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Complement modulation to improve donor organ quality

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Summary
Discussion
Future Perspectives

Summary

This thesis focused on the contribution of the complement (activation) pathways in brain-death-induced organ injury, and potential strategies to reduce complement-mediated organ injury. **Chapter 2** provides an overview of the existing literature on the role of complement activation during different phases of renal transplantation. Therefore, this chapter served as a starting point in order to identify gaps in knowledge that need to be filled in our quest to develop complement-based therapies. This chapter revealed that, based on the current literature, little is known about the role of complement-mediated renal injury in brain-dead donors and during preservation. This contrasts ischemia-reperfusion injury (IRI) as well as renal allograft rejection, about which the role of complement activation is widely recognized.[1–3] Therefore, chapter 2 exposes existing knowledge gaps with regard to (i) the contribution of the different complement activation pathways in brain death-induced renal injury, (ii) the lack of complement modulation strategies to reduce brain death-induced renal injury and inflammation, and (iii) whether complement is activated during preservation of renal grafts via machine perfusion.

In **chapter 3,** the contribution of the different complement (activation) pathways in brain death-induced renal injury and inflammation was elucidated. As a proof of principle, to confirm the role of complement in brain death-induced renal injury and inflammation, C3-deficient mice were subjected to brain death. C3-deficient mice had significantly less tubular damage and tubular cell apoptosis than wildtype (WT) mice after brain death. Further the absence of C3 in mice prevented the upregulation of pro-inflammatory mediators in kidneys during brain death. So, complement activation during brain death mediates renal injury and inflammation. These results are in line with previous studies, which demonstrated that local complement C3 production in the kidney elicits tissue injury.[4,5] Together, these results might explain why increased C3 gene expression levels in kidneys obtained from brain-dead donors are associated with an inferior renal function in the recipient after transplantation.[6,7]

In addition, the role of the C5a-C5aR-axis was investigated by the use of C5a receptor 1- (C5aR1) and C5a receptor 2- (C5aR2) deficient mice. Prior to this study, we hypothesized that C5a-C5aR1 interaction in the kidney induces renal inflammation, which primes the renal graft prior to transplantation. Our hypothesis would explain the inferior function and graft survival of kidneys retrieved from brain-dead donors. Further, we postulated that C5aR2 would act as a non-signaling decoy receptor, as previously described.[8,9] Interestingly, C5aR2-deficiency, and not C5aR1-deficiency, led to significantly less renal injury and inflammation upon brain death. C5aR2-deficient mice had significantly lower gene expression levels of IL-1ß, IL-6, MCP-1, P-selectin, VCAM-1 and oxidative stress markers NOX2 and SOD1 than WT mice subjected to brain death. In addition, absence of C5aR2 led to a lower influx of neutrophils and macrophages upon brain death. In contrast, gene expression levels of IL-6 and MCP-1 were increased in C5aR1-deficient mice compared to WT mice. In addition, C5aR1$^{-/-}$ mice had similar levels of renal apoptosis and renal tubular damage as seen in WT mice after brain death. These results strongly reject our hypothesis, as they demonstrate a predominant role for C5aR2 in brain death-induced renal injury.

In line with our observations during brain death, renal IRI inflicted to kidneys from C5aR2-deficient mice resulted in reduced tubular damage and reduced renal inflammation compared to WT mice.[10] The C5aR2-deficient mice used, were full knock-outs, which means C5aR2 was not expressed on leukocytes and not in the kidney. To differentiate between the contribution of leukocyte-expressed C5aR2 and renal-expressed C5aR2 in renal IRI, bone marrow chimeras were created. These results demonstrated that renal IRI is mediated by C5aR2 expressed on both renal epithelial cells and leukocytes.[10] The contribution of leukocyte- and renal-expressed C5aR2 in brain death is not investigated, but could have implications for future use of inhibitors of these receptors in the clinical setting. For example, targeting leukocyte-expressed C5aR2 prior to transplantation will not be effective during static cold storage (SCS) or machine perfusion. However, before C5aR2 can be considered as a potential target of intervention in brain-dead donors, it is of crucial importance to first verify the role of C5aR2 in human. In human, C5aR2 seems to be a receptor with dichotomous effects as triggering may either promote or attenuate inflammation, most likely dependent on the microenvironmental context.[8,11]

Recently, our research group demonstrated an increased expression of C5aR1 on renal tubular cells in brain-dead donors.[9,12] Our current study showed that the absence of C5aR1 during brain death appears to propagate renal injury, which suggests a potential protective effect of increased C5aR1 expression during brain death. Recent literature uncovered that C5aR1 expression and activation promoted cell proliferation after ischemic injury of cardiomyocytes, which might explain the upregulation of renal C5aR1 in brain death as a cell survival response.[13]

Lastly, the contribution of the complement activation pathways was elucidated. Absence of properdin, essential for AP activation, led to significantly less renal injury than WT mice after brain death, reflected by a lower tubular damage score and lower gene expression levels of IL-1ß, IL-6, IL-8 and MCP-1. Absence of C4, a component of the CP and LP, resulted in less tubular damage and lower renal pro-inflammatory gene expression levels. In addition, C4-deficient mice had significantly lower blood urea nitrogen levels and lower levels of circulating complement than WT mice upon brain death. So, blockage of all early complement components of the CP, LP and AP seems to protect against brain death-induced renal injury and inflammation. However, based on our results, the CP/LP seem to contribute the most to brain death-induced renal injury and inflammation, since C4-deficient mice were able to preserve renal function during brain death. The results seen in our mouse brain death model are further supported by the increased systemic levels of C4d in deceased human donors and the deposition of C4d in human renal biopsies of brain-dead donors.[14,15] These elevated systemic C4d levels were positively correlated with systemic C5b-9 levels in brain-dead donors. Interestingly, there was no association between systemic levels of MBL and sC5b-9, which suggests that the CP, and not the LP, seems to be involved in sC5b-9 release.[16]

