P01

ANTI MICA (MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I RELATED CHAIN A) ANTIBODY, WHETHER TO TREAT OR AVOID IN RENAL TRANSPLANTATION?

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Introduction: Pre-transplantation anti-major histocompatibility complex class-I related chain A (MICA) sensitization is an uncommon event and its role in kidney graft evolution is not completely defined. Even when kidney allografts are well matched for HLA as in living related transplant and anti-HLA antibodies are not detected, graft rejection can still occur. Anti MICA antibody is reportedly associated with poor transplant outcomes and a high risk of acute and chronic rejection in renal transplantation.

Methods: A retrospective study of patients undergone living renal transplantation between years 2000-2014 was performed. Recipients were classified in two groups, pre-transplantation Anti MICA antibody positive group (n=17) and antibody negative comparison group (n=17). Patients with anti HLA antibodies were excluded and only isolated MICA positive patients were included in the study group. Both groups were comparable in recipient age, donor age, donor relation, HLA and immunosuppression.

Results: Patients with pre transplant MICA antibody positivity were associated with increased acute rejection rate as compared to comparison group (47% vs 11.7 %, p value= 0.02). Renal function in MICA positive group and comparison group were comparable over a mean follow up of 6.5 years (mean creatinine 1.58 vs 1.53 mg/dl). Rate of chronic rejection was same in both groups (5.8%). No patient loss or graft loss occurred in either group over mean follow up of 6.5 years.

Conclusions: Isolated Anti MICA antibody positivity is uncommon event. Pre transplant anti MICA antibody positivity is associated with increased acute rejection rates. However chronic rejection rates and renal function are comparable in both groups. Thus our study emphasizes that MICA antibody positive patients may require more aggressive immunosuppression. Role of desensitization has to be defined.

P02

ENHANCED IMMUNOPATHOLOGY EVALUATION OF HUMAN RENAL BIOPSIES USING MULTICOLOR FLOW CYTOMETRY AND CYTOKINE ANALYSIS: A FOCUS ON TRANSPLANTED KIDNEYS

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Introduction: Current histologic processing of renal biopsies provides limited information about immune mechanisms causing kidney injury and disease activity. To overcome this we developed a protocol to reduce a fraction of a renal biopsy to single cells for multicolor flow cytometry and for capture and quantitation of cytokines present in the biopsy. Using this technique we define new criteria for evaluating rejection and renal inflammation that is useful for directing therapy.

Methods: A third of a standard kidney biopsy core is reduced to a cell suspension amenable for multi-color flow cytometry without losing cells or epitopes of interest. The resulting supernatant is used for measuring cytokines with high-sensitivity Milliplex reagents on a Luminex platform. After analysis of hundreds of transplant biopsies we now generate clinically useful reports describing lymphocyte subsets, endothelial antibody and eculizumab binding and IL-6, IL-8 and IL-10 levels.

Results: A ratio of CD8+ to CD4+ T lymphocytes greater than 1.2 in transplanted allografts is associated with rejection, even before it is apparent by microscopy. Elevated numbers of CD45 leukocytes and higher levels of IL-6, IL-8 and IL-10 within the biopsy indicate more severe injury.

Peripheral blood T lymphocyte subsets do not correlate with those found within the renal allograft. In addition, lymphocytes within the kidney express variable degree of activation based on expression of CD69 and HLA-DR, while these markers are low or absent on peripheral blood lymphocytes.

Antibody binding to renal microvascular endothelial cells can be measured by cytometry and corresponds to antibody-mediated forms of allograft rejection. Eculizumab binding to endothelial cells suggests complement activation, which may be independent of bound antibody, or associated with it.

Conclusions: Assessment of leukocyte subsets, renal microvascular endothelial properties and measurement of cytokines within a renal biopsy enhance understanding of pathogenesis, provide disease activity markers and identify potential targets for therapy.

P03

RITUXIMAB IN DIFFICULT PAEDIATRIC NEPHROTIC SYNDROME: AN ACCOUNT FROM EASTERN INDIA

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Introduction: Reports on the utility of rituximab in difficult nephrotic syndrome (NS) have been varied. We retrospectively analyzed the outcome of rituximab, used in a multi centre cohort of difficult nephrotic syndrome (NS) from Eastern India.

Methods: Data was collected for all children with NS who received rituximab from May 2011 to Nov 2015. Steroid resistant (SRNS) and steroid dependent / frequently relapsing (SDNS /FRNS) were identified as per standard definition. Complete response (CR) for SRNS was defined as normalization of serum albumin and urinary protein creatinine ratio (UPCR) whereas for partial response (PR); 50% improvements in these parameters along with albumin at last follow up $\geq$ 2gm/dl. In cases of SDNS/FRNS; CR was defined as stoppage of steroid and absence of relapses for at least a year and PR as discontinuation of steroid without any relapses for at least six month or reduction in steroid threshold by at least 50%.

