Complement modulation in renal replacement therapy

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Chapter 1

General introduction and rationale

Introduction

The traditional view of the complement system consists of a heat-labile component of serum that is important for host defense. At the end of the nineteenth century, efforts from different researchers resulted in the discovery of a factor that "complemented" the lytic activity of serum. By the late 1920s, the first components of the complement system were discovered.[1] Since then, new findings have broadened our understanding of the structural and functional properties of this system.[2] Today, we know complement as a complex system that contributes to health and disease.[3] In short, the complement system can become activated via three pathways: the classical pathway, lectin pathway, and alternative pathway. Regardless of the trigger, all pathways lead to the generation of a C3 convertase. This convertase cleaves C3 into C3a and C3b. The smaller fragment C3a is an anaphylatoxin. The larger fragment C3b binds to the C3 convertase, generating the C5 convertase. Finally, C5 is cleaved by the C5 convertase into C5a and C5b. The smaller fragment C5a is a powerful anaphylatoxin and chemoattractant. The larger fragment C5b initiates the assembly of the membrane attack complex. Newly formed C5b binds first to C6, then to C7 and finally to C8 forming the C5b-6-7-8 complex. Complement component C9 binds to C5b-8 and polymerizes to form a pore in the cell membrane, known as the membrane attack complex. To prevent unintended self-activation, the complement system is kept under tight regulation by a variety of soluble and membrane-bound regulators.[4,5]

The nomenclature of the complement system can seem confusing and conflicting. Initially, the complement components were assigned a number in the order of their discovery rather than the sequence of the activation.[6] Yet, the proteins of the latter discovered alternative pathway were termed factors and assigned different letters, such as factor B and factor D.[7] The lectin pathway was the third and last route to be identified and the names of these proteins are related to their structural properties. For example, mannose-binding lectin (MBL).[8] Furthermore, as complement proteins are activated they are cleaved into smaller fragments. The minor fragment is assigned the letter "a", while the major fragment is assigned the letter "b".[9] For example, C4 is cleaved to C4a, a smaller fragment and to the large fragment C4b.

The majority of complement proteins are synthesized by the liver, however, most tissues and inflammatory cells can also produce complement proteins.[3,10] For instance, the kidney has been shown to synthesize almost all complement components.[11] Moreover, during inflammatory conditions the kidney can contribute up to 15% of the circulating pool of C3.[12] In addition, the kidney has a unique link with the complement system and for unknown reasons this organ is highly susceptible to complement-induced damage.[13] There is a wide range of complement-mediated renal diseases.[14] The complement system can be the direct cause or the aggravating factor in the pathogenesis of renal diseases.[2] Currently, the clinical arsenal of complement inhibitors is limited to C1 esterase inhibitor (C1-INH; various manufacturers) and the monoclonal antibody against C5 eculizumab (Soliris; Alexion Pharmaceuticals). However, more complement therapeutics are expected to follow, since several clinical trials are currently underway to evaluate the therapeutic potential of new complement inhibitors for the treatment of kidney diseases.[15,16] Altogether, this is an exciting and potentially revolutionary time for complementologists as well as for researchers and physicians interested in the complement system.

Scope of the thesis

The aim of this thesis is to assess the adverse effects of complement activation during renal replacement therapy and to explore the benefits and challenges of therapeutic complement inhibition. The complement system is a defense system that in the context of renal replacement therapy becomes hostile. As part of the innate immune system, complement plays an important role in the balance between the susceptibility to infectious and inflammatory conditions. A more active complement system swings the balance toward inflammation and possibly autoimmunity, whereas an inactive system increases the risk for infection. The present thesis attempts to expand the current knowledge on the role of complement in dialysis and renal transplantation and furthermore aims to identify the optimal therapeutic approach to target this system.

