Hycult Scope

Complement activation by virus infection

Virus infection can activate all three pathways of the complement cascade. The classical pathway is activated by the binding of the C1q1r1s complex with antibodies captured by virus-infected cells and by direct binding of C1q to viral surfaces in the absence of immunoglobulin.

The lectin pathway is antibody- independent. Ficolins and MBL complexes with MBL-associated serine proteases (MASPs) and bind to the surface of virus infected cells.

The activation of the alternative pathway occurs on viral particles that is initiated by a lack of factor H binding to the surface. Factor H binding and regulation of the alternative pathway depends on the addition of sialic acid to carbohydrates found on the cell surface, which can be decreased following infection by some viruses. Furthermore, C3 is cleaved into C3a and C3b. C3b is deposited onto virus particles and has multiple antiviral activities.

Complement activation by all three pathways results in several effector functions that contribute to virus inactivation and elimination. These functions include opsonisation of virions by complement components promoting phagocytosis, virolysis by the TCC (MAC), and through the production of anaphylatoxins and chemotactic factors.

References

1. *A.W. Tarr, R.A. Urbanowicz, J.K. Ball. The Role of Humoral Innate Immunity in Hepatitis C Virus Infection. Viruses 2012, 4:1*
2. *C.E. Blue, O.B. Spiller, D.J. Blackbourn. The relevance of complement to virus biology. Virology 2004, 319: 176*

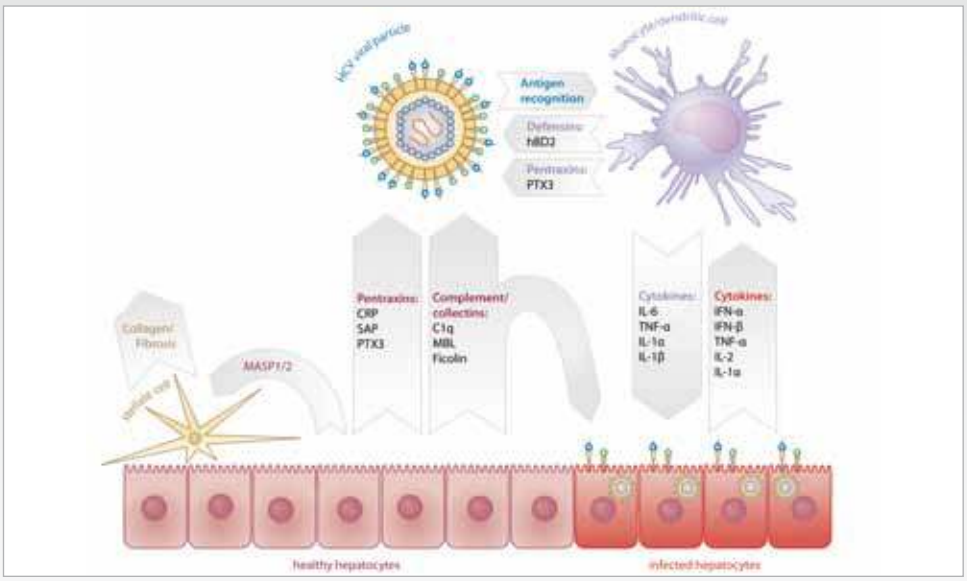

Figure 1. Overview of innate recognition of Hepatitis C Virus (HCV) antigens. Infected cells produce type I interferons, supporting activation of the inflammatory response. Monocytes and dendritic cells recognize viral antigens, resulting in activation of defensins, pentraxins and pro-inflammatory cytokines. These cytokines induce production of acute phase proteins in hepatocytes, which contribute to clearance of viruses and infected cells. The mannose binding lectin (MBL), and L & H ficolins, recruit MBL-associated serine proteases (MASP-1 & MASP-2), triggering the complement cascade and activate liver-resident stellate cells that produce collagen, resulting in progressive fibrosis.

