Complement system activation

and peritoneal membrane alterations:

culprit or innocent by-stander?

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Abstract

Peritoneal dialysis (PD) accounts for approximately 10% of the dialysis population worldwide. Major concern limiting long-term PD success is the loss of the peritoneal membrane function after prolonged exposure to dialysis solutions. The complement system is a major component of the innate immune system, which provides a first-line defense against pathogens. Uncontrolled activation of the complement system directly contributes to the pathophysiology of rare and common kidney diseases, and to a growing number of non-renal diseases. Here we review currently available evidence of complement activation in patients treated with PD and its association with structural and functional alterations of the peritoneal membrane. Mainly, evidences point towards a local, intraperitoneal, production of complement molecules in response to PD exposure. Dialysis fluids, particularly glucose, play a role in complement activation and dysregulation leading to untoward PD related pathophysiological processes such as peritoneal fibrosis, angiogenesis and vasculopathy, and perhaps, encapsulating peritoneal fibrosis (EPS) development.

Those findings could lead to further development and use of anti-complement therapeutics in PD patients to prevent membrane damage.

Key words

Complement system, peritoneal dialysis, angiogenesis, vasculopathy, fibrosis, peritoneal membrane alteration, EPS

Abbreviations

AGE: advanced glycation end product

C1-INH: C1 esterase inhibitor

C4BP: C4b-binding protein

C5aR1: C5a receptor 1 (CD88)

CD46: membrane cofactor protein (MCP)

CD55: decay accelerating factor (DAF)

CD59: MAC-inhibitory protein (MAC-IP)

CR1: complement receptor 1

D/P Cre: dialysate-to-plasma ratio of creatinine

EMT: epithelial-to-mesenchymal transition

EPS: encapsulating peritoneal sclerosis

ESRD: end-stage renal disease

GDP: glucose degradation product

HD: hemodialysis

HUS: hemolytic uremic syndrome

LMWH: low molecular weight heparin

MAC: membrane attack complex (C5b-9)

MASP: mannose-associated serine proteases

MBL: mannose-binding lectin

PD: peritoneal dialysis

PRM: pattern recognition molecule

UF: ultrafiltration

Peritoneal dialysis (PD) accounts for approximately 10% of the dialysis population worldwide. Even though it allows a home-based therapy and is more cost-effective than hemodialysis (HD) with similar patient outcomes, PD is still underused (1;2). Major concern limiting long-term PD success is the loss of the peritoneal membrane function after prolonged exposure to dialysis solutions.

Over time on PD, the peritoneal membrane undergoes structural alterations, including progressive fibrosis, angiogenesis and vasculopathy. These morphological changes are paralleled by acceleration of solute transport, earlier dissipation of the osmotic gradient induced by glucose and, ultimately, loss of ultrafiltration capacity (3;4). In extremely rare but dramatic cases, an excessive fibrogenic response occurs in the membrane, leading to encapsulating peritoneal sclerosis (EPS), which is associated with significant morbidity and mortality (5).

The mechanisms leading to peritoneal remodeling remain ill-defined but likely involve chronic inflammation (6;7). Established triggers of membrane injury include uremia itself, the presence of a catheter, peritonitis episodes, and prolonged exposure to non-physiological PD solutions. High glucose level, glucose degradation products (GDPs) resulting from heat sterilization and low pH are all PD fluid characteristics known to induce fibrotic and vascular changes (8;9). Neutral pH, low GDP solutions have been developed to improve the biocompatibility of dialysis fluids and reduce damage to the membrane. However, recent evidence showed that membrane alterations also occur under so-called biocompatible solutions, suggesting they are not a *panacea* for membrane preservation (10;11).

In this context, recent data points to a putative implication of the complement system in the degradation of the peritoneal membrane exposed to PD.

The Complement system

The complement system comprises more than thirty soluble and membrane-expressed proteins (Fig. 1). It is a phylogenetically ancient component of innate immunity but it also plays a role in adaptive immunity by acting as a bridge between both systems (12;13).

