Published in final edited form as:

Immunobiology. 2012 November ; 217(11): 1097–1105. doi:10.1016/j.imbio.2012.07.012.

Targeted complement inhibition as a promising strategy for preventing inflammatory complications in hemodialysis

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Abstract

Hemodialysis is the most common method used to remove waste and hazardous products of metabolism in patients suffering from renal failure. Hundreds of thousands of people with end-stage renal disease undergo hemodialysis treatment in the United States each year. Strikingly, the 5-year survival rate for all dialysis patients is only 35%. Most of the patients succumb to cardiovascular disease that is exacerbated by the chronic induction of inflammation caused by contact of the blood with the dialysis membrane. The complement system, a strong mediator of pro-inflammatory networks, is a key contributor to such biomaterial-induced inflammation. Though only evaluated in experimental *ex vivo* settings, specific targeting of complement activation during hemodialysis has uncovered valuable information that points towards the therapeutic use of complement inhibitors as means to control the unwelcomed inflammatory responses and consequent pathologies in hemodialysis patients.

Keywords

complement; hemodialysis; inflammation; therapeutics; innate immunity

Introduction

End-stage renal disease (ESRD) is costly in terms of both money spent on patient treatments (in the latest statistics from 2008, the estimated public and private cost for the ESRD program in the U.S. was \$39 billion (NKUDIC 2012) and the reduced quality of life these patients experience. ESRD can occur as a result of several conditions, including diabetes, hypertension, glomerulonephritis, and cystic kidney or urologic disease, to name a few (NKUDIC 2012). Replacement of the diseased kidney(s) with a transplant is a desirable treatment option for ESRD, but unfortunately not one that is readily available. About 17.5 thousand transplants were performed in the U.S. in 2008, meaning that only approximately 3.5% of the over 500,000 patients diagnosed with ESRD were able to receive this treatment (NKUDIC 2012). A majority of patients must instead rely on hemodialysis therapy to remove toxins from the blood. Though hemodialysis is a life-saving procedure in lieu of a kidney transplant, it nevertheless contributes to the reduced quality of life these patients already experience and, more importantly, can lead to life-threatening complications

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(Zimmermann et al. 1999; Yao et al. 2004); the mortality rate for patients undergoing maintenance dialysis is approximately 16% according to the latest statistics (NKUDIC 2012). Often this is a result of the many complications that exist in ESRD patients that can be exacerbated by hemodialysis. These include malnutrition, muscle wasting, oxidative stress, endothelial and immune dysfunction, leukopenia, anaphylactoid reactions, amyloidosis, hypo-/hypertension, headache, fever, sleep disturbances, and increased risk for atherosclerosis and myocardial infarction (Nilsson et al. 2007; Cheung 1990; Cavalli et al. 2010). It is generally accepted that chronic inflammation contributes to these adverse conditions (Galli 2007). Indeed, one of the major causes of mortality in both adult and pediatric ESRD patients is cardiovascular disease (CVD), which is likely a result of chronic inflammation, as levels of C-reactive protein (CRP), IL-6, fibrinogen, and intracellular adhesion molecule-1 (ICAM-1) have been found to be elevated in ESRD patients with CVD (Stenvinkel and Alvestrand 2002; Silverstein 2009). An increased state of oxidation product generation is also seen in hemodialysis patients resulting from the oxidation of sugars and lipids to form reactive dicarbonyl compounds. These small compounds are known to be potent agents that are able to modify amino acid residues to form a complex mixture of compounds known as advanced glycation end products (AGE) and advanced lipoxidation end products (ALE), collectively referred to as advanced oxidation protein products (AOPP) (Galli 2007; Silverstein 2009). Several reports have indicated that the formation of minor concentrations of AOPP can have large functional implications (Shao et al. 2010; Thornalley and Rabbani 2011). Thus, several pro-inflammatory factors are found at higher levels in ESRD and hemodialysis patients, but it can be difficult to identify their sources, assign a definitive role for them in the progression of the disease, or determine their biological mechanisms.