Highlights in Complement Research

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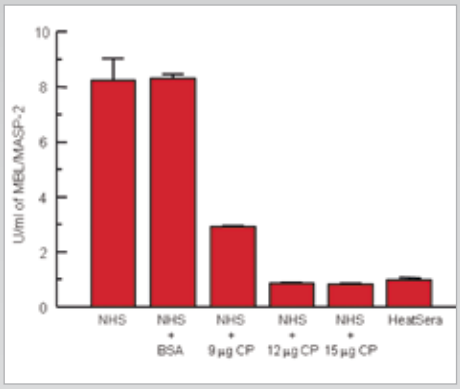

Figure 2 Human astrovirus coat protein (CP) inhibits lectin pathway activation. Units of MBL/MASP-2 were obtained from samples using HyCult Biotech human serum functional MBL/MASP-2 ELISA (HK327). NHS alone, NHS + 15 µg BSA, NHS with the indicated amounts of CP and heat-inactivated serum (HeatSera, negative control for activation) were incubated at 37°C for 1 h. Samples were applied to mannose-coated wells to capture MBL from serum, C4 was then added to the plate, and bound products of C4 activation (expressed as units of MBL/MASP-2) were determined. Data are the means for four independent experiments. Error bars denote SEM.

Identification of a novel inhibitor of lectin pathway activation

Neel K. Krishna and Kenji M. Cunnion, Eastern Virginia Medical School, Norfolk, Virginia USA.

The lectin pathway of the complement system is a critical component of the innate immune response in humans. However, aberrant activation of this pathway has been implicated in numerous inflammatory conditions such as ischemia-reperfusion injury. Our laboratory has recently discovered that the capsid protein (CP) of astrovirus, a gastrointestinal pathogen of humans, can potently suppress the lectin pathway of complement by binding to the lectin pathway initiator molecule, mannose binding lectin (MBL) and interfering with the interaction between MBL and its cognate serine protease, MASP-2. To assess the ability of astrovirus CP to inhibit lectin pathway activation, we utilized the Hycult Biotech human functional MBL/MASP-2 assay (HK327) that measures C4 activation by MBL/MASP-2 in serum. Samples were generated by incubating normal human serum (NHS) with 15 µg BSA or increasing amounts of CP at 37°C for 1 hour. Samples were then applied to the MBL/MASP-2 kit which uses mannan-coated wells to capture MBL from serum. After washing, C4 was added to the plate and bound products of C4 activation (expressed as units of MBL/MASP-2) were measured. NHS alone or in the presence of BSA demonstrated high levels of lectin pathway activation compared to heated serum which was used as control for background signal in the absence of complement activation (Fig. 2). Adding 9 µg of CP decreased MBL pathway activation by 74%, compared with NHS alone (P = 0.02) and adding 12 µg of CP decreased lectin pathway activation by 100%, compared with NHS alone (P = 0.01) (Fig. 2). These findings demonstrate that CP is able to strongly inhibit activation of the lectin pathway. CP and its derivatives may have therapeutic potential to inhibit pathogenic conditions in which aberrant lectin pathway activation is implicated.
Reference: Hair et al; Molec Immunol 2010, 47:792

ELISA Kits

Cat. #	Product
DH001	Hycult Dx Human MBL
DH003	Hycult Dx Complement factor H
HK208-02	MBL-A, Mouse
HK209-02	MBL-C, Mouse
HK210-02	MPO, Mouse
HK211-02	C1q, Mouse
HK323-02	MBL, Human
HK326-02	MASP-2, Human
HK327-02	MBL/MASP-2, Human
HK328-02	TCC, Human
HK334-02	Properdin, Human
HK336-02	L-ficolin, Human
HK339-02	MASP-3, Human
HK340-02	H-ficolin, Human
HK342-02	Complement factor H, Human
HK343-02	Complement Factor D, Human
HK347-02	Pentraxin 3, Human
HK349-02	C5a, Human
HK353	Factor H, 402H/Y variant detection, Human
HK354-02	C3a, Human
HK356-02	C1q, Human
HK358	CRP, Human

