One patient was newly infected with HCV without actual consequences concerning liver function but received a well functioning graft of a 25 year old donor (s. patient and methods). Two patients with underlying HCV infection showed deterioriation of liver function one without correlation to transplantation one with genotype crossing. Of the 20 recipients without HCV infection transplanted with kidneys of anti HCV positive donors with negative HCV PCR none got infected. Mean waiting time of the study group was 12 months shorter than mean waiting time in the Eurotransplant region.

Conclusion: Acceptance of kidneys of anti HCV positive donors increases the donor pool and is safe for recipients with chronic HCV infections. Also recipients without HCV infection can be transplanted safely if PCR is negative. In rare cases especially in old recipients HCV infection can be accepted as consequence of transplantation.

POSTER BOARD NUMBER P4 – 418

809 SYK, A NOVEL THERAPEUTIC TARGET FOR PTLD, DRIVES EPSTEIN BARR VIRUS (EBV)+ B CELL LYMPHOMA GROWTH AND SURVIVAL THROUGH ACTIVATION OF THE PI3K/AKT PATHWAY

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Post-transplant lymphoproliferative disorder (PTLD)-associated Epstein Barr Virus (EBV)+ B cell lymphomas are a serious, and often fatal, complication of solid organ and bone marrow transplantation. Currently, therapeutic options are limited and, apart from reducing immunosuppression, there is no consensus regarding treatment strategies. EBV+ B cell lymphomas associated with PTLD express several viral genes including latent membrane protein 2A (LMP2A). LMP2A mimics a constitutively active B cell receptor (BCR) and provides survival signals to latently infected host cells in part by the recruitment of the Syk tyrosine kinase and activation of downstream signalling pathways, such as the PI3K/Akt pathway. We hypothesized that inhibition of Syk activity would diminish growth of EBV+ B cell lymphoma lines derived from patients with PTLD. Treatment with R406, an orally available inhibitor of Syk, decreased proliferation and induced apoptosis of these lymphomas, as assayed by 3H-thymidine incorporation and Annexin-V/PI staining, respectively. Western Blot analysis for total Syk and autophosphorylated Syk (Y525/526) revealed that Syk is constitutively activated in these PTLD lines. Additionally, treatment with R406 led to a decrease in Syk autophosphorylation at Y525/526, suggesting that Syk is the target of the small molecule inhibitor R406. Syk is the tyrosine kinase exclusively responsible for the phosphorylation of B cell linker protein (BLNK, SLP-65), an important component of the BCR signalling pathway. Treatment of Ramos cells, a human Burkitt's lymphoma line, with R406 led to a decrease in the phosphorylation of BLNK upon BCR ligation as determined by intracellular staining. Together, these data verify that Syk is the target of R406. The PI3K/Akt pathway plays a crucial role in cell growth and survival; in EBV+ B cell lymphomas, activation of the PI3K/Akt pathway leads to, among other things, secretion of the autocrine growth factor IL-10. Akt is constitutively activated in EBV+ B cell lymphoma lines; however, treatment with R406 or Syk siRNA resulted in a significant decrease in levels of phosphorylated Akt, as determined by Western Blot. Additionally, treatment of PTLD lines with R406 or Syk siRNA led to decreased autocrine IL-10 production. These data indicate that Syk mediates its effects on EBV+ B cell lymphoma growth and survival in part through activation of the PI3K/Akt pathway. Thus, these results provide mechanistic insight into how targeting Syk with inhibitors such as R406 may serve as an effective therapeutic strategy for the treatment of EBV-associated PTLD.

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810 GUIDELINES AND PHYSICIAN PRACTICES IN RELATION TO VACCINATION OF BONE MARROW TRANSPLANT (BMT) RECIPIENTS – THE AUSTRALIAN PERSPECTIVE

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Background: Following BMT, not only are patients at risk of infection because of immunosuppression, they also lose immunity acquired from previous immunisation against vaccine preventable infections. As a result of this, bone marrow transplant recipients need re-vaccination against a number of vaccine preventable. The aim of this project is to review available guidelines for the vaccination of bone marrow transplant recipients and examine knowledge of and compliance with local guidelines in a local group of transplant physicians and recipients.

Method: A questionnaire was administered to physicians involved in bone marrow transplantation and bone marrow transplant recipients. This physician questionnaire examined their knowledge of and practices in relation to vaccination of BMT recipients. A questionnaire was also sent out to BMT recipients to examine what vaccinations they had received and how these had been organised.

Results: Two major international guidelines were most commonly used by BMT physicians to guide their practice. There are no national Australian guidelines specifically for this group of patients, and some hospitals have even developed their own. In relation to vaccination practices, there was significant variation amongst physicians. Most physicians felt that vaccinations were important, but did not initiate them or have a system for ensuring that patient vaccinations were carried out. Patient responses echoed this variability even more, and confirmed that many patients had not received appropriate vaccination.

