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Clusterin: modulation of complement function

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Clusterin

Human clusterin (synonyms: complement lysis inhibitor, CLI; sulfated glycoprotein 2, SGP-2; sp-40,40; testosterone-repressed prostatic messenger 2, TRPM-2; gp-III; apolipoprotein J, apo-J; gene name: CLI) was recently discovered as a integral component of the soluble C5b-9 complement complex [1-3], which is assembled in the fluid phase (plasma) from the complement proteins C5b to C9 and vitronectin, upon activation of the complement cascade. Since binding of clusterin to terminal complement complexes at the stage of nascent C5b-7 complexes abolishes their membranolytic potential, clusterin was also called complement lysis inhibitor (CLI).

Cloning and sequencing of cDNAs for human clusterin [1-3] established strong sequence homology with rat sulfated glycoprotein-2 [4]and sheep clusterin [5], a major Sertoli cell-derived glycoprotein, that had been characterized earlier in both rat and sheep testis fluid. Clusterin was also found in human seminal plasma at high concentrations and for this reason the term clusterin has been adopted by several investigators for the human protein.

Clusterin from blood plasma and seminal plasma is a heterodimer, consisting of two non-identical subunits, which both, in the human, display a molecular mass of approximately 35 kDa. Approximately 30% of the mass corresponds to N-linked carbohydrates. Ten cysteines give raise to 5 disulfide bridges linking the two subunits (for a review, see [6]).

Like all other components of terminal complement complexes, clusterin circulates as a normal constituent in human blood plasma. Clusterin of plasma is specifically bound to high density lipoproteins (HDL) and may represent an important regulatory apolipoprotein (called apo-J) of HDLs [2, 7]. Besides liver and testis, the clusterin gene is expressed in many other tissues and in different cell types, in epithelial cells, e.g. those

lining the proximal tubules of the kidney, in neuronal cells and in some cells of the diffuse endocrine system.

The lytic MAC

The C5 convertases, generated by either the classical or the alternative pathway, initiate the activation of the terminal components of the complement system. Assembly of the late components leads to the formation of MAC (Membrane attack complex) or TCC (Terminal complement complex). Since the pores formed by the MAC permit the passive exchange of small molecules, ions and water while macromolecules remain inside, the cell's normal mechanism for controlling the water balance is disrupted. Furthermore, the insertion of the large diameter transmembrane channel allows a lethal amount of calcium to enter the cell, leading to cell death independent of osmotic lysis.

Regulation of the lytic MAC activity

The self-amplifying destructive properties of the complement cascade make it essential that the activated components are rapidly inactivated to ensure that the attack does not spread to nearby host cells. Inactivation of the MAC is achieved by at least two mechanisms:

- the formation of the MAC is inhibited by two membrane proteins called homologous restriction factor (HRF) and CD59. Both of these proteins are linked to the membrane via a phosphatidylinositol anchor. CD59 (also called membrane inhibitor of reactive lysis, or MIRL) is a 18 to 20 kDa glycoprotein that inhibits MAC formation probably by interfering with the C9 to C8 interaction and C9 polymerization [8];
- the insertion of the terminal complement components into lipid membranes is inhibited by vitronectin, also called S-protein [9]. It functions by binding to the C5b-7 complex and preventing the complex from inserting into lipid membranes. The resulting fluid phase SC5b-7 still binds C8 and C9 but extensive polymerization of C9 does not take place [10]. The vitronectin containing complexes are rapidly cleared from the circulation via vitronectin receptors present on a wide variety of cells including macrophages [11].

Molecular mechanisms of complement inhibition by clusterin

The ability of the MAC to lyse cells is also modulated by clusterin which inhibits MAC formation in a similar manner as vitronectin. Both clusterin and vitronectin are stochiometric constituents of the water-soluble, non-lytic MAC (sTTC).