Inflammation related to hemodialysis

The underlying disease in ESRD patients undoubtedly contributes to the long-term inflammation that can lead to morbidity and mortality; often inflammatory factors can accumulate in the tissues of those with ESRD due to decreased renal function and lack of proper clearance (Stenvinkel and Alvestrand 2002). But inflammation caused by the dialysis procedure is also thought to contribute to these morbidity and mortality rates; the five-year survival rate for all dialysis patients is only 34.5% (NKUDIC 2012). Higher levels of pro-inflammatory cytokines, especially CRP and IL-6, have been associated with and can predict increased all-cause and cardiovascular mortality for those receiving hemodialysis (Kimmel et al. 1998; Zimmermann et al. 1999; Yeun et al. 2000; Rao et al. 2005; Bologna et al. 1998). Part of the issue may be that the procedure does not appear to reduce the elevated pro-inflammatory and oxidative stress markers already found in patients with impaired kidney function. CRP and IL-6 (inflammatory markers) and carbonyl content (oxidative stress marker) were found to be elevated in the serum/plasma of pre-dialysis ESRD patients, but hemodialysis did not significantly alter the amounts of these factors even after 12 months of therapy (Pupim et al. 2004). A corollary of this result would be that hemodialysis does not induce an inflammatory state above that which already exists in ESRD patients, and the work of others seems to corroborate this supposition (Grooteman et al. 1997; Engelberts et al. 1994; Mege et al. 1994; Pereira et al. 1994). However, long-term studies such as the one above may not show a difference in inflammatory markers because hemodialysis likely induces an acute, intradialysis inflammatory response. Many studies have shown increases in various inflammatory markers, including IL-6, IL-1, CRP, and the fractional synthetic rates of albumin and fibrinogen, during, immediately after, or up to 2-24 hours after hemodialysis treatment (Caglar et al. 2002; Haubitz et al. 1990; Schouten et al. 2000; Lonnemann et al. 1987; Herbelin et al. 1990; Kaizu et al. 1998; Haeffner-Cavaillon et al. 1993). Thus, rather than create a constant state of elevated inflammatory mediators, hemodialysis more often induces inflammation during the procedure or up to a day

afterwards, which then abates until the next hemodialysis session; this would preclude detecting an increase in overall inflammation in long-term studies. However, the constant induction of inflammation for ESRD patients on maintenance hemodialysis, who usually undergo three or more treatments per week for long periods of time, would create a “chronically acute” inflammatory condition, resulting in the activation of immune cells and processes that contribute to their morbidity and mortality (Hakim 1993).

Besides timing, another possible reason for discrepancies in studies on the inflammatory potential of hemodialysis may be the “bioincompatibility” of some materials used for the procedure, especially the dialysis membrane. Bioincompatible membranes are considered at least partly responsible for the higher levels of pro-inflammatory mediators seen after hemodialysis. However, the complexity of the disease, the hemodialysis treatment, and the analyses render a reliable evaluation challenging and lead to conflicting results. Indeed, while some studies have shown little or no influence of the type of dialysis membrane on inflammatory markers (Herbelin et al. 1990; Herbelin et al. 1991; Cavaillon et al. 1992; Mege et al. 1994; Grooteman et al. 1997), others have suggested that membrane type can have a significant effect on inflammation (both the extent and mediators induced) as well as mortality risk (Schouten et al. 2000; Kaizu et al. 1998; Pereira 1997; Schindler et al. 2000; Memoli et al. 2000; Canivet et al. 1994; David et al. 1993; Uda et al. 2011). Early membranes made from cellulose (e.g., cuprophane) were major activators of the complement system, a strong inducer of inflammation, and resulted in leukopenia (Craddock et al. 1977a; Craddock et al. 1977b; Chenoweth et al. 1983). Modern membranes with increased biocompatibility consist of modified cellulose (e.g., cellulose acetate) or synthetic materials, such as polysulfone (PS), polymethylmethacrylate, polyamide, polyacrylonitrile, polyethersulfone, or polyethylene-co-vinyl alcohol. However, even though these improved membranes substantially reduced acute complement activation in patients receiving hemodialysis treatment, they can still elicit significant complement activity and related inflammation that can contribute to hemodialysis-associated morbidity. Notably, the hydrophobic nature of most modern membranes results in greater binding of complement and other plasma proteins, which can initiate complement activation (see below), yet may also explain the decreased systemic complement activity observed after use of these membranes, since activation products could remain bound to them (Hakim 1993; Cheung 1990; Uda et al. 2011; Girndt et al. 1999). A better understanding of complement-related effects in hemodialysis is thus essential for developing improved membranes and treatment options.