The complement cascade can be triggered through the classical, lectin or alternative pathways. Classical and lectin pathways are initiated when pattern recognition molecules (PRM) recognize distinct structures. In the classical pathway, C1q is the PRM that binds to immune complexes or other molecules such as C-reactive protein or structures found on foreign (*e.g.* microbial) and apoptotic cells. Lectin pathway is activated when mannose-binding lectins (MBL), ficolins or collectin-11 bind to carbohydrate motifs found on various pathogens. The alternative pathway is continuously activated at a low rate through spontaneous hydrolysis of C3, thus requiring a strict regulation. It is also referred to as the "tick-over" segment of the complement system. However, it may also be activated through properdin, a PRM binding to structures found on foreign and apoptotic cells.

All three pathways converge to C3-convertase production and eventually to generation of effector proteins. For instance, those molecules allow clearance of microbial antigens via opsonization by C3b, promote inflammation via the anaphylatoxins C3a and C5a and induce non-nucleated cells lysis (*e.g.,* red blood cells or bacteria) via the membrane attack complex (MAC) C5b-9. C3b also initiates the "amplification loop" leading to more C3-convertase formation through interactions with factor B and properdin.

Complement regulation is crucial to prevent damage to self-cells and occurs at several steps of the cascade, through either soluble or membrane-bound regulators. Overactivation or dysregulation, triggered by acquired or genetic factors, has been involved in numerous pathological processes such as auto-immune and inflammatory disorders,

atypical HUS, cancer, reaction to biomaterials or drugs, or allograft rejection in transplantation(14-16).

Is the complement system activated in PD patients?

The potential role of the complement system in PD has been suggested by a series of studies, both in humans (Table 1) and in experimental models (Table S1), but is still far from being thoroughly elucidated.

Currently, it is established that molecules belonging to the complement system such as C3 to C9 and factor D are present in PD effluent (17;18). Likewise, up to 18 complement proteins including C3, C4, C9, factors D, B, H and I have been found in the PD effluent by proteomic analysis (19-23). Altogether, those data indicate either a clearance of those molecules from the circulation, a local intraperitoneal production or a combination of both phenomenon.

In addition, increased systemic concentration of various complement components has been reported in PD patients but was actually ascribed to an increased liver synthesis in reaction to ESRD pro-inflammatory state and/or an adaptive reaction to peritoneal clearance of those molecules (24). However, blood levels of complement proteins also depend on which pathway is looked at as, for instance, Lam et al documented a reduced systemic MBL concentration in PD patients in comparison with healthy subjects and thus proposed that loss via the peritoneal route could exceed its production (25).

Several lines of evidence support complement system activation directly within the peritoneal cavity of PD patients and that this phenomenon is a consequence of PD itself rather than being secondary to the uremic state.

Theoretically, complement system could be activated within the peritoneal cavity since many effectors (*e.g.* C3, C4, C5, C6 to C9) as well as their regulators (MCP, CD55 and CD59) have been found to be produced and expressed by mesothelial cells originating from

healthy non-uremic, uremic and PD subjects (26-29). A low dialysate-over-plasma ratio of C3, C4 and factor P, along with increased levels of C3d in the dialysate, suggests intraperitoneal complement activation (18). Local intraperitoneal activation of the complement system is further supported by the absence of correlation between plasma and dialysate levels of C3 (28); and by the presence of the large molecular size C5b-9 (>1000kDa) in PD effluent (17;28). Finally, Bartosova et al recently demonstrated activation of complement and increased deposition of both classic and alternative pathway proteins (C1q, C3d and C5b-9) in peritoneal parietal and omental arterioles, from PD children treated with low GDPs solutions in comparison to ESRD and healthy controls (30). Peritoneal production of complement proteins could also originate from other tissue-resident cells such as immune cells, since peritoneal chronic inflammation is commonly found in patients exposed to PD. Although it has been demonstrated that those cells are able to secrete complement components, to the best of our knowledge, it has not been specifically studied in the peritoneal membrane (31).

Modified expression of the complement regulators within the peritoneum has also been proposed as one of the potential mechanism responsible for complement activation (32). Reduced CD59 and particularly CD55 expression was noted on mesothelial cells of PD patients in comparison with non-PD subjects (28;29). Local CD55 level did not correlate with systemic expression. Furthermore, decreased CD55 expression was associated with high C5b-9 level in PD fluid, suggestive of complement activation (28).

