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Commentary

Taming hemodialysis-induced inflammation: Are complement C3 inhibitors a viable option?

Dimitrios C. Mastellos[a], Edimara S. Reis[b], Ali-Reza Biglarnia[c], Meryl Waldman[d], Richard J. Quigg[e], Markus Huber-Lang[f], Marc A. Seelen[g], Mohamed R. Daha[h], John D. Lambris[b,*]

[a] Division of Biodiagnostic Sciences and Technologies, National Center for Scientific Research 'Demokritos', Athens, Greece
[b] Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, USA
[c] Department of Transplantation, Skåne University Hospital, Malmö, Lund University, Lund, Sweden
[d] Kidney Disease Section, Kidney Diseases Branch, NIH, Bethesda, USA
[e] Division of Nephrology, Department of Medicine, Jacobs School of Medicine & Biomedical Sciences, University at Buffalo, USA
[f] Institute for Clinical and Experimental Trauma-Immunology, University Hospital of Ulm, Ulm, Germany
[g] Division of Nephrology, Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, Netherlands
[h] Department of Nephrology, Leiden University Medical Centre, Leiden, Netherlands

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ABSTRACT

Owing to an increasing shortage of donor organs, the majority of patients with end-stage kidney disease remains reliant on extracorporeal hemodialysis (HD) in order to counter the lifelong complications of a failing kidney. While HD remains a life-saving option for these patients, mounting evidence suggests that it also fuels a vicious cycle of thromboinflammation that can increase the risk of cardiovascular disease. During HD, blood-borne innate immune systems become inappropriately activated on the biomaterial surface, instigating proinflammatory reactions that can alter endothelial and vascular homeostasis. Complement activation, early during the HD process, has been shown to fuel a multitude of detrimental thromboinflammatory reactions that collectively contribute to patient morbidity. Here we discuss emerging aspects of complement's involvement in HD-induced inflammation and put forth the concept that targeted intervention at the level of C3 might constitute a promising therapeutic approach in HD patients.

1. Overview

Hemodialysis (HD) is a life-saving renal replacement modality that has been consolidated in clinical practice as a mainstay of treatment for end-stage renal disease [3,4,23]. "Hemodialysis" originates from the ancient Greek words "hema" (blood) and "dia-lysis" (separation) which fittingly describe the process by which blood is separated through a semipermeable membrane. Despite recent technological advances in biomaterial design and surface functionalization, HD circuits can have a broadly negative impact on key sentinel systems of the intravascular innate immune response, including the complement, contact and coagulation systems [4,18]. Concerted activation of these blood-borne defense systems is believed to fuel a chronic inflammatory response in HD patients which is strongly associated with an elevated risk for cardiovascular disease (CVD) [4]. In fact, chronic hemodialysis treatment is associated with a 10–50-fold higher risk of premature mortality than that of the age-matched general population, with CVD as a leading cause of death [12]. Biomaterial-induced contact activation of plasma proteins occurs early during HD and leads to local generation of inflammatory mediators close to the biomaterial surface. Inflammation is further propagated by soluble mediators that are generated during HD and transported from the extracorporeal circuit back into the patient together with activated leukocytes (e.g. macrophages/neutrophils) and platelets [5]. These early 'priming' events are thought to culminate longitudinally in the undesirable activation of the endothelium, e.g., the cardiovascular endothelium, which gradually loses its anti-thrombotic and anti-inflammatory properties, leading to atherogenesis and arteriosclerosis [4].

An estimated 2.6 million people are treated for end-stage kidney disease (ESKD) worldwide [23]. The majority of these ESKD patients remains dialysis-dependent for their entire life-span or until a compatible donor organ can be found. Given the alarmingly increased shortage of donor organs, ESKD patients are forced to rely on HD for extended time periods, facing serious complications due to a chronic

* Corresponding author.
E-mail address: lambris@pennmedicine.upenn.edu (J.D. Lambris).