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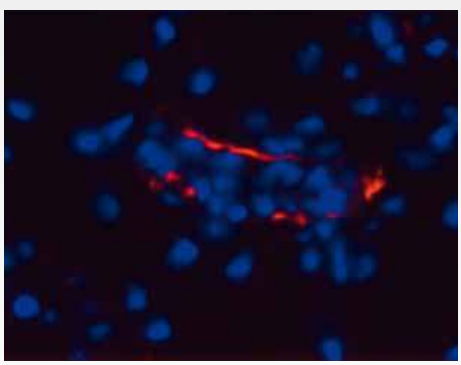
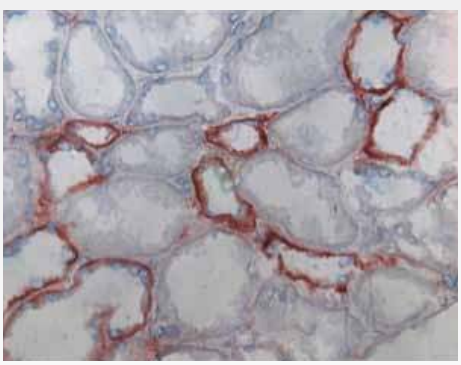
Novel insights in localization & expression of C5aR and C5L2

Maaike B. van Werkhoven, Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, The Netherlands. Molecular Immunology 2013, 53:237-245

Distinct renal expression patterns for C5L2 and C5aR have been detected. The findings mentioned in the article suggest a functional role for renal C5L2 rather than being a C5a decoy receptor.



C5L2 in human renal tissue. Staining of frozen section with antibody to human C5L2 (Cat.# HP9036).



C5aR in human renal tissue. Staining of distal tubuli or proximal tubuli (*) in frozen section with antibody to human C5aR(Cat.# HM2094).

Nucleosomes and C1q bound to glomerular endothelial cells serve as targets for autoantibodies and determine complement activation

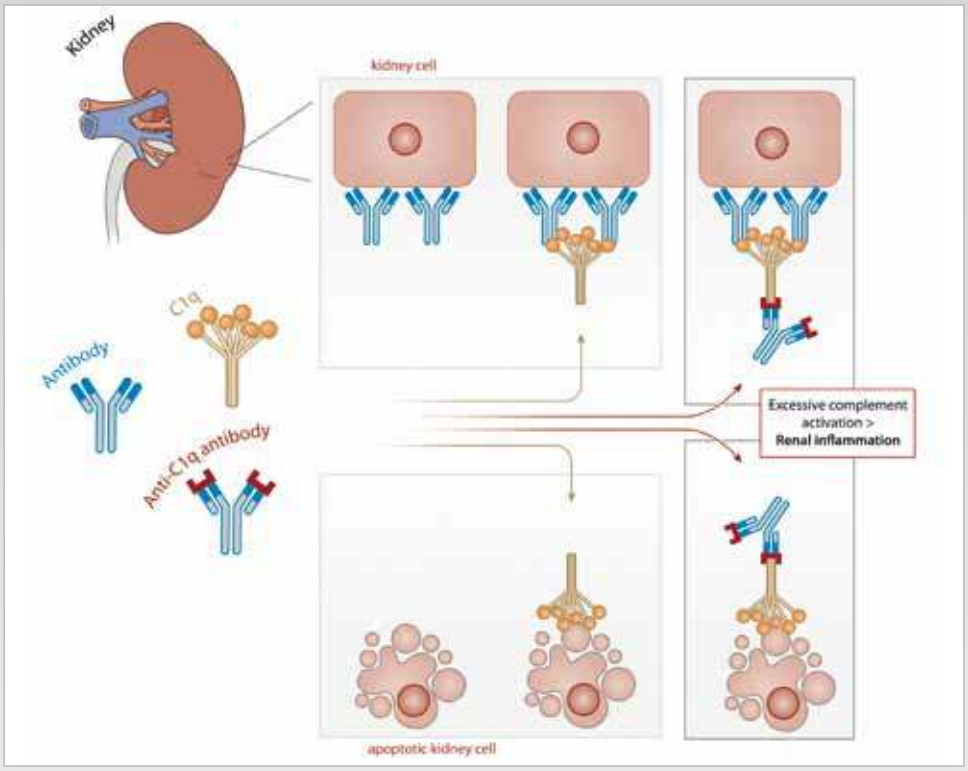


Figure 3: During renal inflammation, immune complexes and apoptotic cells provide binding sites for C1q. Binding of C1q to these substrates results in the generation of neoepitopes that are recognized by anti-C1q antibodies. Binding of anti-C1q antibodies amplifies complement activation, resulting in exaggerated tissue inflammation. (Pickering, M. Nat. Rev. Rheumatol 2010. 6, 490-493)

Joseph O'flynn, Department of Nephrology, Leiden University Medical Centre.