Purified clusterin inhibits C5b-9 mediated haemolysis *in vitro*. When the cytolytic complement complex is generated from purified components C5b-6, C7, C8 and C9 in the presence of clusterin, the efficiency of haemolysis is suppressed in a concentration-dependent manner. Half-maximal inhibition is observed at a concentration of around 1 μg/ml which is below the physiological concentration of clusterin in human serum. No inhibition is observed when C5b-7-erythrocyte intermediates are preformed before clusterin addition, indicating that clusterin interferes before the membrane attachment of nascent C5b-7.

Inhibition of complement-mediated cytolysis was originally explained by the competition of hydrophobic plasma proteins for the hydrophobic membrane seeking sites of C5b-7. The amino acid sequence of clusterin contains a structural domain at the amino-terminus which appears to be suitable for interaction with hydrophobic segments. The terminal 76 residues display all features of amphipathic a-helices and show homologies with proteins known to form a-helical coiled-coil structures. This N-terminal region may also be responsible for clusterin's propensity to associate with lipids and to form with apolipoprotein A-I a unique class of cholesterol-rich HDL.

Although a fraction of clusterin dissociates from the sTTC in the presence of detergents (in contrast to vitronectin), most of the protein remains bound. This precludes the possibility that clusterin is associated with the terminal complement complexes merely by the intermediates of lipids derived from clusterin comprised in the HDL complex. Using ligand blotting in the presence of Tween 20, high affinity binding sites for C7, the β-chain of C8 and the b fragment of C9 are detectable. This interaction is inhibitable with polymerized C9 suggesting that only the 'activated' forms of the late complement complex expose the binding sites. This precludes binding of clusterin to circulating complement proteins in plasma [12].

Direct interaction of clusterin with C9 is also apparent from the fact that clusterin inhibits Zn^{2+}-induced C9 polymerization. The potential of C7, C8β and C9 to bind clusterin suggest that the binding site is conserved in the three molecules. Indeed, all terminal complement proteins, with the exception of C8γ and C5, belong to a highly conserved family of pore-forming proteins which also includes the T-cell-derived lytic protein perforin. Besides various cysteine-rich domains, all members contain a conserved sequence proposed to form two amphipathic helices interacting with the lipid bilayer of target cells. This structural motif is found in the clusterin-binding fragment of C9, making it a good candidate region for clusterin-terminal complement protein interaction. On the other hand, vitronectin binds to the cysteine-rich region of C9a which bears homology to a module found in the LDL-receptor and, thus, the simultaneous binding of vitronectin and clusterin to the TTC is possible. However, association and inhibition of the soluble late complement complex *in vitro* is not dependent on the presence of the second inhibitory complex; clusterin alone efficiently abrogates the lytic activity in the absence of vitronectin and vice versa. Moreover, the sTTC devoid of clusterin or vitronectin is found in the respective protein-depleted serum. The combined inhibitory activity may, however, be required to reduce inappropriate complement activity *in vivo*.

Regulation of complement function *in vivo*

The final phase in the activation of the complement cascade is the formation of the MAC. The MAC is responsible for the lysis of bacteria and foreign cells *in vivo*, and its importance in the defence against infections is highlighted by the occurence of recurrent infections (*Neisseria meningitidis* or *Neisseria gonorrhoeae*) in patients with deficiencies of terminal complement pathway components.

Under some circumstances the consequences of complement activation *in vivo* may be deleterious rather than beneficial. Complement activation is an important cause of tissue injury in certain diseases such as those mediated by antigen-antibody complexes for example. In certain of these diseases immune-complexes (IC) may form in tissues. This is the case in the thyroid of patients with autoantibodies to the thyroid-stimulating hormone receptor (Grave's disease) or at motor nerve end-plates in patients with autoantibodies to acetylcholine receptors (myasthenia gravis). In both these diseases MAC has been suggested to be involved. Deposits of terminal complement components have been observed at the site of complex formation in both diseases, increased levels of MAC are found in the plasma of patients with Graves disease, and in an animal model of myasthenia gravis the disease is prevented in the absence of C6. In other diseases antigen-antibody complexes are found in

the circulation. This is the case in systemic lupus erythematosus (SLE), a disease characterized by autoantibodies against intracellular antigens. As in the previously described diseases, MAC seems to be associated with the pathogenesis of lesions in SLE, since IC deposits have been shown to be associated with MAC be it in the patients skin or kidneys.