The role of the complement system in hemodialysis-related inflammation

The complement system, an integral pillar of humoral innate immunity, has traditionally been seen as the first line of defense against pathogens. However, it is now known that complement is involved in a multitude of homeostatic processes that go well beyond the killing of bacteria and plays a role in many pathological conditions (Ricklin et al. 2010). Complement activation follows three major initiation pathways: classical (CP), lectin (LP), and alternative (AP). The CP is activated by the C1 complement protein complex (consisting of C1q, C1r, and C1s) binding to antibody-antigen complexes, pathogen- and damage-associated molecular patterns (PAMP, DAMP), and pattern recognition receptors (PRR) such as pentraxins (e.g., CRP, PTX3, serum amyloid protein). The LP is triggered when PRR such as mannose-binding lectin (MBL) or ficolins bind to PAMP or apoptotic host cells, activating MBL-associated serine proteases (MASP). CP- and LP-related initiation events all lead to the cleavage of C4 and C2 (into C4a/C4b and C2a/C2b, respectively) and the formation of the CP C3 convertase on foreign or host cell surfaces. At the same time, the AP may be triggered spontaneously or induced by the PRR properdin, generating distinct AP C3 convertases. The C3 convertases can cleave the abundant plasma protein C3, the

central component of complement, into the anaphylatoxin C3a and opsonin C3b. Interaction of surface-bound C3b with factor B (fB) and factor D (fD) leads to the formation of additional C3 convertases, resulting in rapid amplification of the response. C3b and its degradation products (iC3b, C3dg) bind to various complement receptors (CR) for signaling or targeting of cells for phagocytosis and clearance. Finally, continuous deposition of C3b also enables the generation of C5 convertases that cleave C5 into C5a (a potent anaphylatoxin) and C5b. The C3a and C5a anaphylatoxins signal through the G-protein coupled C3a receptor (C3aR) and C5a receptor (C5aR; CD88), respectively. C5a and its desarginated form (C5a^{desArg}) can also bind to the C5L2 receptor, though the functional implication of this interaction has not yet been fully resolved and may be cell-specific (Klos et al. 2009). C5b initiates the terminal pathway of complement by binding to C6, eventually forming the terminal complement complex (TCC), consisting of C5b, C6, C7, C8, and C9. This complex can incorporate into the lipid bilayer of membranes of susceptible cells, creating a pore that may result in leakage and cell activation or cell lysis. Finally, other mechanisms may directly activate complement, including cleavage of C3 or C5 by extrinsic proteases related to the fibrinolytic, kinin, and coagulation cascades, systems known to interact with the complement pathway (Markiewski et al. 2008; Oikonomopoulou et al. 2012; Amara et al. 2010). While complement can potentially target any surface, regulators of complement activation (RCA) expressed on host cells or in the blood protect host cells against complement attack through the prevention of convertase formation, the acceleration of convertase decay, or the promotion of C3b and C4b degradation (Ricklin et al. 2010; Zipfel and Skerka 2009). In addition, C1 inhibitor (C1-INH) controls CP activation and various regulators (CD59, clusterin, vitronectin) share the ability to prevent TCC formation. This regulation of complement usually maintains a balance to ensure complement acts where and when needed to prevent the development of pathogenic situations in the host (Ricklin et al. 2010; Ricklin and Lambris 2007).