Complement system activation in PD patients seems to be triggered by exposure to PD fluids, and particularly by the glucose load and presence of GDPs. Up and downregulation of factors C3 and C4 by human mesothelial cells originating from healthy individuals was observed when exposed to different glucose concentrations. This effect was unrelated to hyperosmolarity and didn't differ when using lactate or bicarbonate solutions while exposure to advanced glycation end products (AGEs) marginally reduced C3 secretion (27).

Glucose effect on complement activation was also supported by Bartosova's data indicating a correlation between the level of dialytic glucose exposure and the abundance of complement fragments (*e.g.* C1q and C5b-9) deposition in the parietal peritoneal arterioles. A same trend was noted between C1q depositions and level of GDPs exposure. Interestingly such relationship wasn't observed in omental arterioles, less exposed to PD fluids, thus supporting the potential role of both glucose and GDPs on complement dysregulation (30). In addition, Sei et al didn't find a difference in CD55 expression between neutral dextrose and icodextrin use (28).

The pathophysiological role of hypertonic glucose on complement activation is further supported by several lines of evidence from studies in patients with diabetes (33). First, inactivation of complement regulatory protein CD59 by glycation is associated with C5b-9 depositions in diabetic tissues therefore suggesting a role in the development of diabetic vasculopathy (34). Second, hyperglycemia is responsible for lectin pathway activation through increased MBL recognition and binding to neoepitopes emerging from protein glycation (35). Of note, there is no available data so far regarding MBL presence or production within the peritoneum.

The implications of the complement system activation in PD

Complement system activation is suspected to play a causative role in the genesis of both structural and functional changes observed in the peritoneal membrane in response to PD exposure.

Pharmacological inhibition of complement regulatory proteins in rat caused abundant deposition of both C3b and C5b-9 on the peritoneal surface and was associated with accumulation of inflammatory cells; peritoneal edema; and severe peritoneal injury, including omental adhesions, sclerotic changes within the retroperitoneum and peritoneal effusion (36). Peritoneal injuries were enhanced with lower pH and higher glucose

concentration exposure (36). In contrast, inhibition of the complement system at the local or systemic level efficiently prevented complement product deposition and peritoneal membrane alterations (37;38).

Angiogenesis, leading to an increased effective peritoneal surface area available for solute transport, is a common finding after long-term PD exposure (39). In 1984, Miller et al showed that molecules activating the complement system increase microvascular protein leakage across the rat peritoneal membrane (40). More recently, reduced mesothelial expression of membrane-bound regulator CD55 (but not CD46 or CD59) was associated with fast transport status in PD patients (29). Similar findings were found in another study in addition to a correlation between levels of C5b-9 in PD effluent and peritoneal solute transport rate (28). The observation by Bartosova et al of a strong correlation between complement system up-regulation within altered peritoneal vessels suggested the complement system may be involved in PD-associated vasculopathy (30).

Complement system activation has also been involved in fibrogenic processes occurring in various settings such as ischemia-reperfusion injury in organ transplantation (41) or renal fibrosis (42). It still remains unclear if fibrosis is induced by complement driven inflammatory injury rather than by a direct profibrotic effect. In favor of the latter is the suggestion of an epithelial-to-mesenchymal transition (EMT) undergone by tubular cells in response to C3 (43;44). It also appears that C5a is a central mediator in fibrosis mechanisms through production of profibrotic factors such as TGF-β (45). Unfortunately, specific studies linking complement activation and fibrosis in PD are actually scarce although mesothelial EMT has been suspected to participate in peritoneal fibrosis development (46). Raby et al suggested C5a implication in rodent and cellular models as C5aR -/- mice developed less fibrosis in bacteria-induced peritoneal fibrosis. Similarly, blockage of C5aR inhibited inflammatory and profibrotic factors production by peritoneal leukocytes isolated from PD effluent (45). Finally, Bartosova et al also demonstrated a positive correlation between

complement factors depositions in peritoneal arterioles of PD patients and TGFβ-SMAD pathway effectors, two key mediators of fibrogenesis (30).

