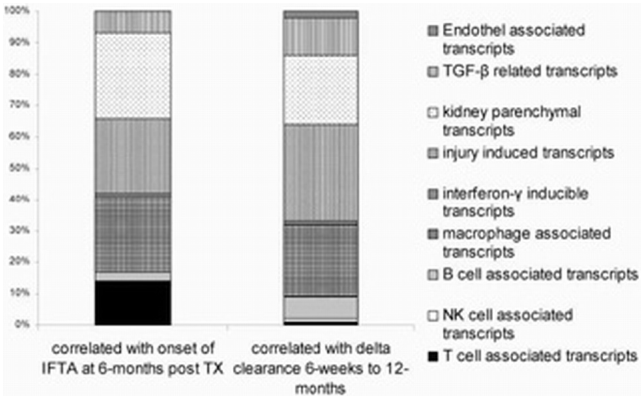
We tested the hypothesis that molecular changes in protocol biopsies (PB) from stable renal allografts predict future adverse events. We developed a system of summarizing genome wide expression data as pathogenesis based transcript sets (PBTs), representing and quantifying major biological processes: infiltration by cytotoxic T, B, NK cells and macrophages; endothelial changes, interferon-γ effects, TGF-β regulated transcripts; kidney transcripts, and response to injury. In 81 six-week PB analyzed by microarrays, all measured transcripts were correlated with: (1) onset of interstitial fibrosis and tubular atrophy (IFTA) in a sequential protocol biopsy at 6 months; (2) change in GFR between 6 weeks and one year post transplant; (3) diagnosis of acute rejection between 6 weeks and one year.
The top 100 PBT annotated transcripts correlating ($r>0.25$, $p<0.03$) with the later onset of IFTA were macrophage associate (27%), injury induced (24%), and transcripts with differential expression in tubular epithelial cells during rejection (24%), with 14% of transcripts being T cell associated and 7% TGF-β related, but not interferon-γ transcripts. The results were similar for loss of func-



tion: most of correlating transcripts ($r>0.3$, $p<0.01$) were injury induced (31%), macrophage associated (23%), and kidney parenchymal transcripts (22%), but not T cell or interferon-γ associated. The prevalence of future biopsy proven rejection showed similar correlations ($r>0.3$, $p<0.01$): transcripts associated with kidney parenchyma (26%), macrophages (21%), and injury (17%). In addition, 17% of the transcripts were cytotoxic T cell associated.
Thus in six-week protocol biopsies, the molecular changes that correlate most strongly with the future endpoints are not related to T cell infiltration and interferon-γ effects (i.e. rejection) but to renal injury response, loss of epithelial function, and macrophages.

O-340 ALLOSPECIFIC B CELLS CAN RECEIVE EFFECTIVE HELP FROM CD4 T CELLS THAT RECOGNIZE AN UNRELATED ALLOANTIGEN

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Alloantibody responses often incorporate additional specificities against non-donor MHC antigens; how such ''bystander' activation occurs is poorly understood. In theory, antibody specificity is restricted by the requirement for T cell help delivered through 'cognate' recognition of processed target antigen that is presented following BCR internalization. Here we examine whether CD4 T cells that recognize additional mismatched alloAg can provide help for anti-MHC class I alloantibody responses.
B6 ($H2^b$) mice challenged with BALB/c ($H2^d$) hearts produced strong anti-K^d IgG alloantibody responses. T cell deficient B6 (TCR KO) allograft recipients reconstituted with 10^7 monoclonal TCR Tg CD4 T cells specific for self-restricted K^d peptide (provide cognate help through recognition of processed allopeptide on K^d-specific B cells) mounted a 3-fold greater anti-K^d response. Surprisingly, TCR KO female mice reconstituted with Mar CD4 T cells (specific for self restricted H-Y peptide) developed an anti-K^d IgG alloantibody response, following male, but not female, BALB/c heart transplantation. Anti-K^d responses were also observed in TCR KO allograft recipients reconstituted with TEa CD4 T cells (that recognise self-restricted donor I-E peptide). Finally, female TCR KO mice reconstituted with Mar T cells and challenged with male B6 APC (that provoked strong Mar responses) did not develop alloantibody to female BALB/c hearts. Thus although helper T cells can recognise a different antigen from allospecific B cells, both antigens need to be expressed on the graft for effective humoral immunity. We hypothesise that as alloantigen-specific B cells internalise target alloantigen, neighbouring donor proteins are also captured, processed and presented to helper T cells.

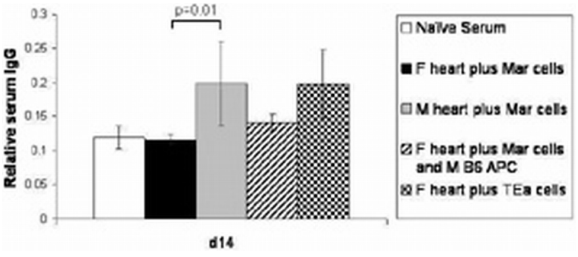

Figure 1. Serum levels of anti-Kd IgG in TCR KO mice following Balb/c heart graft.