Conclusion: This study highlights the need for national post-BMT vaccination guidelines in Australia. It also suggests that systems need to be developed for ensuring that appropriate post-BMT vaccinations are actually performed regularly.

MINI-ORAL SESSION 28: IMMUNOBIOLOGY 4: IMMUNOMODULATION 2

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811 SILENCING OF HLA CLASS I EXPRESSION BY LENTIVIRUS VECTOR-MEDIATED RNA INTERFERENCE RESULTS IN RESISTANCE OF PRIMARY HUMAN CELLS TO ALLOREACTIVE T CELL-MEDIATED CYTOTOXICITY

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Background: Recent advances in vector technology now allow the possibility of genetically engineering graft tissues to express reduced levels of HLA. Reducing HLA expression could help to overcome the limitations imposed by polymorphisms which restrict availability of suitable donors, complicate logistics of procuring and delivering matched tissues and organs, and necessitate life-long immunosuppression. We have previously shown that lentiviral vectors can be used to deliver short hairpin RNAs (shRNA) that knock down HLA expression in a class- or allele-specific manner in established human cell lines, and thereby allow evasion from immune recognition by alloreactive T cells. Here we have further characterized and quantitated the effects of dose-dependent gene transfer and shRNA-mediated knockdown of HLA in human cell lines and primary cells on their ability to be recognized by alloreactive cytotoxic T lymphocytes (alloCTL).

Materials & Methods: Lentiviral vectors for shRNA targeting Class I-specific and HLA-A0201 allele-specific sequences were employed to transduce 293T human embryonic kidney cells and CD34+ human primary hematopoietic progenitor cells. Transduced target cells were analyzed for sensitivity to cytolysis by incubation with HLA A2-activated alloCTL at an E:T ratio of 10:1.

Interferon-gamma production from the alloCTLs was measured by ELISA, and viability of target cells was determined by MTS assay, annexin V staining, and complement-mediated cytotoxicity assay.

Results: With higher multiplicities of infection (MOI=10-30), cell surface expression of HLA-A2 and HLA-ABC was reduced in a dose-dependent manner by 50% or >80%, respectively, compared to cells transduced with control vector. Additionally, relative to target cells transduced with control vector, target cells transduced with HLA-ABC shRNA and HLA-A0201 shRNA vectors induced 2.5- and 4.5-fold less interferon production from alloCTL, respectively, and exhibited >2-fold enhanced resistance to alloCTL-mediated killing ($p<0.05$). However, there were no significant differences in survival of shRNA vector-transduced target cells compared to control vector-transduced cells after incubation with non-HLA-restricted LAK cells derived from the same donor.

Conclusions: Reducing expression of HLA in a class- or allele-specific manner may be as effective as utilization of powerful non-specific immunosuppressive drugs, and would represent a fundamental shift in the concept of achieving histocompatibility, by engineering the graft rather than immunosuppressing the host. While efficient gene transfer to entire solid organs remains a technical hurdle, application of this strategy can be readily envisioned for ex vivo transduction of cellular transplants such as bone marrow and stem cells, pancreatic islet cells, and keratinocytes.

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812 HUMAN PLASMACYTOID DENDRITIC CELLS INDUCE PROFOUND HYPORESPONSIVENESS AND SUPPRESSIVE CAPACITY IN ALLOGENEIC T-CELLS

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Background: A major barrier for induction of liver transplant tolerance in humans is their large repertoire of allo-reactive memory T-cells, which rapidly respond to allo-antigens, even when these are presented by non-professional antigen-presenting cells. We investigated whether human plasmacytoid dendritic cells (PDC) can induce hyporesponsiveness and suppressive capacity in allogeneic T-cells containing memory T-cells.

Methods: Purified PDC from human blood, were stimulated with Toll-Like Receptor (TLR)-7 agonist loxoribine (LOX) or TLR-9 ligand CpG A. After 20 hours, allogeneic T-cells containing $CD45RA^+$ naïve and $CD45RO^+$ memory cells, were added. T-cell proliferation, cytokine production, CD25 and Foxp3-expression were determined after 7 days. Hyporesponsiveness was assessed in re-stimulations with LPS-matured monocyte-derived DC (MoDC), and suppressive capacity was determined by adding graded numbers of PDC-primed T-cells to responder T-cells stimulated by mature MoDC, either derived from the same donor as PDC, or from a third party.

Results: Toll-like receptor (TLR)-stimulated PDC primed allogeneic T-cells to produce IL10 (CpG-PDC: 668±260 pg/ml; LOX-PDC: 715±369 pg/ml). After stimulation with CpG-PDC or LOX-PDC 13±2% and 14±2%, respectively, of allogeneic $CD4^+$ T-cells acquired Foxp3 and CD25-expression. CFSE-dilution showed that TLR-stimulated PDC induced proliferation of $CD4+Foxp3^{hi}$ T-cells. No $Foxp3^{hi}$ T-cells were generated when $CD25^+$ T-cells were depleted from allogeneic T-cells prior to their stimulation with PDC, showing that their enrichment was due to expansion from pre-existing Treg. T-cells primed by TLR-stimulated PDC were hyporesponsive upon restimulation with mature MoDC derived from the same donor (90% decrease in proliferation compared with fresh T-cells), and suppressed responder T cells stimulated by mature MoDC in a dose-dependent and donor-specific fashion (55% inhibition of proliferation at suppressor:responder ratio of 1:2). Suppression was abrogated by anti-IL10 receptor antibody.

