Evidence for Immune Activation in Kidney Transplant Recipients Who Develop Post-transplant De Novo Anti-HLA Antibodies: Interim Report of NIH CTOT-02

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The NIH CTOT-02 study is a multi-center prospective blinded randomized controlled trial where unsensitized kidney transplant recipients, 3 to 36 months post-transplantat, are screened for development of de novo anti-HLA antibodies. Confirmed positive subjects are then randomized (1:1) to receive rituximab or placebo. To date >400 subjects have been enrolled in the screening phase of the study. Subjects were divided into those who developed anti-HLA antibodies (Ab +) and those who remained negative (Ab -). An interim analyis of baseline characteristics was perfomred, and p-values were calculated using t-test for continuous measures and chi-square or Fisher exact tests for categorical measures. Serum from all the Ab + subjects was also compared with a group of Ab - subjects for a battery of cytokines/chemokines (18) by Milliplex human immunoassay kit, and differences assessed by the Mann-Whitney U test.
To date 26 of 404 subjects (6.4%) are confirmed as Ab +, and the mean time to confirmed Ab + conversion was 14.5 months. The mean age of Ab + subjects was 55.6 vs. 49.6 for Ab - (p=0.023). 53.8% of Ab + subjects were female vs. 29.1% Ab - (p=0.019). 50% of Ab + subjects received a living related allograft compared to 24.6% of Ab - subjects (p=0.047). Interestingly, Ab + subjects were also more likely to have had induction therapy with thymoglobulin (p=0.001). Importantly, analysis of serum cytokine/chemokine profile showed a highly significant difference between the Ab + and Ab - groups with Ab + subjects producing more IFN-gamma, IL-1ra, IL-2 , IL-5, IL-6, IL-7, IL-8, TNF-alpha, IL-10, IL-12(p70), IL-13 and IL-15. Multivariate analysis using logistic regression identifies IL-8, IP-10 and TNF-alpha (all $p < 0.005$) as the best set of serum levels predictive of antibody conversion. ROC analysis of that model results in an area under the curve of 0.93, and sensitivity and specificity of 0.92 and 0.86, respectively. We conclude that development of post-transplant anti-HLA antibodies is mechanistically associated with evidence of immune activation that can potentially be measured and utilized in the future to define immunological risk factors, and ultimately predict long-term outcome early post-transplantation.