Our results challenge the tenet of 'linked' antigen recognition between the BCR and helper TCR as a critical requirement for T-dependent antibody responses and provide a mechanism to explain how alloantibody specificities diversify after transplantation.

O-341 INDOLEAMINE 2,3-DIOXYGENASE (IDO) AND Treg SUPPORT ARE CRITICAL FOR CTLA4Ig MEDIATED TOLERANCE INDUCTION TO SOLID ORGAN ALLOGRAFTS

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Purpose: Costimulatory blockade of CD28-B7-interaction with CTLA4Ig is a well-established tolerance induction strategy. Although previous in-vitro studies confirm that CTLA4Ig up-regulates IDO expression in DCs, the precise mechanisms of CTLA4Ig and IDO interaction remain unclear. Here we studied if concerted immunomodulation in-vivo by CTLA4Ig, IDO and Tregs accounts for indefinite survival of murine cardiac allografts.
Methods: C57BL/6 IDO (WT/knock outs) mice received BALB/c hearts. Group 1 [No treatment], Group 2 [Donor-specific transfusion (DST)], Group 3 [CTLA4Ig], Group 4 [CTLA4Ig+DST], Group 5 [CTLA4Ig+DST+ IDO inhibitor 1-methyl-tryptophan (1-MT)] and Group 6 [CTLA4-Ig+DST+ αCD25 mAb]. 1-MT was delivered in slow release pellets (at surgery or POD 50). Serum-enzyme-activity of IDO (kyn/trp) was analyzed by HPLC. Quantitative PCR was used for mRNA expression of IDO1/IDO2, Foxp3 and granzyme B. Anti-donor Abs were screened by FACS. Histopathology (H&E) and immunohistochemistry (for IDO,Foxp3,CD4,CD8,CD20,CD68 and C4d) of tissues was performed.
Results: Graft survival: Group 1 [7.7$\pm$1.9 d], Group 2 [10.7$\pm$1.3 d], and Group 3 [47.7$\pm$29.8 d]. Group 4: Indefinite graft survival [$>$100 d] and tolerance without chronic rejection in IDO WT but acute rejection [16.5$\pm$5.9 d] in IDO knock out recipients. Group 5:IDO inhibition with 1-MT, either at transplant or at POD 50, abrogated CTLA4Ig+DST tolerance induction. Group 6:αCD25 mAb depletion of Tregs prevented CTLA4Ig+DST tolerance induction. Tolerant recipients had significantly higher IDO activity as compared to non-tolerant animals, which markedly correlated with intragraft IDO and Foxp3 levels on immunostaining. IDO1/IDO2 mRNA expression was similar in tolerant and non-tolerant recipients. Anti-donor Abs were absent in all long-term survivors.
Conclusion: This study provides the first direct in-vivo evidence that CTLA4Ig induced tolerance to murine cardiac allografts is critically dependent on synergistic cross-linked interplay of IDO and Tregs. These results have important implications for the clinical development of this costimulatory blocker.

O-342 ACTIVATION STATUS OF NK CELLS DRIVES DICHOTOMOUS T CELL RESPONSE

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NK cells have recently been shown to have phenotypic features of CD11c+ dendritic cells (DCs). However, the precise factors that regulate such attributes in NK cells and their significance in T cell response are poorly understood.
In order to examine the DC-like phenotypic and functional features of CD3-NK1.1+ NK cells, we created homozygous B6.CD11c-GFP reporter mice, in which the expression of CD11c is genetically linked to co-expression of GFP and diphtheria toxin receptor. Furthermore, we used TCR-transgeneic B6.OTIIa-Foxp3-GFP-knockin mice to study the role of NK cells as antigen presenting cells (APCs) in T cell response in vitro.
Using a polychromatic approach we found that NK cells in naive B6 mice express low levels of CD11c, B220, CD86, and MHCII, in line with recent reports. Importantly, further studies in wild-type B6 and B6.CD11c-GFP mice showed that those markers are upregulated on NK cells upon activation with IL-15 or TLR ligands (i.e. PolyI:C, ODN1668) in vivo. Thus, we hypothesized that NK cells by themselves may directly function as APCs that regulate antigen-specific T cell response in vitro. Using our OTIIa-Foxp3-GFP-knockin model, we found that naive NK cells induce modest proliferation of GFP- T effector cells (Teffs) at levels comparable to plasmacytoid DCs (pDCs), whereas activated NK cells largely failed to induce Teffs expansion, due to the killing of proliferating Teffs in a Perforin-dependent fashion. Intriguingly, we also found that naive NK cells are powerful inducers of GFP+ regulatory T cells (iTregs) when compared to pDCs and conventional DCs; whereas activated NK cells largely prevent the generation of iTregs in an Perforin-dependent manner.
Thus, NK cells play a dichotomous role in T cell response contingent on their activation status, giving them a novel role in directly regulating peripheral T cell homeostasis in autoimmunity and transplantation.