CONTRIBUTION OF RENAL SECRETED COMPLEMENT C3 TO THE CIRCULATING POOL IN HUMANS 253

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Accumulating evidence indicates that complement C3 produced within the kidney is an important mediator of renal inflammatory and immunological injury. Meaningful interpretation is limited by the lack of data on the amount of C3 the kidney can produce in normal and inflamed states. This was investigated by utilizing the conversion of complement proteins from recipient to donor allotype (C3S to C3F) that occurs following renal transplantation.

We examined the C3 allotypes of 80 consecutive renal donor-recipient pairs (148 individuals) by amplification refractory mutation system analysis. The extent of allotype conversion in C3 F/S mismatched recipients was quantified by enzyme-linked immunosorbent assay at different stages post-transplantation and confirmed by Western blotting. Twenty-one of the 80 donor-recipient pairs were informative (C3 FF or FS donors; C3 SS recipients). In the early post-operative period, the level of donor-derived C3F was undetectable, increasing to 9.6% of the total circulating C3 at times of acute allograft rejection. When graft dysfunction occurred from causes other than rejection, namely acute tubular necrosis, cyclosporin toxicity, thrombosis, urinary tract or cytomegalovirus infection, its levels remained undetectable. After stable graft function was attained (3-13 months post-transplant), donor-derived C3 contributed to 4.5% of the total C3 pool.

Our findings demonstrate that the kidney in the resting state is a significant source of extrahepatic C3. Its heightened local synthesis during rejection episodes suggests a possible pathogenic role of C3 in this immunological process. The concept of local C3 synthesis may have potential implications for strategies directed against intrarenal inflammatory injury.

PROXIMAL TUBULAR KIDNEY CELLS EXPRESS A FUNCTIONAL ALTERNATIVE PATHWAY AND RESPOND TO C3a RECEPTOR LIGATION. 254

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It is not known if the pathways of complement are functional in the human kidney. We have used RT-PCR to investigate the potential for the kidney to synthesise all proteins of the alternative pathway, [factors B, D, I and H, properdin and C3] both in vivo and in vitro. Poly dT cellulose was used to purify mRNA from medulla and glomeruli isolated from healthy areas of kidneys removed because of malignancy, and from HK2 tubular epithelial cells in culture. The integrity of the resultant products was determined by their apparent size upon electrophoresis, and by the formation of predicted fragments after cleavage with an appropriate restriction endonuclease. Freshly isolated and cultured human glomeruli, and fresh medulla all produce mRNA for all components of the alternative pathway as do cultured HK2 cells both under normal conditions and on cytokine stimulation. Immunoprecipitation of ^{35}S-methionine labelled supernatants showed HK2 cells produce C3, factor B and factor H proteins by de novo synthesis as well as C3a, which was detected by RIA.

We further demonstrated that glomeruli and HK2 cells express mRNA for the C3a receptor. Ligation of this receptor with C3a lead to an increase in intracellular IP3 levels, and to tyrosine phosphorylation of at least 2 proteins in a pertussis-toxin sensitive fashion. C3a desarg also induced phosphorylation of these proteins. We conclude that human renal glomerular, medullary and tubular epithelial cells have the potential to synthesise all proteins required for activation and regulation of the alternative complement pathway. The binding of urinary C3a to the C3a receptor has the potential to activate tubular epithelial cells, while their ability to produce C3a could lead to autoactivation via this receptor. These findings could have implications for renal immunopathology.