The latest stage of peritoneal fibrosis is encapsulating peritoneal sclerosis (47). In a rodent model of peritonitis induced by peritoneal scraping and zymosan, a yeast cell membrane component, peritoneal inflammation and damage, as well as fibrin exudation, considered as an early sign of EPS, were correlated with complement activation (48). Another rodent model supposed to mimic human EPS (zymozan-induced peritonitis with pretreatment with well-known GDP, methylglyoxal), showed increased C3b and C5b-9 depositions while C5a inhibition was able to reduce fibrotic and encapsulating changes as well as complement factors depositions (49). The pertinence of those models still needs to be validated. Nevertheless, a proteomic analysis of PD effluent revealed increased levels of complement factors B and I up to five years prior to EPS development compared to patients with stable membrane function (23).

Coagulation and complement systems are interconnected and this also applies to PD. Signs of complement and coagulation cascades activation were noted after exposure to PD fluids or in case of peritonitis, both in rat models but were also reduced after either complement or coagulation inhibition by anti-C5a molecule or low molecular weight heparin (LMWH) respectively, and inversely after enhanced complement activation (50-52). However, few data on coagulation system's implication in PD are available and the relevance of coagulation and complement systems interconnection on various PD aspects still remains unknown. Still human peritoneal mesothelial cells can produce several coagulation factors ; among them, plasminogen activator inhibitor 1 (PAI-1) could play a role in peritoneal membrane damage such as fibrosis and angiogenesis as well as peritonitis and EPS (53, 54).

Lastly, complement system activation has been demonstrated in several rodent models of peritonitis (36; 37; 49). In a retrospective analysis, high levels of complement

proteins, particularly C5b-9, in PD effluent of patients suffering from early stages of peritonitis were also associated with poor prognosis, defined as withdrawal from PD (52). Although, it is still unclear whether increased complement activation is itself responsible for the poorer outcomes or if it is the signature of a more severe infection associated with a worse prognosis. On the other hand, it has also been proposed that reduced complement components levels due to intraperitoneal activation could impair host defense and thus increase the risk of peritonitis (32)

In summary, experimental and clinical data suggests a pathophysiological role for the complement system in the development of angiogenesis, submesothelial fibrosis, inflammation and abnormal coagulation processes within the peritoneal membrane exposed to PD (fig 2). However, to the best of our knowledge, complement system role in outcomes such as membrane function and PD failure have not been clearly evaluated so far and further studies will be required to determine if it could have a clinical impact on PD patients care.

Potential therapeutics

Complement system inhibition has been a major field of interest in recent years and several drugs targeting different steps of the cascade are being developed to treat various disorders. Many of them are now under clinical trial evaluation but so far, only two types of inhibitors are used in clinical settings: C1 esterase inhibitors in hereditary angioedema, and eculizumab, a monoclonal antibody specifically inhibiting C5, which is currently approved for the treatment of atypical HUS, paroxysmal nocturnal hemoglobinuria and myasthenia gravis (55).

Complement inhibition in PD has obviously only been studied in experimental animal models and mostly used to prove complement system implication in PD-related pathogenic processes. C5 has been the main target probably because of its central role in

the cascade, leading to MAC production. Overall, studies using C5 inhibitors showed reduction in tissue damage, inflammation, fibrosis development and increased ultrafiltration (38; 45; 51). Reduction of EPS signs after C5 inhibition in a rat model mimicking human EPS could also be the first step towards future research for an efficient treatment of this serious complication (49).

On the other hand, one should keep in mind that complement inhibition also weakens the host immune system and therefore alters its response to infection. For instance, eculizumab increases the risk of meningococcal infection although this can be partially reduced by vaccination prior to treatment initiation (55). The increased risk of infections will need to be balanced if we ever consider using complement inhibitors in PD patients. Administration through the peritoneal route might potentially allow selective inhibition of local complement system and partially preserve the host immune system.

Aside from direct anti-complement therapy, other potential therapeutics includes LMWH, which has been proved to improve ultrafiltration, inhibit formation of thrombin, and potentially block C5a activity when added to PD fluid in a rat model (50).

Preventing complement activation is another way to look at the problem. This may be undertaken for instance through development of PD solutions with osmotic agents alternative to glucose more biocompatible solutions or addition of cytoprotective supplement such as alanyl-glutamine to PD fluids (56). Although, complement activation in PD patients using solutions currently defined as biocompatible would need to be further studied first.