Conclusion: TLR-stimulated human PDC induced profound hyporesponsiveness nand suppressive capacity in allogeneic T-cells, including memory T-cells. Cellular immunotherapy with PDC from donor blood may be considered as a promising approach to silence the allo-reactive repertoire of liver transplant recipients.

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813 HOST MHC CLASS I-DEPENDENT IMMUNITY IMPAIRS ISLET XENOGRAFT PROLONGATION

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Background: Recent studies show an unexpected role for host MHC class I-dependent immunity and NK cells in promoting islet allograft tolerance. In this study, we determined whether induced islet xenograft prolongation also showed a reliance on MHC class I-dependent pathways. In particular, we tested whether NK1.1+ NK/NKT cells and/or CD8 T cells were required for the long-term (>100 day) survival of rat islet xenografts following a short course of combined anti-CD154 plus anti-LFA-1 monoclonal antibody therapy.

Methods: 450 WF (RT1u) islets were grafted beneath the kidney capsule of streptozotocin-induced diabetic C57Bl/6 (B6; H-2b), B6 NKT cell-deficient CD1-knockout, or CD8 T cell-deficient CD8-knockout mice. Recipients were either untreated or treated with combined anti-CD154 (MR1; 250 μ;g d –1,2,7,9) plus anti-LFA-1 (KBA; 200ug d 0,1,7,14). In addition, some animals were also depleted of NK1.1+ cells prior to transplant (PK136; 500ug d –1). Long-term (>100 day) xenograft survival was confirmed by nephrectomy of the graft-bearing kidney with subsequent return to hyperglycemia.

Results: WF rat islet xenografts were rapidly rejected in all untreated wild-type B6, CD1-deficient, and CD8-deficient recipients (all rejecting within 13 days) indicating that neither CD1-dependent NKT cells nor CD8 T cells are required for rejection. In wild-type B6 mice, anti-CD154/anti-LFA-1 treatment resulted in prolonged, but not indefinite xenograft survival (6/6 grafts rejecting in 62.3 ± 14 days). Such xenograft prolongation was not significantly enhanced in treated CD1-deficient mice (6/9 grafts rejecting in 57.9 ± 12 days; p = NS). In contrast, while anti-NK1.1 treatment itself did not prolong xenograft survival, adjunct depletion of NK1.1+ cells greatly enhanced the efficacy of anti-CD154/anti-LFA-1 therapy with 5/6 xenografts surviving >100 days ($p = 0.015$ relative to anti-CD154/anti-LFA-1 therapy alone). Furthermore, anti-CD154/anti-LFA-1 therapy showed greater efficacy in CD8-deficient animals (6/6 xenografts surviving >100 days; $p < 0.01$ relative to wild-type).

Conclusions: While previous studies show that the depletion of NK1.1+ cells inhibits islet allograft survival, this same approach greatly augments the survival of islet xenografts. Thus, the role of NK1.1+ cells can play opposite roles in the prolongation of islet allografts versus xenografts. Also, while CD8 T cells are not required for acute xenograft rejection, they do constitute a resistance to induced xenograft prolongation in this model. Thus, both NK1.1+ cells and CD8 T cells represent MHC class I-dependent immune pathways that can impede long-term islet xenograft prolongation.

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814 CD300A AND CD300C ON PLASMACYTOID DENDRITIC CELLS ARE DOWN-REGULATED BY TLR7 AND TLR9 LIGAND INDUCED TYPE I INTERFERON

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The success of transplantation relies on the ability to control immune responses. The CD300 family of leukocyte surface molecules are immunoregulatory glycoproteins that balance the immune response. In humans, there are 6 members of the family, CD300a-f. Both CD300a and CD300c are broadly expressed on leukocytes, CD300b, d, and f are expressed on myeloid cells and dendritic cells, whilst CD300e is restricted to monocytes. Plasmacytoid dendritic cells (pDC) constitute a distinct population of DC in the peripheral and secondary lymphoid organs. Human pDC numbers predict for the success of haematopoietic stem cell transplantation and acute graft versus host disease (GVHD). High numbers of pDC in a graft facilitate engraftment. In this study we analysed the expression of CD300a and CD300c on pDC. After activation with the ligands for Toll-like receptors (TLRs), particularly TLR7 and TLR9, pDC secrete large amounts of type I interferons and differentiate into mature DC, expressing co-stimulatory molecules and other cytokines. CD300a and CD300c cell surface molecules are expressed by pDC and their expression was down-regulated by CpG ODN binding TLR9. Because these CD300 molecules