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