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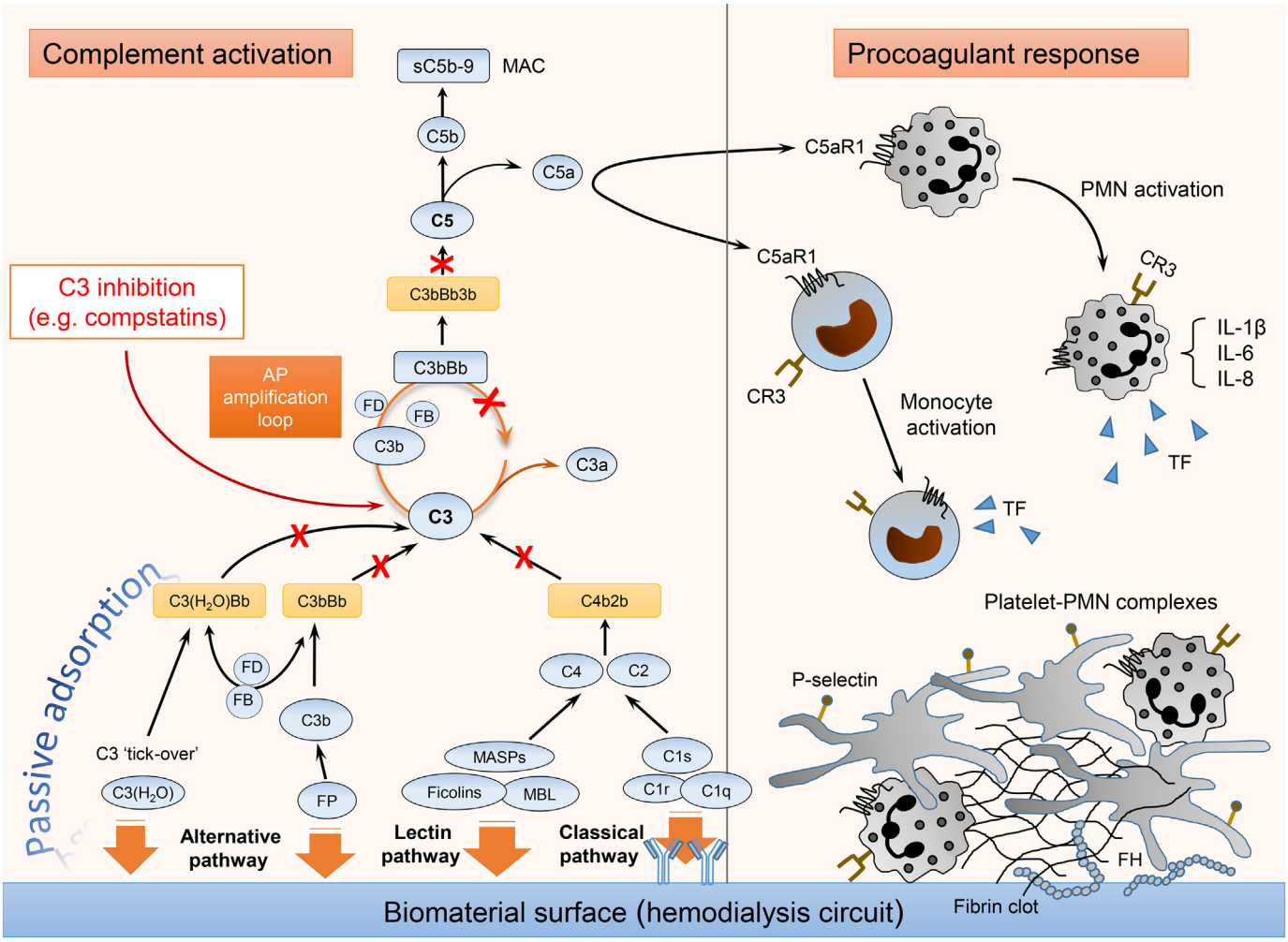

