findings, we conclude: 1) maternal delivery of anti-NK cell antibody effectively eliminates fetal NK cell activity for one month after a single dose; 2) early fetal NK cells function as an immune barrier to allo-IUHCT even prior to the expression of receptors for MHC class Ia antigens; and 3) pan-NK cell depletion may disrupt the normal pattern of NK cell self-education in prenatal chimeras. Further study of allo-specific immune responses by early gestational fetal NK cells is needed to develop targeted fetal NK cell depletion as an immunotherapeutic strategy in the clinical application of IUHCT.

30.10. Impact of Gestational Age on Targeted Amniotic Cell Profiling in Experimental Neural Tube Defects.
E. C. Pennington,[1] K. L. Rialon,[1] B. Dionigi,[1] A. Ahmed,[1] D. Zurakowski,[1] D. O. Fauza[1]; [1]Children's Hospital Boston - Surgery, Boston, MA, USA

Introduction: Neural tube defects (NTDs) can present significant diagnostic and ethical dilemmas during the prenatal period, including the perspective of termination of pregnancy. It has been previously shown that the relative proportions of neural and mesenchymal stem cells in the amniotic fluid at term correlate with the type and size of experimental NTDs, suggesting that such targeted quantitative amniotic cell profiling may become a useful diagnostic tool in the prenatal counseling and management of these anomalies. In this study, we sought to determine the impact of gestational age upon this form of amniotic cell profiling. **Methods:** After IACUC approval, 28 time-dated pregnant Sprague-Dawley dams underwent prenatal exposure to 60mg/kg of all-trans retinoic acid on gestational day 10 (E10) for the induction of fetal NTDs (term = E22). Animals were euthanized at different gestational ages, namely E16, E17, E19, and E21, at which time amniotic fluid samples were procured for analysis and fetuses examined for the presence and type of NTDs. Each individual fresh sample of amniotic fluid (n=127) underwent quantitative multicolor flow cytometry for the detection of cells concomitantly expressing Nestin and Sox-2 - primary markers of neural stem cells - as well as of cells concomitantly expressing CD29 and CD44 - markers of mesenchymal stem cells. Data were expressed as the ratio of positive cells to the overall number of cells. Statistical analysis was by nonparametric Kruskal-Wallis ANOVA, with significance set at $p<0.05$. **Results:** Neural tube defects were identified in 77.2% (115/149) of the fetuses - either an isolated spina bifida (n=64), an isolated exencephaly (n=23), or a combination of the two defects (n=16). There was a statistically significant impact of gestational age on the proportions of both amniotic-derived mesenchymal stem cells (aMSCs; $p=0.01$) and amniotic-derived neural stem cells (aNSCs; $p<0.01$) in fetuses with isolated spina bifida, with higher values at E19 compared to E16, E17 and E21. No such impact was noted in normal fetuses ($p>0.10$ for both cell types); in fetuses with exencephaly ($p>0.10$ for both cell types); or in fetuses with combination defects ($p>0.10$ for both cell types). Gestational age had no effect on the aNSC/aMSC ratio in any group (isolated spina bifida - $p=0.24$; exencephaly - $p=0.18$; combination defects - $p=0.65$; normal controls - $p=0.08$). **Conclusions:** Targeted quantitative amniotic cell profiling varies with gestational age in experimental isolated spina bifida. This finding should be considered prior to the eventual clinical application of this methodology as a prospective diagnostic adjunct in the prenatal counseling and management of these anomalies.

TRANSPLANT/IMMUNOLOGY 1: TRENDS IN REJECTION, IR INJURY AND TOLERANCE

31.1. SARS Patey Prize Winner: Availability of T Cell Help Determines Alloantibody Levels And Graft Outcome In A Murine Model Of Antibody-Mediated Rejection.
M. Chhabra,[1] C. J. Callaghan,[1] S. Rehakova,[1] M. C. Negus,[1] J. A. Bradley,[1] G. J. Pettigrew[1]; [1]Cambridge University, Cambridge, United Kingdom

Introduction: The mechanisms by which alloantibody mediates acute or chronic allograft rejection remain unclear. Here we hypothesise that the availability of T cell help determines the outcome of antibody-mediated rejection. **Methods:** MHC-mismatched BALB/c hearts were transplanted into T cell-deficient ($TCR^{-/-}$) CB57BL/6 recipients. T cell help was provided by transfer of either high (10^5 cells) or low (10^3 cells) numbers of TCR-transgenic TCR75 CD4 T-cells that recognise Class I donor H2-K^d antigen as self-restricted processed peptide via the indirect pathway. Alloantibody production was determined by anti-H-2K^d ELISA. CB57BL/6 $Rag2^{-/-}$ recipients that lack both T and B cells were used as control. **Results:** Reconstitution of $TCR^{-/-}$ recipients of BALB/c hearts with 10^5 TCR75 T cells resulted in strong alloantibody responses and acute graft rejection (MST 9 days, n=9). Reconstitution with 10^3 CD4 T cells resulted in much more modest alloantibody production, but was nevertheless associated with endothelial complement deposition, development of progressive allograft vasculopathy and gradual graft loss (MST 50 days, n=6). In contrast, BALB/c hearts survived indefinitely, with minimal allograft vasculopathy, when transplanted into control $Rag2^{-/-}$ recipients reconstituted with either high or low numbers of TCR75 T cells. Notably, passive transfer of $Rag2^{-/-}$ recipients with immune serum from $TCR^{-/-}$ recipients reconstituted with 10^5 TCR75 T cells resulted in acute heart graft rejection and florid intragraft complement deposition. **Conclusions:** The magnitude of the alloantibody response determines whether humoral alloimmunity effects acute or chronic rejection, and is in turn determined by the availability of T cell help. MST – mean survival time. The availability of T cell help is a critical factor in determining the size of the alloantibody response and its consequent ability to mediate acute or chronic graft damage in a newly-developed model of antibody-mediated rejection.

31.2. Mesenchymal Stem Cells Enhance Bone Marrow Engraftment and Induce Immunological Tolerance.
P. Agarwal,[2] M. Rashighi,[2] B. Rajeshkumar,[2] R. F. Saidi[1]; [1]Brown University School Of Medicine, Providence, RI, USA; [2]University Of Massachusetts Medical School, Worcester, MA, USA

Introduction: Successful transplantation currently requires long-term immunosuppression for essentially all patients. This is associated with multiple complications including infection, malignancy and other toxicities. Immunologic tolerance is considered the optimal solution to these limitations. A disadvantage of the current tolerance induction regimen is the requirement for a toxic conditioning making it applicable only for recipients of living donor allografts. In addition, the regimen is not applicable to patients who are too sick to tolerate the pre-transplant conditioning regimen. **Methods:** We studied to achieve tolerance to skin grafts (SG) through mixed chimerism (MC) by simultaneous skin graft and non-myeloablative donor bone marrow transplantation (DBMT) +/- meschenchymal stem cell (MSC). Wild type C57BL6 mice and Bal/C were be used as donors and recipients, respectively. On the day of SG, bone marrow +/- MSC from donors will be infused (250 $X10^6$ bone marrow cells per animal) via tail vein. All recipients will receive rapamycin from day 0 to day 9 and CTLA-4 Ig without radiation. **Results:** DBMT+MSC combination led to stable mixed chimerism and donor-