The role of Autoantibodies against C1q and nucleosomes in Lupus Nephritis

Various studies indicate a role for both anti-nucleosome and anti-C1q autoantibodies in glomerulonephritis in patients with systemic lupus erythematosus. However, a causal relationship between these autoantibodies and the development of lupus nephritis has not been fully established. Since injury of the endothelium is a major target in lupus nephritis we assessed the interaction of C1q and nucleosomes with glomerular endothelial cells in vitro, in the presence or absence of autoantibodies against these antigens.

C1q and nucleosomes bind glomerular epithelial cells in vitro

In our study we demonstrated a direct and dose-dependent binding of both nucleosomes and C1q to immortalized human glomerular endothelial cells (GEnC) in vitro, which in part is mediated by cell surface heparin sulfate. We demonstrated that nucleosomes and C1q serve as targets for monoclonal and polyclonal antibodies as well as for anti-nuclear autoantibodies from patients with systemic lupus erythematosus. An additive effect of anti-C1q autoantibodies on anti-nucleosome mediated complement activation was observed. Furthermore, we showed that the activation of complement on glomerular endothelial cells is mediated by the classical pathway since the deposition of C3 on GEnC is abrogated by MgEGTA and does not occur in C1q-depleted serum.

Conclusions

Taken together, our studies demonstrate a direct binding of both nucleosomes and C1q to glomerular endothelial cells in vitro. The subsequent binding of autoantibodies against nucleosomes in patients with systemic lupus erythematosus is potentially pathogenic and autoantibodies against C1q seem to have an additional effect. The complement activation was shown to be mediated by classical pathway and C1q, which is bound either directly or indirectly via autoantibodies to the endothelial glomerular cells.

Antibodies

Cat. #	Product
HM1044	C1q, Mouse, mAb 7H8, 100 µg
HM1045	C3, Mouse, mAb 11H9, 100 µg
HM1065	C3b/iC3b/C3c, Mouse, mAb 2/11, 100 µg
HM1073	C5, Mouse, mAb BB5.1, 100 µg
HM1078	C3b/iC3b/C3c, Mouse, mAb 3/26, 100 µg
HM1096BT	C1q, Mouse, mAb JL-1, Biotin, 50 µg
HM2056	Mannose receptor, Human, mAb 15-2, 100 µg
HM2074	C3a/C3a des-Arg, Human, mAb 2991, 100 µg
HM2077	C5/C5a, Human, mAb 557, 100 µg
HM2089	H-ficolin, Human, mAb 4H5, 100 µg
HM2091	L-ficolin, Human, mAb GN5, 100 µg
HM2094	C5aR, Human, mAb S5/1, 100 µg
HM2167	TCC, Human, mAb aE11, 100 µg
HP8014	C6, Mouse, pAb, 100 µg
HP8015	C5L2, Mouse, pAb, 100 µg
HP8033	C4d, Mouse, pAb, 100 µg
HP8034	C4d, Rat, pAb, 100 µg
HP9036	C5L2, Human, pAb, 100 µg
HP9039	M-ficolin, Human, pAb, 50 µg

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Clinical associations of H-ficolin in neonates

Maciej Cedzynski, Laboratory of Immunobiology of Infections, Institute of Medical Biology, Polish Academy of Sciences.

Innate immune mechanisms, including complement activation, are particularly important during the neonatal period. Preterm births, low birthweight and infection are the main causes of perinatal morbidity and mortality. The family of human ficolins comprises three oligomeric lectins: M-ficolin, L-ficolin and H-ficolin, being pattern-recognizing molecules. They act as opsonins (L- and H-ficolin) or as a phagocytic receptor (M-ficolin). All of them activate complement via the lectin pathway.