Fig. 1. Biomaterial-triggered complement activation drives early thromboinflammatory changes during hemodialysis. Passive adsorption of multiple plasma proteins onto the HD filter or dialysis tubing, including antibodies and pattern recognition molecules such as ficolin-2, MBL, properdin, C1q and C3($H_2$0), may trigger simultaneously multiple routes of complement activation in the HD circuit. All routes of complement activation converge at the level of C3 and lead to the generation of proinflammatory effectors and immunostimulatory molecules such as C3a and C5a anaphylatoxins and of cell-activating soluble C5b-9 complexes (sC5b-9). Sequestering of fluid phase complement regulators, such as factor H, to the biomaterial surface through possible entrapment in the adsorbed plasma protein mesh or fibrin coat, favors the forceful amplification of complement C3 fragment deposition on the surface via the AP amplification loop. Moreover, C5aR1 stimulation on monocytes and neutrophils leads to upregulation of adhesion receptors and increased expression of the complement phagocytic receptor CR3. C5aR1-mediated activation of monocytes/neutrophils foster a procoagulant environment via the de novo release or increased surface expression of TF which in turn initiates the intrinsic coagulation pathway leading to thrombin activation, fibrin formation and platelet-neutrophil complex formation via CR3-iC3b tethering in the vicinity of the HD biomaterial surface. Targeted inhibition of C3 by members of the compstatin family of peptidic inhibitors blocks the cleavage of C3 by all convertases, abrogates the generation of downstream proinflammatory effectors (C3a, C5a, sC5b-9) and attenuates the procoagulant response which is elicited following blood-biomaterial surface contact. The 'X' sign denotes points of therapeutic intervention with a C3 inhibitor.

and insidious inflammatory response that ensues upon contact of whole blood with the HD circuit's biomaterial surface. Therefore, HD-reliant ESKD patients define a population that faces unmet clinical challenges, being in need of more effective options to treat HD-associated pathological changes and co-morbidities that are fueled by their failing kidneys [4,23].

2. Complement activation during HD

It is well known that the complement system is readily activated in the vasculature upon contact with foreign material, thereby amplifying a host response that can release 'danger' signals, leukocyte/endothelium priming factors and proinflammatory mediators in the circulation [7]. In this respect, exposure of biomaterial surfaces (e.g., HD filters, extracorporeal perfusion circuits or implants) to whole blood constituents can rapidly trigger complement activation that will, in turn, induce an vicious cycle of thrombo-inflammation, leading to procoagulant responses that have detrimental consequences for organ function [5]. Recent advances in surface nanopatterning and biopolymer technology have attempted to bridge the gap of incompatibility in such systems, but nevertheless, clinical complications remain, largely as the result of recurring biomaterial-induced inflammatory episodes that exacerbate chronic underlying pathologies [26]. Hemodialysis-induced complement activation has been associated with such thromboinflammatory responses, which likely increase the burden of disease (e.g., the risk of cardiovascular disease) [4,20].

Despite significant progress in biocompatibility of HD membranes, undesirable complement activation within the HD circuit remains a significant challenge with detrimental proinflammatory consequences. Short-term effects of complement activation in HD include promoting inflammation and coagulation at the endothelium-vasculature interface [4]. In addition, long-term complications of dialysis, such as infection, fibrosis and cardiovascular events, are also linked to imbalanced or inappropriate activation of the complement system [18].

Interestingly, several studies have shown that even modern "biocompatible" HD filters trigger relevant levels of complement activation at the level of C3 and also induce Tissue Factor (TF) expression, thereby contributing to a thromboinflammatory milieu that can increase morbidity in ESRD patients [13,15]. Of note, it has been shown that during HD, C3 activation peaks as early as during the first 10–15 min, marked by elevated levels of C3a, whereas terminal pathway activation, resulting in C5a and C5b-9 formation occurs at a later stage of dialysis [1,21]. During a single HD session soluble C5b-9 (sC5b-9) levels and C3d/C3 ratios in the plasma increase up to 70% [19]. Yet, this is most likely an underestimation of the amount of complement activation, since these values represent fluid phase activation and tend to overlook surface deposition of activated fragments.