The activation of complement by hemodialysis filters (and other biomaterials) is considered to occur mainly through the AP triggered by plasma proteins such as albumin, C3, or IgG that form a protein layer on the material; thus the hydrophobic nature of individual hemodialysis membranes and their resulting tendency to bind plasma proteins may have an influence on this activation (Nilsson et al. 2007; Ekdahl et al. 2011; Nilsson et al. 2010; Cheung 1990). When C3 gets adsorbed to plasma protein-coated surfaces, it changes its conformation into a C3b-like state that leads to convertase formation and subsequent initiation and amplification of the complement response (Fig. 1A) (Andersson et al. 2002; Andersson et al. 2005). Early studies on the influence of cellulose-based hemodialysis membranes on complement activity indeed indicated initiation through the AP, as leukopenia was induced in the absence of Ca²⁺ (which prevents CP initiation), the AP components C3b and fB were detected on membrane surfaces, and a lack of increase of C4 was observed during dialysis (Craddock et al. 1977a; Craddock et al. 1977b; Cheung et al. 1989; Chenoweth et al. 1983). However, recent work suggests the CP and LP may also be involved in hemodialysis membrane-induced activation of complement through binding of C1q to membrane-adsorbed IgG (Fig. 1B), based on a decreased rate of initial C3b deposition and AP activation on cuprophane membranes when C4 is absent, and by ficolin-2 deposition on PS membranes (Fig. 1C) (Nilsson 2001; Lhotta et al. 1998; Mares et al. 2009; Mares et al. 2010). Regardless of the initiation pathway, activation of complement leads to the generation of effector components (C3a, C5a, C3b, iC3b, C3d, C4b, TCC) that can promote chemotaxis and immune cell recruitment (Nilsson et al. 2007; Cheung 1990; Fujimori et al. 1998; Deppisch et al. 1990; Hauser et al. 1990). These components also activate leukocytes (polymorphonuclear cells [PMN], monocytes, and mast cells), which release pro-inflammatory cytokines and oxidative agents such as reactive oxygen species and myeloperoxidase, and also upregulate receptors on these cells that promote adhesion and interaction with platelets and vascular and pulmonary endothelial cells and increase vascular

permeability; activated leukocytes also release acute phase proteins such as β_2 -microglobulin (a significant contributor to amyloidosis) that can further activate complement and bind to activated endothelial cells (Fig. 1D) (Haeffner-Cavaillon et al. 1993; Nilsson et al. 2007; Uda et al. 2011; Galli 2007; Hakim 1993). Activated leukocytes have also been associated with increased concentrations of plasma AGEs (Fig. 1D), and several studies have shown elevated levels of these AOPPs in hemodialysis patients (Thornalley and Rabbani 2011; Agalou et al. 2005; Thornalley et al. 2003; Thornalley 2006). Complement proteins themselves are glycated and may contain AGEs, which are suspected to influence complement activity (Cheng and Gao 2005; Acosta et al. 2000; Davies et al. 2005; Zhang et al. 2011; Austin et al. 1987; Hair et al. 2012; Niemann et al. 1991; Thornalley et al. 1999).

Recent work by our group has emphasized the ability of modern hemodialysis membranes to induce both complement and inflammatory activities despite their improved biocompatibility, as well as the potential contributions of this activity to thrombotic complications seen in hemodialysis patients. Increased complement activation and a substantial increase in the protein levels of pro-inflammatory cytokines were detected in blood from ESRD patients undergoing hemodialysis, and the sera of hemodialyzed ESRD patients induced the production of functionally active tissue factor (TF), the primary initiator of coagulation, by blood leukocytes (Kourtzelis et al. 2010); the production of TF is perhaps not surprising given the known interactions between the complement and coagulation cascades (Oikonomopoulou et al. 2012). In an attempt to define the mechanism connecting complement activation and inflammation during hemodialysis, we developed an *ex vivo* model to simulate blood filtration using commercially available and clinically relevant PS hemodialysis filters under very controlled conditions (Kourtzelis et al. 2010). Blood from healthy donors used in this extracorporeal system showed a strong increase in complement activation, activation of PMN, and increased levels of pro-inflammatory markers such as IFN- γ , IL-1RA and G-CSF (Fig. 2). Thus, despite improvements in the biocompatibility of modern hemodialysis membranes and other biomaterials, these products still induce significant complement activity and inflammation. The studies mentioned here serve to confirm the connection between this biomaterial-induced complement activation and downstream pro-inflammatory activities. However, broader examinations of complement activation on distinct materials are still needed to better elucidate the mechanisms involved in these processes and to identify potential targets for therapeutic intervention.