The MAC can be generated in two ways (complement activation on a phospholipid membrane or assembly in the fluid phase), and its lytic potential is tightly controlled by membrane bound (CD59 and HRF) and fluid phase inhibitors (clusterin and S-protein/vitronectin). Clusterin is clearly a potent fluid phase inhibitor of the MAC *in vitro*, and besides its very probable physiological role in limiting bystander lysis at sites of terminal complement activation, it may also be involved in the modulation of tissue injury in certain diseases mediated by complement.

Clusterin in renal pathology

The terminal complement is known play an important role in several renal diseases [13]. Goodpasture's syndrome is not the only example. MAC deposits have been observed in the glomeruli of patients with SLE nephritis, IgA nephropathy, post-streptococcal glomerulonephritis (GN) as well as in certain membranous and membrano-proliferative glomerulonephritis (MPGN). Direct involvement of MAC in renal disease has been best demonstrated in experimental Heymann's nephritis; an animal model which clinically and histologically resembles human membranous GN [14]. This disease is characterized by the presence of granular subepithelial glomerular deposits of IgG, C3 and MAC, and little inflammatory infiltrate or mesangial proliferation. By reconstituting passive Heymann's nephritis (PHN; induced by heterologous antibodies against glomerular antigens) in an isolated perfused rat kidney, Cybulsky *et al.* [15] have demonstrated that perfusion of the antibody treated kidney with C8-deficient serum prevented the appearance of proteinuria, thus directly associating MAC with the pathogenesis of this nephropathy. In these glomerulopathies the damage caused by MAC may be indirect, by increasing local release of reactive oxygen species; direct, by destroying glomerular epithelial cells, damaging the glomerular basement membrane and thus disrupting the glomerular permeability barrier; or a combination of both. As we will see, terminal complement inhibitors like clusterin are associated with this process.

Human clusterin (then called SP-40,40) was initially identified by monoclonal antibodies directed against glomerular immune deposits as a novel component of complement and immune deposits in human glomerulonephritis [16]. Clusterin was shown to be present only in fluid phase MAC namely SC5b-9 isolated from inulin-activated normal human serum (NHS), but not in lytic MAC (C5b-9) extracted from antibody coated sheep erythroctyes that had been lysed by addition of NHS. Clusterin was thus shown to co-localize with terminal complement deposits in diseased glomeruli *in vivo*, and incorporate into the fluid phase lytically inactive form of MAC *in vitro*. Taken together with the later *in vitro* demonstration of clusterin's capacity to inhibit complement mediated cytolysis [1], this discovery was of great interest as it suggested that at least part of the MAC resulting from local complement activation in glomeruli was probably being locally inactivated.

Two recent studies were undertaken in face of these interesting results so as to assess the association of clusterin with a variety of human nephropathie. Both studies were performed on human renal biopsies and compared the glomerular deposition of clusterin and S-protein to that of immunoglobulins (Ig) and other complement components; essentially C3 and the MAC. Clusterin was shown to co-localize with glomerular deposits of Ig, MAC and S-protein. This is not always the case however. The second study, in which a total of 118 renal biopsies were studied, clearly demonstrated that clusterin preferentially co-localizes with MAC deposits associated with Ig. In addition, MAC deposited in glomeruli with sclerotic or non-Ig-mediated lesions, in tubules and in blood vessels was only rarely associated to clusterin. It seems therefore that the molecular composition of MAC differs between these different localizations. At the site of glomerular Ig deposits, at least a portion of total MAC deposits appear to be cytolytically inactive since associated with clusterin. In contrast, in the case of sclerotic tissue or in tissue undergoing remodelling in the absence of immunoglobulin, MAC appears to lytically active (not associated to inhibitors). This may mean that clusterin intervenes to control the extent of terminal complement mediated damage induced by immune mechanisms, but does not get involved in the processes of sclerosis and remodelling in which MAC may be playing a physiological role.