PRODUCTION OF RAT CD59 AND CHIMERIC CD59-CRRY AS ACTIVE SOLUBLE PROTEINS IN *PICHIA PASTORIS*. 255

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Crry (CR1-related gene/protein) is a rodent complement regulator that combines the activities of human DAF and MCP towards C3/C5 convertases. CD59 is a conserved protein inhibitor active towards C8 and C9. We have previously produced rat Crry as a recombinant soluble (rs) protein in *P. pastoris*. In this study we produced functionally active rsCD59 and a chimeric rsCD59-Crry protein in *P. pastoris*. The GPI anchor addition site of CD59 (Asn-79) was either replaced by a stop codon to produce rsCD59, or with the sequence of the first 5 short consensus repeats of Crry to produce rsCD59-Crry. These constructs were cloned in pPIC9 which was integrated into *P. pastoris* strain GS115 genomic DNA. Proteins were generated by fermentation with methanol stimulation of the *AOX1* promoter and purified from the fermentation media by affinity chromatography on an anti-CD59 mAb 6D1 column. By N-terminal amino acid analysis, the CD59 was entirely native protein, beginning with amino acid 1 (Leu) of the known sequence. By immunoblotting, rsCD59 reacted with anti-CD59 mAb 6D1, rsCrry reacted with anti-Crry mAb 5I2, and rsCD59-Crry was reactive with both 6D1 and 5I2. In a standard classical pathway assay, all 3 rs proteins had inhibitory activity, with 50% inhibition at 0.44 μM (rsCrry), 0.50 μM (rsCD59-Crry) and 4.6 μM (rsCD59). In an assay examining inhibition of C5b-9 in which C5b-7 were first formed followed by purified C8 and C9, rsCD59 and rsCD59-Crry were active with 50% inhibition at 1.0 μM (rsCD59) and 0.58 μM (rsCD59-Crry). If serum diluted in EDTA was used as a source of C8 and C9, 50% inhibition of lysis occurred at 4.6 μM (rsCD59) and 1.6 μM (rsCD59-Crry). The degree of inhibition was independent of whether C8 and C9 were of rat or human origin. Therefore, we have produced rsCD59 and rsCD59-Crry in *P. pastoris*. rsCD59 retains inhibitory activity towards C5b-9, while rsCD59-Crry appears to have the combined activities of Crry and CD59. In a hemolytic assay, the inclusion of CD59 to Crry is of no additional benefit to Crry, likely illustrating the overall importance of the C3/C5 convertase step. Yet, in a C5b-9 assay, inclusion of Crry to CD59 increases potency 1½-3 times. This may be in part because the Crry in rsCD59-Crry targets the inhibitor to C3b on the cell surface, which is where C5b-9 is generated.

CHARACTERIZATION OF COMPLEMENT RECEPTORS FROM RAT GLOMERULAR EPITHELIAL CELLS 256

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The mouse *Cr2* gene gives rise to two alternatively spliced mRNA transcripts that are translated into mouse complement receptor (CR)1 and CR2. Consequently, both proteins share 15 short consensus repeats (SCR), which is the fundamental unit of the regulators of C activation proteins. In the mouse, CR1 and CR2 are present on B lymphocytes and follicular dendritic cells. In contrast, CR1 is more widely distributed in humans, and is present on glomerular epithelial cells (GEpC). We have been interested in the presence of CRs in rat glomeruli and have found a 200-kDa C3b-binding protein in cultured rat GEpC, which we considered to be rat CR1. Furthermore, 200-kDa proteins reactive with anti-human CR1 Ab are present both in cultured GEpC and in rat glomeruli. Lastly, 4.5 kb mRNA from rat GEpC and glomeruli were identified by northern blotting using nucleotide probes derived from human CR1 and from rat Crry (CR1-related gene y).

In this study, our aim was to clone the cDNA for rat GEpC CR1. A GEpC cDNA library was constructed in lambda ZAP II. Initially, this was screened with a nucleotide probe derived from rat Crry. However, with this approach, only cDNA for Crry were obtained, likely reflecting the abundance of Crry in GEpC. Subsequently, a PCR-based approach was used with the cDNA library as the template. An array of primers were systematically designed from the known mouse CR1/CR2 nucleotide sequence not homologous with Crry. The PCR products obtained were cloned in pCR2.1 and sequenced. Thus far, 3 kb of cDNA has been obtained by this "PCR walking" approach. The nucleotide sequences are 92% similar to those of cDNA for mouse CR1/CR2.

Thus, rat GEpC appear to have a CR1-like protein on the basis of its capacity to bind C3b, its reactivity with anti-human CR1 Ab, and the presence of a 4.5 kb mRNA that hybridizes with cDNA for rat Crry and human CR1. It seems likely that the cDNA we have isolated in this study is that for this CR1-like protein from rat GEpC, which is highly homologous to mouse CR1/CR2. Further characterization of rat GEpC CR1 will allow studies to determine its role *in vivo*.