The potential for complement-modulating therapeutics in hemodialysis

Various types of anti-inflammatory interventions for patients with kidney disease and on hemodialysis, some targeting CVD risk factors, have been proposed or tested and met with mixed success (Wanner et al. 2005; Besarab et al. 1998; Cano et al. 2007; Jamison et al. 2007; Suki et al. 2007; Hung et al. 2011; Himmelfarb et al. 2007; Stenvinkel et al. 2006). Thus, there is still a strong need to develop therapeutic options that will aid in limiting inflammatory conditions in hemodialysis patients. The extent of complement activation due to hemodialysis depends in part, as mentioned, on the type of membrane used. Despite reduced induction of complement activity by newer modified cellulose or synthetic membranes, however, hemodialysis patients are still consistently exposed to low-level complement activity during their frequent treatment sessions, which likely contributes to their chronically acute inflammatory state as described above. As complement acts upstream of many inflammatory pathways, modalities designed to reduce complement activity, and in turn beneficially regulate related cytokine and coagulation networks, would be expected to improve the overall condition of hemodialysis patients and reduce inflammation-related complications. Other factors further increase the appeal of targeting the complement system to tame hemodialysis-related inflammation. First, the complement network involves some

fifty different proteins (Ricklin et al. 2010), thereby potentially offering a variety of targets to modulate the response in a material-tailored manner. Second, local and systemic administrations of complement inhibitors have so far proven to be safe in a number of clinical trials, including some involving biomaterial-induced complement activation in the context of cardiopulmonary bypass (CPB), where consequent induction of inflammation can lead to significant morbidity and mortality (Lazar et al. 2007; Warren et al. 2009; Thiara et al. 2011). In a study in which patients undergoing CPB surgery received soluble CR1 (TP10) as a complement inhibitor immediately before the procedure, there was not only a decreased incidence of patient death and myocardial infarction but also less need for prolonged intra-aortic balloon pump support for patients (Lazar et al. 2004). Finally, as complement activation during hemodialysis is restricted to the time when blood is in contact with the filter membrane, complement-targeted intervention is only needed during the procedure, restoring complement activity to full capacity between sessions, which reduces concerns of long-term immunosuppression.

Heparin coating of biomaterials has been studied as an alternative anti-coagulation strategy in hemodialysis, and this has been used in various studies as a complement inhibition strategy through active adsorption of factor H (fH) and enhancement of C1s/C1-INH complexes (Fig. 1E) (Cronin and Reilly 2010; Davenport 2011; Andersson et al. 2003; Lappegard et al. 2004; Moen et al. 1997; Mollnes et al. 1995). However, this procedure is costly and complex and has not shown benefits significant enough to justify its use as yet in hemodialysis (Cronin and Reilly 2010; Davenport 2010; Davenport 2011; Andersson et al. 2003; Lappegard et al. 2004). Further, heparin is known to interact with various plasma proteins and may cause undesirable effects due to its 'broadband' specificity. An alternative approach would be the direct targeting of natural complement inhibitors to biomaterials. Under physiological conditions, healthy human cells are protected from complement attack by regulators that are either expressed on the cell surface or circulate in the blood (Ricklin et al. 2010; Zipfel and Skerka 2009). The RCA family is particularly important, as these regulators act at the key step of activation/amplification. Whereas CR1, decay accelerating factor (DAF), and membrane cofactor protein (MCP) are membrane-bound, C4b-binding protein (C4BP) and the abundant fH (200-800 $\mu\text{g/ml}$ in plasma) are soluble regulators that target CP and AP convertases, respectively. Importantly, both C4BP and fH tame complement activation in circulation but also recognize specific patterns (e.g., glycosaminoglycans, sialic acid) on host cells and support complement inhibition on surfaces (i.e., self-recognition). A well-maintained balance between complement activation and regulation is crucial for the immunosurveillance, housekeeping, and defensive functions of complement, and any disturbance may lead to disease (Ricklin et al. 2010; Ricklin and Lambris 2007). Surfaces lacking regulators or self-recognition pattern, such as microbial intruders and artificial biomaterials, will likely trigger amplification of complement responses. Intriguingly, some human pathogens expose RCA-capturing molecules as part of their immune evasion strategy (Lambris et al. 2008). For example, M proteins of *Streptococcus pyogenes* bind C4BP (Jenkins et al. 2006; Persson et al. 2006; Thern et al. 1995), *Neisseria meningitidis* expresses a fH-binding protein (Madico et al. 2006; Schneider et al. 2006; Schneider et al. 2009), and Staphylococcal proteins (Efb, Sbi) have been implicated in the enhancement of fH binding to C3b (Chen et al. 2010; Haupt et al. 2008). Whereas the ability of such proteins to bind complement inhibitors can be explored in the context of targeting specific complement regulators to biomaterials, their coating with bacterial RCA-capturing proteins appears unfeasible due to size and immunogenicity concerns. M protein-derived peptides have indeed been shown to recruit C4BP to polystyrene surfaces and reduce complement activation (Engberg et al. 2009), but their length of some fifty amino acids renders cost-effective production challenging. Therefore, the development of small molecules for coating biomaterials is highly desired and considered very promising (Andersson et al. 2006; Andersson et al. 2001). In line with this