Part A of this thesis focuses on the role of the complement system in dialysis. **Chapter 2** provides a comprehensive overview of the role of complement as a driver of inflammation in patients subjected to dialysis (i.e., hemodialysis and peritoneal dialysis). Furthermore, this chapter summarizes the role of complement activation in pathologies associated with thrombo-inflammatory responses during dialysis. More importantly, possible strategies to inhibit complement during dialysis and subsequently minimize undesired morbidity and mortality are discussed in detail. In **Chapter 3** systemic complement activation was studied in hemodialysis patients and compared to healthy controls. We hypothesized that significant complement activation still occurs with modern hemodialysis membranes, despite the advanced nanostructured materials and enhanced biocompatibility profiles. In addition, complement levels may also help to identify hemodialysis patients who are at risk to develop cardiovascular disease. Therefore, we investigated MBL levels of hemodialysis patients in relation to cardiovascular outcome. In **Chapter 4** the link between the complement system and outcome in hemodialysis patients is further explored by measuring complement activation at different time points during one dialysis session (pre-dialysis, intra-dialytic, and post-dialysis). In addition, inflammatory and pro-thrombotic markers were studied to determine if complement, inflammation, and coagulation are involved in the increased cardiovascular risk of hemodialysis patients. In addition to complement activation via blood-to-membrane interaction, other factors in dialysis also modulate the complement system such as the underlying disease, infection and modern medicines (i.e. nanoparticles, liposomes and monoclonal antibodies). In **Chapter 5** a fresh look is provided at hypersensitivity reactions seen to intravenous iron. Treatment with intravenous iron is common in patients undergoing hemodialysis. However, these preparations can cause hypersensitivity reactions. A new concept of the mechanism behind these hypersensitivity reactions to these preparations has arisen, the concept of complement activation-related pseudo-allergy (CARPA).

Part B of this thesis focuses on the role of the complement system in kidney transplantation. Complement is involved in different stages of the transplantation process: in the donor, during preservation, in reperfusion and at the time of rejection. The importance of the complement system in renal ischemia-

reperfusion injury and acute rejection is widely recognized, however, its contribution to the pathogenesis of tissue damage in the donor remains underexposed. **Chapter 6** outlines what is currently known about complement activation in brain-dead organ donors. The role of local and systemic complement is discussed and how complement activation during brain death contributes to the pathogenesis of transplant injury. Moreover, the therapeutic strategies that have been tested to target complement in brain-dead donors are examined. In **Chapter 7** the inhibitory capacity of C1-inhibitor on the three complement pathways was tested since conflicting data exist on the effect of C1-inhibitor on the alternative pathway. C1-inhibitor has been used extensively for the prophylaxis and treatment of hereditary angioedema and clinical trials are currently under way to evaluate the beneficial effects of C1-inhibitor on renal ischemia-reperfusion injury and renal antibody-mediated rejection. In **Chapter 8** the potential of donor treatment with C1-inhibitor was evaluated in a rat model of brain death. C1-inhibitor treatment was administered after the induction of brain death, to mimic the clinical situation that would involve treating human brain-dead donors after the diagnosis of brain death but prior to procurement for transplantation. In **Chapter 9** a mouse brain death model was used to examine the contribution of complement activation to inflammation and injury in brain dead organ donors. In addition, complement deficient mice were used to dissect the pathway responsible for complement activation in brain death and to determine the effect of inhibition of C5a in brain death. Furthermore, activation of the complement cascade is also an important mediator of renal ischemia-reperfusion injury. The role of C5a receptor 1 (C5aR1) in renal ischemia-reperfusion injury has been extensively studied and inhibition of C5a and C5aR1 protects kidneys from ischemia-reperfusion injury. However, the role of C5a receptor 2 (C5aR2) in this injury is less clear. Initial studies proposed the hypothesis that C5aR2 functions as a decoy receptor. In **Chapter 10** the contribution of C5aR2 to renal ischemia-reperfusion injury was determined by using C5aR2 knockout mice. To further investigate the contribution of renal-expressed C5aR2 versus leukocyte-expressed C5aR2 to renal ischemia-reperfusion injury, bone marrow chimeras were created. In addition, an *in vivo* migration assay was performed to determine the role of C5aR2 in leukocyte migration.

In **Chapter 11,** the final chapter, single nucleotide polymorphisms (SNPs) in complement genes were studied in relation to renal graft survival in humans. Analyses of polymorphisms in complement genes form an elegant way to study the effect of alterations in the complement system on long-term outcome. In the past, complement deficiencies were thought to be rare and of little clinical importance. However, since then various gene variants have been identified that result in either functional or quantitative differences. Ideally one would want to look at the total make-up of the complement genes since multiple polymorphisms are not rare. The total inherited set of complement genes is called the Complotype and is believed to determine the individual's ability to activate and regulate the complement system. Therefore, instead of looking at the effect of single SNPs, this chapter looked at the Complotype of donor-recipient pairs. Furthermore, the potential of the Complotype to improve risk stratification and prediction of renal allograft loss was determined.

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Part A

COMPLEMENT MODULATION IN DIALYSIS