Conclusion

Complement system is triggered by PD and is implicated in peritoneal membrane alterations after long-term exposure to PD. However, implication of each factor and pathway has not been accurately defined and conflicting data persists. Evidences point

towards a local, intraperitoneal, production of complement molecules in response to PD exposure. Dialysis fluids, particularly glucose, seem to trigger complement activation.

Current knowledge mostly relies on a small amount of studies presenting with recurrent bias: old and/or small studies, or narrowed populations (such as diabetic subjects), animal models, or *in vitro* cellular model using mesothelial cells, not always originating from PD patients, where it remains unclear if they really reflect *in vivo* mesothelial cells behavior. Better understanding of the complement system involvement in PD is required before potential therapeutics targeting its molecules can be studied in clinical trials. Furthermore, the effect of newer, more "biocompatible", PD solutions on complement system also needs to be clarified in future research.

Based on available literature, it would not be surprising that the complement system is a culprit in peritoneal membrane alterations rather than an innocent bystander. Careful evaluation of the mechanisms of complement activation during PD should be thoroughly evaluated, as well as the potential of complement inhibition to mitigate deleterious effects on the membrane.

Legend to figure 1

Schematic view of the complement system.

Complement system is initiated through three pathways: classical, lectin and alternative. In the classical pathway, C1q binds to immune complexes or other molecules (C-reactive protein, structures found on foreign and apoptotic cells). C1q then activates C1r and C1s, thus leading to C2 and C4 cleavage. Resulting cleavage products, C4b and C2a, form C3-convertase which cleaves C3 into C3a and C3b. Lectin pathway is initiated when MBLs, ficolins or collectin-11 bind to carbohydrate motifs, thus activating MASPs and resulting in C3-convertase creation also through C2 and C4 cleavage. Alternative pathway, the "tick-over" pathway, is continuously activated at a low rate through spontaneous hydrolysis of C3 but may also be activated through Properdin binding to structures found on foreign and apoptotic cells. C3b binds to factor B, previously cleaved by factor D, to form C3-convertase (C3bB). Increased C3b levels lead to C5-convertase formation (C4bC2aC3b or (C3b)2Bb), which cleaves C5 into C5a and C5b. Next, C5b interacts with C6-C9 to generate the MAC (C5-b9), which induces cell lysis. C3b also has two other roles: clearance of microbial antigens through opsonization and initiation of the "amplification loop" when interactions with factor B and Properdin leads to more C3-convertase formation. C3a and C5a are both anaphylatoxins. Complement regulation occurs at several steps of the cascade through either soluble or membrane-bound regulators. C1 esterase inhibitor prevents early activation of all three pathways. Factors H and I control both C3 and C5-convertases whereas C4BP acts at the C4 level. CR1, DAF, MCD, CD59 and Clusterin are all membrane-bound regulators. CR1 and DAF regulate activation of C3 and C5-convertases. MCP acts as a co-factor to factor I. CD59 and Clusterin prevent MAC generation.

Legend to figure 2

Schematic representation of the various mechanisms implicating complement system dysregulation in the development of peritoneal membrane alterations. Exposure to high-glucose based dialysis solutions (together with recurrent peritonitis episodes) may induce complement system activation and/or defects in complement regulatory proteins production, both subsequently leading to complement system *overactivation*, local inflammation and cellular damage, and *in fine*, to structural alterations of the peritoneal membrane including vascular proliferation, vasculopathy and fibrosis, and ultimately, membrane failure. Alternative osmotic agents and/or dialysis solutions might prevent complement activation while complement inhibitors might inhibit overactivation of the complement system.

Legend to Table 1

Published studies investigating the complement system in human peritoneal membrane

HPMC: human peritoneal mesothelial cells. MC: mesothelial cell. SMF: stable membrane function. D/P Cre: dialysate-to-plasma ratio of creatinine. BSA-AGE: bovine serum albumin modified by advanced glycation.

Legend to Table S1

Published studies investigating the complement system in animal peritoneal membrane

KO: knock out. CRegs: complement regulators. IV: intravenous. IP: intraperitoneal. CVF: cobra venom factor. LPS: lipopolysaccharide. LMWH: low molecular weight heparin. TAT: thrombin-antitrombin complex. CINC-1: cytokine-induced neutrophil chemoattractant.

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