concept, a phage-display peptide library was recently screened against fH and several clones with high binding affinity were identified. One of the clones, the 5C6 peptide, showed no binding to either terminus of fH that is involved in regulatory activities, and it is therefore expected to capture fH in the broad middle region of this elongated regulator. Indeed, 5C6 did not interfere with either the decay acceleration or cofactor activity of fH but showed strong binding affinity towards it. More importantly, immobilized 5C6 was able to capture fH and potently suppress complement activation induced by the contact of human plasma with polystyrene surfaces (Fig. 1F) (Wu et al. 2011). Such RCA-capturing coatings may be particularly attractive for biomaterials that reside in the body (e.g., implants) as they impair complement activation directly on the affected surface and may reduce the need for chronic treatment on a systemic level.

In the case of hemodialysis, however, soluble complement inhibitors may be an equally attractive alternative to the modification of hemodialysis membranes. The repetitive but temporary periods of exposure of blood to foreign materials during hemodialysis makes this a particularly suitable application for this approach. Several different complement inhibitors have been developed, some of which are currently used in the clinic for specific pathologies or are undergoing clinical trials (Ricklin and Lambris 2007; Qu et al. 2009; Ricklin and Lambris 2012). Purified human C1-INH, currently the only complement-associated protease inhibitor on the market, is a heavily glycosylated plasma protein that has been safely and effectively used for the treatment of hereditary angioedema (HAE) (Wagenaar-Bos and Hack 2006; Agostoni et al. 1980; Kirschfink and Mollnes 2001; De et al. 2003; Longhurst et al. 2007). C1-INH acts by blocking the C1r and C1s esterase activities (Pensky et al. 1961), but also inhibits other serine proteases such as MASP, kallikrein, and coagulation factors XI and XII. Studies have suggested that C1-INH may be a beneficial therapeutic for other complement-related disorders besides HAE, such as ischemia/reperfusion injury (Kirschfink and Mollnes 2001; Caliezi et al. 2000; Nielsen et al. 2007; Lauterbach et al. 2007). Potential complement activation through the CP and LP during hemodialysis suggests that C1-INH could also be used therapeutically in hemodialysis patients to attenuate complement-related complications (Fig. 1E). However, the comparatively high cost of C1-INH must be taken into account and may preclude an economical use in hemodialysis given the frequency of treatments that would be required.

Given the strong involvement of C5a in inflammatory signaling, inhibition at the C5 level appears an attractive option. The C5 antibody Eculizumab efficiently prevents the generation of both C5a and TCC and is successfully used in the clinic to treat paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome (Risitano et al. 2011; Loirat and Fremeaux-Bacchi 2011). Similar to the case of C1-INH, however, the high costs of this therapeutic antibody would likely prevent an application in hemodialysis. In contrast, C5aR antagonists are usually small molecule drugs and can be produced more cost-effectively (Bray 2003; Vlieghe et al. 2010). C5a is a potent activator of leukocytes (through C5aR expressed on myeloid and nonmyeloid cells) that can contribute to hemodialysis inflammatory responses and CVD, and therefore its inhibition could significantly reduce their actions (Fig 1G). Several C5aR antagonists have been shown to inhibit pro-inflammatory complement activity in experimental and preclinical studies. Currently, the most promising C5aR antagonist candidate is PMX-53, a cyclic peptidomimetic less than 1 kDa in size (Qu et al. 2009). This compound has been used in various animal models, and has been shown to be safe and well tolerated in phase I clinical trials for rheumatoid arthritis and psoriasis (Hezmee et al. 2012; Köhl.J 2006).