The role of clusterin has also been assessed in an animal model of nephritis. Heymann nephritis, as described above, is a model in which MAC plays an important role in the mediation of proteinuria. In this rat model, clusterin was also shown to co-localize with IgG, C3 and MAC along the glomerular capillary walls [17]. In addition, in decomplemented animals in which proteinuria and glomerular MAC deposition was prevented, no clusterin deposits were observed. Analysis of this model and human GN strongly suggest that clusterin is actively implicated in the modulation of terminal complement mediated damage that is a consequence of immune-mediated complement activation. This experimental approach was recently taken one step further by Saunders *et al.* who have analysed the effect of clusterin depletion on glomerular injury using an isolated perfused rat kidney model of passive Heymann nephritis [18]. In this experimental model, proteinuria was significantly increased in kidneys perfused with clusterin depleted plasma as compared to fibronectin depleted plasma. Although definitive conclusions on the *in vivo* role of clusterin will probably have to await the availability of clusterin knockout mice, this report strengthens the suggested role of clusterin as an inhibitor of terminal complement mediated cell damage *in vivo*.

Clusterin in other diseases associated with complement activation

Complement activation and generation of MAC are associated with a series of other diseases *in vivo*, but two of them, Alzheimer's disease and atherosclerosis, are of particular interest due to their frequence, the consequences they have on public health and the existing evidence suggesting that clusterin may be involved.

Alzheimer's disease is a devastating chronic degenerative disease of the central nervous system that affects millions of individuals of all races and ethnic backgrounds. The disease is clinically characterized by progressive dementia and diffuse cerebral atrophy. The pathogenesis of Alzheimer's is still unclear but evidence suggests that certain proteins or protein fragments are abnormally precipitated as amyloid, and this is accompanied by neuritic lesions and cell death. Classical pathway components of complement have been identified in senile plaques and amyloid. MAC is also detectable in Alzheimer's, but essentially on dystrophic neurites, senile plaques, neuropil threads and tangled neurons. Interestingly, clusterin seems to co-localize with MAC, staining being localized to dystrophic neurites and neurophil threads in Alzheimer's disease brain

tissues using antibodies [19]. Moreover, clusterin mRNA has been identified in brain tissue and the level of clusterin gene expression is increased in Alzheimer's disease [20]. Although MAC is not the only factor involved in the pathogenesis of Alzheimer's disease, identification of MAC in degenerating neurons and their processes associated with the upregulation of inhibitory proteins such as clusterin is of considerable interest. One interpretation may be that during the process of remodelling involving upregulation of phagocytic receptors on microglia and complement fixation to senile plaques a certain amount of bystander lysis occurs. Clusterin which is locally upregulated may well be involved in limiting the extent of lysis.

Several lines of evidence suggest that the complement system is involved in the development of atherosclerosis [21]. The MAC has been detected in areas of intimal thickening and in fibrous plaques of the human aorta [22], and it has been suggested that the complement system is activated at these sites by deposits of crystalline cholesterol. The implication of terminal complement is further suggested by the marked reduction of cholesterol-induced atheroma in rabbits deficient in C6 [23]. Although no study has so far investigated whether clusterin co-localizes with MAC in arterial lesions, it is certain that clusterin is locally available, and thus may well also be implicated in atherogenesis. Indeed clusterin is not only present in high quantities in human serum, but is also present in the alpha-granules of platelets [24], synthesized by endothelial cells (Unpublished personal observation) and aortic smooth muscle cells [25], all of which are known to be involved in atherosclerosis.

Since the identification of clusterin (alias SP-40,40 or cytolysis inhibitor) as a potent inhibitor of complement mediated cytolysis *in vitro* in 1989, considerable progress in the understanding of its function *in vivo* has been made. It seems highly probable that one of clusterin's *in vivo* functions is to control terminal complement mediated damage, and we hope that clusterin gene deletion through homologous recombination will enable definite conclusions to be made.

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