H-ficolin concentrations and FCN3 gene 1637delC dimorphism:
This study included 613 newborns. Cord sera H-ficolin concentrations and corresponding FCN3 gene 1637delC frameshift mutation were investigated to find possible clinical associations of H-ficolin insufficiency/deficiency. H-ficolin levels positively correlated with gestational age as well as birthweight. Both pre-term deliveries and low birthweight (independently of gestational age) were significantly associated with low H-ficolin concentrations (less than 8.6 µg/ml) but not with heterozygosity for 1637delC frameshift mutation. The presence of the variant allele, however, influenced protein level significantly. The median gestational age in low-H-ficolin babies (36 weeks) was significantly lower than in H-ficolin-sufficent babies (39 weeks). Mean birthweights between H-ficolin-insufficient and -sufficient groups differed as much as by 668 g (>20% of average birthweight in a cohort). No association of low protein levels or heterozygosity for FCN3 gene mutation with perinatal infections was found. In a single case of total H-ficolin deficiency (1637delC homozygote, no H-ficolin detectable in cord serum) an infection with Streptococcus agalactiae has been confirmed. However, this premature neonate was moreover mannan-binding lectin-deficient (O/O MBL2 genotype, no MBL detectable, lack of MBL-dependent lectin pathway activity) and had low L-ficolin concentration.

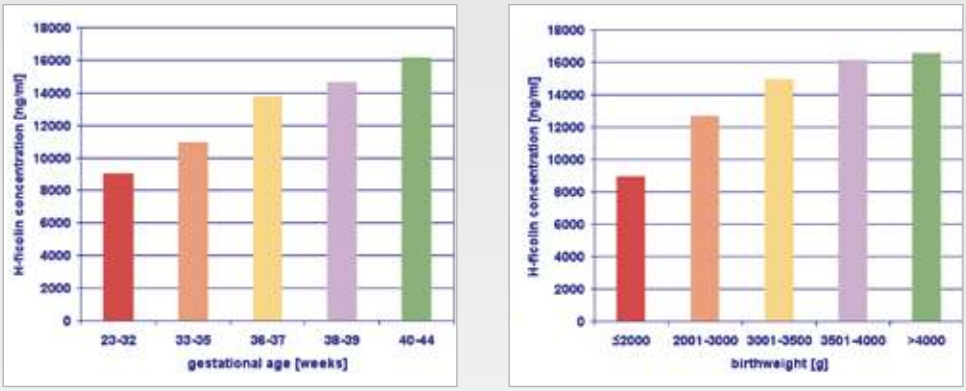

Figure 4
A) H-ficolin concentrations (medians) in cord sera, depending on gestational age.
B) H-ficolin concentrations (medians) in cord sera, depending on birthweight.

Conclusions:
Data from the largest group of neonates studied so far provided evidence for an association between H-ficolin insufficiency and short gestational age and low birthweight. Heterozygosity for the deletion at position 1637 seems not to have major clinical importance. One case of the rare homozygosity for this mutation was described. This condition might be a risk factor for perinatal infection, however more cases have to be studied.

Reference: Michalski M. et al.: H-ficolin (ficolin-3) concentrations and FCN3 gene polymorphism in neonates, Immunobiology 2012, 217, 7, 730–737.

Proteins

Cat. #	Product
HC1101	C5a, Mouse, Recombinant, 50 µg
HC1102	C5a des Arg, Mouse, Recombinant, 50 µg
HC2101	C5a, Human, Recombinant, 50 µg
HC2102	C5a des Arg, Human, Recombinant, 50 µg
HC2122	C1, Human, Natural, 200 µg
HC2123	C1q, Human, Natural, 1 mg
HC2124	C2, Human, Natural, 50 µg
HC2125	C3, Human, Natural, 250 µg
HC2126	C3a, Human, Natural, 50 µg
HC2127	C3a desArg, Human, Natural, 50 µg
HC2128	C4, Human, Natural, 250 µg
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