Expression of human CD47 in pig glomeruli prevents proteinuria and prolongs graft survival following pig-to-baboon xenotransplantation by Takeuchi K et al.

Supplemental Document

A. Immunosuppression

A total of 10 baboons were divided into three groups as follows (Table 1). Eight baboons received kidneys and vascularized thymic grafts (K+VT) from the same donors [1-3], and two received a kidney one month after intra-bone bone marrow transplantation (IBBM Tx followed by delayed KTx) [4].

All animals received a "basic induction regimen" before K+VT XTx. Rabbit ATG was administered on day -3 at 20 mg/kg and -2 at 10 mg/kg to deplete T cells. Rituximab at 20mg/kg was given 1-2 weeks before transplantation for the depletion of B cells. Following XTx, either anti-CD40L or anti-CD40 mAb was started on POD 2 at 50 mg/kg/day for the first two doses (PODs 2 and 4), and then decreased to 20 mg/kg/day, three times per week in the first month, followed by twice weekly from the second month onwards and scheduled to taper down to once a week beyond POD 120. Eight of 10 received anti-CD40 mAb and 2 baboons received anti-CD40L mAb (Table 1). Mycophenolate mofetil (MMF) was given on day -6 at 110 mg/kg except for the delayed KTx recipients, which received MMF at 70 mg/kg/day, tapered to 10 mg/kg weekly and then finally kept at 30 mg/kg/day. Rapamycin was given intramuscularly (IM) from the 4th post-operative week at a dose of 0.1 mg/kg/day to minimize the rapid growth of pig organs in baboons [5,6].

In order to prevent post K-XTx proteinuria, the second dose of Rituximab at 10 mg/kg was given during renal graft vein/arterial anastomosis to mask SMPDL-3b in kidney xenografts [7] in all animals.

CTLA4-Ig 10 mg/kg was started once a week to decrease the upregulation of CD80 on podocytes when 2+ proteinuria developed in the first post-operative month [5]. Anti-IL6R Ab 10 mg/kg once a week from POD 15 and low dose steroid at 2 mg/kg/day in the first 2 weeks and then 1 mg/kg/day to minimize TSP-1 upregulation contingent on hCD47 expression in kidney grafts and porcine thymocytes on VT grafts in 3 baboons.

B. Assays to assess anti-pig non Gal preformed nAb cytotoxicity (CDC)

Our K+VT XTx protocol included pre-screening of baboon sera using complement-dependent cytotoxicity (CDC) assays on GalT-KO PBMC [4,8]. CDC of baboon sera against porcine PBMC was measured. Briefly, the detection of lysed cells was performed using the fluorescent viability stain 7-actinoaminomycin D (7-AAD, Sigma) in place of trypan blue. Analysis of cells stained with 7-AAD was collected for $2x10^5$ cells per sample using FCM (FACS CANTO II, BD Biosciences, CA).

C. Histology

Excised kidney graft tissue samples were divided into two sections. Formaldehyde fixed tissue samples were stained using either hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) stains. Coded samples were examined by light microscopy, and rejection was diagnosed by a board-certified pathologist [9-11]. Frozen samples were used for immunohistochemistry. Anti-human IgM, IgG, C3, C4d, and C5b conjugated to FITC (all from DAKO, Carpentaria, CA), were used for immunohistochemistry to assess antibody binding and complement activation in the graft [5]. To confirm hCD47 and Nephrin expression, donor kidneys and baboon kidneys were used and stained with anti-human CD47 (Santa Cruz, Dallas, Texas), and anti-Nephrin antibody (Abcam, Cambridge, UK, and Santa Cruz, Dallas, Texas). Immunofluorescence staining was performed on frozen sections to assess IgM, IgG, and Complement deposits as well as TSP-1 upregulation (Abcam, Cambridge, UK) and SIRP α (Bio legend, San Diego, CA).

D. Quantitative analysis of hCD47 in donor kidneys

Formalin-fixed and paraffin embedded tissue samples were used to assess hCD47 expression in glomeruli. Kidney samples from contralateral donor kidney or graft kidney were used. After deparaffinization and rehydration, heat mediated antigen retrieval was performed using Diva Decloaker® (Biocare Medical, Pacheco, CA). After blocking by avidin/biotin (Vector Laboratories, Burlingame, CA) and Protein block®(Agilent, Santa Clara, CA), anti hCD47 antibody (BD Biosciences, San Jose, CA) was incubated for 1 hour in room temperature, followed by biotin conjugated anti-mouse IgG and Alexa 488

conjugated streptavidin (Fisher Scientific, Waltham, MA). As a negative control, 1% BSA in PBS was used. The mean fluorescence intensity (MFI) of glomeruli was measured by ImageJ software (https://imagej.nih.gov/ij/), and the average and SD of MFI was shown in the figure. Two samples that had beyond mean+2SD in negative control were excluded from analysis due to the quality of samples. A two-tailed unpaired t-test was performed. A P value of <0.05 was considered statistically significant.

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