Complement inhibition by PMX-53 still leaves the pro-inflammatory activities of the C3a anaphylatoxin intact, as well as the continuous deposition of C3b and downstream formation of the TCC, which may activate leukocytes and participate in red blood cell hemolysis

during hemodialysis (Deppisch et al. 1990; Hakim 1993). Thus, a broader inhibition of complement at the level of C3 may be desirable. The peptidic complement inhibitor compstatin and its derivatives inhibit cleavage of C3 to C3a and C3b, thereby simultaneously blocking C3b-opsonization by all pathways, the amplification loop, and most downstream complement activities. This cyclic tridecapeptide likely acts by disrupting protein-protein interactions during convertase formation (Fig. 1H) (Ricklin and Lambris 2008; Janssen et al. 2007), and shows narrow species specificity for primate C3 (Sahu et al. 2003). Rational design and screening efforts have led to a panel of compstatin analogs with improved inhibitory properties (Katragadda et al. 2006; Qu et al. 2011; Magotti et al. 2009). Compstatin-mediated complement inhibition improved outcomes in several experimental disease models, and no adverse effects from treatment were detected in any of these studies (Holland et al. 2004; Qu et al. 2009; Soulika et al. 2000; Silasi-Mansat et al. 2010; Chi et al. 2010). One compstatin analog (POT-4, Potentia Pharmaceuticals) was found to be safe in phase I clinical trials and is currently being evaluated in a phase II trial for the local treatment of age-related macular degeneration (AMD) by Alcon (AL-78898A; (Alcon Research 2012)). Importantly, compstatin has been shown to inhibit complement activity, leukocyte activation and binding, and cytokine induction in response to blood contact with artificial surfaces (Nilsson et al. 1998; Lappégard et al. 2008; Schmidt et al. 2003; Kourtzelis et al. 2010). The most recently disclosed compstatin analogs showed large improvements concerning target binding affinity (picomolar range), solubility and/or pharmacokinetic profiles (elimination half-life of up to 12 h), thereby rendering them much more suitable for systemic administration (Qu et al. 2012). Given the high activity of current compstatin analogs and the general advantages of small peptide drugs, such as low and predictable toxicity, limited immunogenicity, and largely reduced production costs (as low as \$1 per gram per amino acid) (Bray 2003; Vlieghe et al. 2010), compstatin has strong potential as a drug candidate for use in hemodialysis to limit complement-related complications.

The promise of complement inhibition to reduce hemodialysis-related complications has been recently demonstrated by studies using ESRD sera and the extracorporeal blood circulation model described earlier. Treatment of sera from ESRD patients receiving hemodialysis with PMX-53 prevented the increase in PMN-produced TF normally observed when C5aR signaling is present. Incubation of sera from both ESRD patients and healthy donors with fibers from hemodialysis filters normally induces TF-dependent pro-coagulant activity in PMN, but pre-treatment with either PMX-53 or compstatin reduced this activity. Finally, in the extracorporeal system, pre-treatment of blood with compstatin inhibited complement activation and TF expression in PMN and reduced levels of several inflammatory markers such as IFN- γ , IL-1RA, and G-CSF (Fig. 2). Thus, complement inhibitors were shown to be effective at attenuating both inflammatory and thrombotic events induced by hemodialysis or filter materials.

Concluding remarks

Accumulating evidence from the past 30 years has shown the involvement of complement in hemodialysis-related inflammation. Though huge progress has been made towards the selection and manufacturing of dialysis filters with better biocompatibility, these efforts have proven to be insufficient at completely eliminating complement activation and related inflammation. Thus, patients undergoing maintenance hemodialysis still suffer from poor quality of life and low long-term survival rates, warranting further analysis of the mechanisms of filter-specific induced complement initiation, the multiple downstream effects of this activation, and the potential to use complement inhibitors to block the unwelcomed effects resulting from chronically acute inflammation to potentially improve their long-term prognosis. The complement system offers ideal therapeutic targets as its

activation and, therefore, required inhibition, is only temporary, occurring during the hemodialysis procedure, thus allowing full recovery of immune functions between treatments. Further, the development of complement therapeutics targeting specific proteins and pathways may benefit from the reduced production costs of small peptides compared to other types of drugs, ultimately leading to the development of practical, cost-effective treatments to improve the condition of those undergoing hemodialysis. Clearly, the attenuation of inflammatory complications related to hemodialysis through the inhibition of complement activity already shows high promise and may result in urgently desired novel treatment options for patients suffering from ESRD.