Results: 34 children (56 % male) were identified (SRNS =12, SDNS/FRNS =22). Among SRNS all had failed steroid (S), mycophenolate(M) as well as calcineurin inhibitor (CNI) except two who were CNI naïve prior to rituximab. Among the SDNS/ FRNS group, 11 (50%) children had failed all drugs (S, M, CNI & cyclophospha-mide) and the rest were not exposed to CNI. Majority were minimal

change nephrotic syndrome (MCNS) (62%) followed by focal segmental glomerulosclerosis (FSGS, 27%) and IgM nephropathy (11%). Median age was 7.8 (Range 2.5 − 16.5) years with median follow up post rituximab 33 (Range 1.5 to 87) months. Rituximab was given as infusion at 375 mg / m2 . Each cycle constituted of 2 injections at an interval of 1-2 weeks followed by confirmation of B cell depletion. Single cycle achieved total B-cell depletion in all. 5 cases (all SRNS) received 2. Median duration for normalisation of CD 19 was 4.65 (3 − 7) months. Among the SRNS, serum albumin rose from mean 1.87 (SD ± 0.53) to 2.63 (SD ± 1.1) g/dl, (p=0.06) and UPCR fell from mean 19.3 (SD ± 12.04) to 9.8 (SD ± 10.96) p= 0.15. 20% of SRNS (n=2) achieved CR another 20% PR. Steroid threshold among SDNS/FRNS fell from mean 0.47 (SD ± 0.19) to 0.17 (SD ± 0.23) mg/kg, p =0.0003 and dose of steroid at last follow up fell men 0.88 (SD ± 0.56) to 0.22 (SD ± 0.43) mg/kg, p =0.0009. 50% of SDNS/FRNS (n=11) did not have any relapse during the follow up period and median time to first relapse was 6.6 (Range 0.3 − 25) months.

Conclusions: Rituximab was demonstrated to be useful with significant benefit particularly in the SDNS group.

P04

A NOVEL MOUSE MODEL OF MEMBRANOUS NEPHROPATHY INDUCED WITH HETEROLOGOUS RABBIT ANTI-THSD7A ANTIBODIES

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Introduction: Phospholipase A2 receptor 1 (PLA2R1) and thrombospondin type-1 domain-containing 7A (THSD7A) are podocyte membrane proteins that have been identified as target antigens for autoimmunity in membranous nephropathy (MN). The investigation of the pathogenicity of the involved autoantibodies has been hampered by the fact that PLA2R1 is not expressed on rodent podocytes. On the other hand, THSD7A is expressed on mouse podocytes and shares over 90% of sequence homology with the human protein. We have recently demonstrated that anti-THSD7A antibodies isolated from a patient with THSD7A- associated MN can cause morphological and clinical MN in mice, allowing further investigations of disease pathogenesis. However, THSD7A-associated MN is a rare entity, incapacitating patient antibodies for larger experimental procedures.

Methods: We generated polyclonal antibodies against human and mouse THSD7A in rabbits using cDNA immunization. IgG from the immunized rabbits and from preimmune control rabbits was affinity-purified and intravenously injected into mice. Urine was collected daily and investigated for the development of proteinuria by measurement of albumin-to-creatinine ratios. Mice were sacrificed five, nine, and fourteen days after injection of rabbit IgG and kidneys were analyzed using confocal and light microscopy.

Results: Purified IgG from immunized rabbits recognize, like patient autoantibodies, (a) conformation-dependent epitope(s) present in both native human and mouse THSD7A in vitro. Moreover, two hours after intravenous injection into mice, rabbit IgG is bound along the glomerular filtration barrier. Two days later, mice develop

proteinuria that rapidly increases and reaches around 10 g/g after 5 days and 200-300 g/g albumin-to-creatinine after 14 days with some mice developing a severe nephrotic syndrome with ascites and hyperlipidemia. In immunofluorescent analysis, granular rabbit IgG is found subepithelially along the glomerular filtration barrier after 14 days and immunohistochemistry for rabbit IgG shows the classic picture of human MN. Mice injected with purified IgG from rabbit serum that was taken before THSD7A-immunization fail to develop any of these changes.

Conclusions: Our study introduces a heterologous mouse model that allows further mechanistic investigations of the molecular events leading to membranous nephropathy.

P05

IDENTIFICATION OF GLYCOSAMINOGLYCANS THAT SPECIFICALLY INHIBIT THE LECTIN PATHWAY OF COMPLEMENT

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Introduction: The complement system can be activated via three pathways, the classical (CP), alternative (AP) and lectin pathway (LP). Studies have shown a role for complement in renal diseases such as hemolytic uremic syndrome, C3 glomerulopathy and renal transplantation. It is well known that heparin and some other glycosaminoglycans (GAGs) inhibit complement activation. We tested the pathway specific complement inhibiting potential of >70 GAGs. The results showed that some GAGs had a specific inhibitory effect on the LP only. In this work we unravel the inhibitory mechanism of GAGs on the LP.

Methods: GAGs were tested in the Wieslab complement screening assay for a dose-dependent inhibition assay of the complement pathways. Inhibition of ficolin-3 mediated LP activation by GAGs was tested in the Wieslab ficolin-3 assay. To assess whether GAGs inhibit the MBL − mannan interaction, GAGs were added to diluted serum and incubated on mannan coated plates where after deposition of MBL was measured. To test the inhibition of GAGs on MASP activity, the GAGs were added to purified C4 and incubated on MBL-MASP coated plates, C4 activation was measured by detection of C4b. To assess whether the MBL-MASP complex binds to heparin, serum was incubated on heparin-albumin coated plates and binding of MBL, MASP-1 and MASP-2 was measured.

Results: In the Wieslab complement screening assay, (LMW-)heparin dose-dependently inhibited all three pathways of complement. Depolymerization of heparin followed by size-fractionation of the resultant oligosaccharides revealed hexasaccharides and tetrasaccharides to exclusively inhibit the LP, without any inhibition potential for the CP and AP. Heparin (oligo's) also show a dose-dependent inhibition of the ficolin-3 mediated LP activation, however failed to inhibit the binding of MBL to mannan, indicating inhibition by GAGs of MASP1/2 enzymes. This was proven in the MASP-1/2 inhibition assays. Heparin (oligo's) inhibited the C4 cleavage by MASP-1/2 in a dose-dependent way. Comparison of defined heparin-derived tetrasaccharides with various sulfation revealed the fully N-, 2-O, 6-O-sulfated fragment the most potent MASP-1/2 inhibitor. Vice versa the MBL-MASP complex shows