Several studies have attempted to dissect the pathways responsible for complement activation in HD. Early evidence for complement AP activation during HD was provided in a study by Cheung et al., employing cellulose HD membranes [2]. Initially, the involvement of the CP or LP was excluded, since it was reported that plasma C4d concentrations remained unaffected during HD [9]. However, others were able to show C4 activation by cellulose membranes [10]. The increase in C4d levels correlated with the rise in C3d levels, implying that the CP or LP is (at least partly) responsible for the complement activation seen in HD. More than two decades later, a role for the LP in triggering HD-associated complement activation was demonstrated using polysulfone membranes [11].

In summary, complement activation in HD is mainly triggered by binding of the LP pattern recognition molecules, MBL and ficolin-2, to the HD membrane which results in LP activation. The biomaterial-triggered complement response is further augmented by the surface adsorption of C3/C3b and/or properdin which trigger AP activation and further amplification of complement fragment deposition. The latter is supported by the evidence that in C4-deficient patients, systemic complement activation and C3b deposition on the HD membrane are reduced during dialysis but not abolished [14]. Collectively, these results support the parallel contribution of several complement pathways to HD-induced systemic inflammation, while indicating a crucial role for the AP in amplifying the complement-driven proinflammatory response elicited during HD (Fig. 1).

A second mechanism that could modulate complement activation during HD is the loss of complement inhibitors via absorption to the HD membrane. Polysulfone membranes have been shown to absorb factor H and clusterin [16]. Factor H is the main fluid-phase regulator of the AP, while clusterin prevents terminal pathway activation, thereby blocking the formation of C5a and C5b-9 [22]. The loss of these inhibitors would cause dysregulation of the AP, leading to further complement activation in the fluid phase (i.e., in the circulation) in HD patients.

3. Clinical consequences of HD-associated complement activation

Deregulated complement activation has been implicated as a driving factor that contributes to the clinical deterioration and comorbidities manifested by HD patients over time [4]. This poor clinical course is likely driven by the sustained activation of downstream proinflammatory and procoagulant/thrombogenic pathways which are triggered by distinct complement fragments and receptors on innate immune cells, vascular endothelial cells and platelets. These pathways are considered important determinants of HD-associated pathology [3,13,17]. For instance, generation of the anaphylatoxins C3a and C5a during HD promotes the recruitment and activation of leukocytes [24]. Leukocyte activation culminates in the oxidative burst of neutrophils/macrophages and the release of oxidative enzymes and proinflammatory cytokines or chemokines such as IL-1β, IL-6, IL-8, TNF-α. MCP-1 and interferon-γ [18].

Furthermore, complement activation in HD patients results in the upregulation of adhesion molecules both on endothelial cells and leukocytes, especially complement receptor 3 (CR3). The C5a-activated leukocytes will then bind C3 fragments (iC3b) deposited on the membrane via CR3, leading to leukopenia [17]. Likewise, CR3 on neutrophils is also important for the formation of platelet–neutrophil complexes, which can contribute to both inflammatory and thrombotic processes [8]. It has been demonstrated that C5a generation during HD leads to the expression of TF and granulocyte colony-stimulating factor in neutrophils, shifting HD patients to a procoagulant phenotype [13]. Consistently, plasma C3 levels have been shown to positively correlate with a denser clot structure in HD patients [25].

Chronic, low-level inflammation and persistence of a thrombogenic state are considered integral pathogenic drivers of cardiovascular disease [4,6]. Accordingly, complement activation has been associated with a heightened risk of HD patients to develop cardiovascular disease [4,15]. Plasma C3 levels, prior to a HD session, were found to be higher in patients who developed a cardiovascular event (CV-event) than in HD patients who remained event-free. Moreover, an association was found between C3 levels and the development of CV-events [15]. A similar trend of higher C3 levels in HD patients who developed a CV-event has recently been reported in a study by Poppelaars et al. [20]. Overall, these studies have highlighted the important contribution of all complement pathways, and especially AP- amplified C3 activation, in the early inflammatory response in HD patients. They also formulate the basis for developing complement-targeted therapeutics as a means of ameliorating HD-associated pathology and improving the clinical course of patients that rely on life-long, maintenance HD.