Abbreviations

ESRD	end-stage renal disease
CVD	cardiovascular disease
CRP	C-reactive protein
IL-6	interleukin-6
ICAM-1	intracellular adhesion molecule-1
AGE	advanced glycation end product
ALE	advanced lipoxidation end product
AOPP	advanced oxidation protein product
PS	polysulfone
CP	classical pathway
LP	lectin pathway
AP	alternative pathway
PAMP	pathogen-associated molecular pattern
DAMP	damage-associated molecular pattern
PRR	pattern recognition receptor
MBL	mannose-binding lectin
MASP	MBL-associated serine protease
fB	factor B
fD	factor D
CR	complement receptor
C3aR	C3a receptor
C5aR	C5a receptor
TCC	terminal complement complex
RCA	regulator of complement activation
C1-INH	C1-inhibitor
PMN	polymorphonuclear cell
TF	tissue factor
IFN-γ	interferon-gamma

IL-1RA	interleukin-1 receptor A
G-CSF	granulocyte-colony stimulating factor
CPB	cardiopulmonary bypass
fH	factor H
DAF	decay accelerating factor
MCP	membrane cofactor protein
C4BP	C4b-binding protein
HAE	hereditary angioedema
PK	pharmacokinetic
AMD	age-related macular degeneration

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Figure 1. Membrane-induced activation of complement and inhibition strategies

(A, B) Binding of C3 to a membrane-bound plasma protein layer results in a conformational change leading to the formation of AP C3 convertases (A). Binding of C1q to deposited IgG (with subsequent binding of C1r/C1s) activates the CP, while direct binding of ficolin may lead to LP activation through the binding and actions of MASP, both resulting in CP C3 convertase formation (B). Cleavage of C3 and C5 by the convertases produces the C3a and C5a anaphylatoxins, respectively, and the downstream TCC. (C) C3b degradation products formed as a result of convertase and cofactor activity can bind directly to membrane-bound proteins. (D) All of the above events activate leukocytes and lead to the production of pro-inflammatory and damaging mediators and effects. (E) Heparin coating of membranes can lead to the binding of C1 inhibitor (C1-INH), which can prevent the release of C1r/C1s and block the actions of MASP. Soluble C1-INH can also act to reduce the activity of these proteases. (F) The fH-binding molecule 5C6 is able to bind to surfaces and capture the fH protein, which acts as a co-factor to factor I (not shown) to cleave and inactivate C3b and prevent AP activation of complement. (G) C5aR antagonists such as PMX-53 act downstream of the surface bound inhibitors, preventing the binding of C5a to its target receptor, and thus inhibiting the activation of leukocytes that would normally occur during hemodialysis. (H) Another soluble inhibitor, compstatin, offers more complete complement inhibition by acting at the level of the C3 convertases to prevent the cleavage of C3, which blocks downstream effects such as the generation of anaphylatoxins, TCC, and C3 degradation products and, ultimately, leukocyte activation and pro-inflammatory consequences. Abbreviations (not mentioned in the text): H, heparin; Conv, convertase(s).

Figure 2. Suggested model for hemodialysis membrane induction of complement-mediated inflammation and thrombosis

Hemodialysis was shown activate complement, with production of the TCC evident. Neutrophils, which normally express low levels of the C5aR and pro-coagulant TF, were also activated by hemodialysis, with a corresponding increase in expression of CD11b and internalized C5aR (but decrease of surface C5aR), likely the result of increased binding by C5a, which leads to internalization of the ligand-receptor complex. There was also an increase in the production and secretion of TF and several pro-inflammatory cytokines by these cells, as well as TF-dependent pro-coagulant activity, suggesting that complement activation as a result of the hemodialysis procedure eventually leads to increased inflammation and thrombosis. Confirming this, pre-treatment with compstatin, which blocks C3 convertase activity and prevents C5a and TCC production, and/or the C5aR antagonist PMX-53, which blocks C5a binding to the C5aR, prevented the increases in CD11b expression, IFN- γ , IL-1RA, G-CSF, and TF production/secretion, as well as TF-dependent pro-coagulant activity.