3.1. Therapeutic targeting of complement in HD-associated inflammation

To date, therapeutic strategies to mitigate HD-induced complement activation have mostly relied on: i) new biomaterials that increase the biocompatibility of HD filters, thus reducing their complement-activating capacity, ii) the use of anticoagulants that indirectly inhibit complement CP- or LP-dependent proteases and iii) developing and testing targeted complement therapeutics in preclinical settings of biomaterial-induced inflammation [4,18,21]. The clinical promise of complement modulation during HD was recently underscored in a study following up complement activation and inflammatory biomarkers in a cohort of HD patients with increased risk of CVD [20]. Prominent C3 activation during HD (i.e., C3d/C3 levels) was correlated with increased CVD risk, while these CVD-prone patients exhibited a higher IL-6/IL-10 ratio and increased levels of TNF-α and von Willebrand Factor (vWF) during the HD session. More importantly, administration of a single dose of C1-esterase inhibitor (C1-INH) (a CP- and LP-directed complement inhibitor with broad substrate specificity) in an ex-vivo perfusion model of HD, resulted in attenuated C3 activation and decreased levels of key pro-inflammatory and prothrombotic markers implicated in CVD predisposition [20]. These findings further corroborate a causal association between HD-induced complement activation and proinflammatory/thrombogenic responses in HD patients. Furthermore they strongly argue for a pivotal role of C3 activation in driving the proinflammatory reaction that is triggered early during blood-biomaterial surface contact.

3.2. C3 inhibition as an effective means to attenuate HD-associated thromboinflammation

Biomaterial-induced thromboinflammation is an early hallmark of pathological changes following extended HD treatment. Notably, this thromboinflammatory reaction has been shown to be attenuated in an ex vivo model of biomaterial-induced inflammation when C3 activation is inhibited by the second-generation compstatin analog 4(1MeW)/POT-4 [13].

Recently, these findings were recapitulated in a refined, clinically relevant, non-human primate (NHP) model of HD-induced inflammation [21]. Cynomolgus monkeys were subjected to HD using a circuit of

pediatric hemodialysis filters, and significant complement activation was observed even after a single HD session. Of particular note, a single intravenous bolus injection of the third-generation compstatin analog Cp40 prior to HD completely abrogated HD-induced complement activation and led to elevated levels of the anti-inflammatory cytokine IL-10 [21]. The time-restricted dosing scheme of Cp40 in hemodialysis allows for rapid recovery of complement activity between sessions, thereby pointing to a safe and affordable treatment option that may alleviate inflammatory and subsequent atherogenic complications in ESRD patients. Furthermore, the availability of surface-targeting moieties and coating strategies that can direct soluble C3 regulators to surfaces [27] offers alternative routes for modulating complement activation and containing inflammatory responses evoked by HD filters and other medically applied biomaterial surfaces (e.g., biomedical implants).

Finally, the emerging correlation between C3 inhibition and a reciprocal increase in anti-inflammatory IL-10, which has been consistently observed in both NHP and human (ex-vivo) models of HD, likely offers a unifying mechanistic basis for considering C3-targeted intervention as an effective and comprehensive strategy for preventing complement-mediated proinflammatory activation in chronic HD patients.

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Disclosure statement

J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors for the treatment of complement-mediated inflammatory disorders (including third-generation compstatin analogs such as AMY-101), and inventor of patents or patent applications that describe the use of complement inhibitors for therapeutic purposes, some of which are developed by Amyndas Pharmaceuticals. J.D.L. is also the inventor of the compstatin technology licensed to Apellis Pharmaceuticals (4(1MeW)7W, also known as POT-4 and APL-1) and of PEGylated derivatives such as APL-2. The other authors declare no competing interests.

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