Altogether, **chapter 3** shows that deficiency of upstream complement components of the CP/LP (partially) prevents against brain death-induced renal injury and inflammation. Overall, these findings suggest an important role for the upstream complement effector

functions (i.e. C3a and opsonization by C3b) in the induction of renal damage and inflammation upon brain death. So far, the effector functions of complement activation fragment C4a have remained elusive. C4a exerts its effector functions via protease-activated receptors (PAR)1 and PAR4.[17] Despite structural similarities with anaphylatoxins C3a and C5a, the functional profile of C4a remained unknown due to the complicated interpretation of the available data.[18] Several studies reported a role for C4a in smooth muscle contraction, chemotaxis and vascular permeability.[18–20] However, in all these studies there was possible contamination of C4a with C3a and C5a, which complicated the interpretation of these results, since observed effects might also be induced by C3a or C5a. Preliminary data from our research group suggests that C4a provokes cellular responses that reflect the physiologic role of an anaphylatoxin. In a pilot study, conditionally human immortalized glomerular endothelial cell line (CiGEnC) cells were incubated with purified C4a, which resulted in increased concentrations of IL-6 in supernatants of human CiGEnC cells (Supplementary Figure 1). These results might suggest that C4a might have functional properties similar to anaphylatoxins C3a and C5a. So not only the blockage of the CP/LP could explain the protective effect seen in C4-deficient mice upon brain death, but also the absence of C4a could play an important role.

An important question when treating brain-dead donors is whether other donor organs will benefit from the same treatment. A brain-dead donor could potentially donate heart, intestines, liver, lungs, and kidneys. In contrast to kidneys, less is known about the contribution of complement-mediated injury in heart, liver, lungs, and intestines upon brain death. Only the role of complement-mediated heart injury during brain death is described in literature. Atkinson *et al.* demonstrated that C3-deficient mice had significantly less cardiac damage than WT mice subjected to brain death, reflected by reduced serum levels of cardiac troponin I.[21] In addition, significantly less cardiac inflammation was seen, as demonstrated by a lower influx of leukocytes and lower gene expression levels of IL-1β, ICAM-1, TNF-α, and VCAM-1 after brain death.[21,22] In accordance, preliminary data from our mouse brain death model demonstrated that C3-deficiency resulted in significantly lower cardiac gene expression levels of IL-6 and MCP-1 (data not shown). Similar results were obtained in absence of complement C4 but not in absence of properdin, which points towards a role of the CP/LP in complement-mediated cardiac inflammation during brain death. In addition, Atkinson *et al.* looked at complement deposition patterns in human heart biopsies from brain-dead donors, which revealed co-localization of complement C3d and IgM complexes. These results also suggest an involvement of the complement CP in brain death-induced heart injury.[21]

So far, less is known about complement-mediated liver injury during brain death. In our mouse brain death model, preliminary data showed that livers obtained from C3-deficient mice had significantly lower gene expression levels of IL-6, TNF-α and BAX/Bcl-2 ratio (data not shown). C4-deficiency resulted in significantly lower IL-6 and TNF-α gene expression levels in livers after brain death. In addition, both C3- and C4-deficient brain-dead mice had significantly lower serum plasma levels of alanine transaminase (ALAT) and lactate

dehydrogenase (LDH). In contrast, properdin-deficient mice were not protected against brain death-induced liver injury.

In **chapter 4,** the contribution of the complement activation pathways in brain death-induced lung injury was examined. This study demonstrates that brain death results in the deposition of complement in lungs, as reflected by the increased C9 deposition in lungs of brain-dead WT mice compared to sham-operated WT mice. Lungs of C3-deficient mice were partially protected against the upregulation of pro-inflammatory genes and had a significantly lower influx of neutrophils than WT mice upon brain death. Properdin-deficient mice had significantly less histological lung injury after brain death, but complement levels and neutrophil influx were not affected. In C4-deficient mice, both systemic and local complement levels were significantly reduced compared to WT mice after brain death. Further, histological lung injury was significantly attenuated in C4-deficient brain-dead mice, reflected by less alveolar septal thickening, hemorrhage, intra-alveolar edema, and over-inflation. Lastly, lack of C4 resulted in a lower influx of neutrophils and lower gene expression levels of pro-inflammatory cytokines. Therefore, brain death-induced lung injury and inflammation in mice is predominantly mediated via the CP and/or LP. In contrast to other donor organs, it is possible to administer complement therapeutics in donor lungs via nebulization. The use of a nebulizer results in the direct drug delivery to the lung parenchyma, without affecting other donor organs. In addition, it is easily used and accessible, which makes it a promising approach. This is demonstrated by a study that administered a complement C3aR antagonist to a brain-dead donor via nebulization.[23] The C3aR antagonist was administered 30 minutes before organ procurement and resulted in significantly less lung inflammation, as demonstrated by reduced gene expression levels of pro-inflammatory cytokines IL-6, MCP-1 and TNF-α. So, nebulization could be a therapeutic strategy to reduce complement-mediated lung injury in brain-dead donors without affecting the other organs prior to procurement.

So, **chapter 3 and chapter 4** demonstrate a predominant role for the CP and/or LP in brain death-induced injury in hearts, livers, lungs, and kidneys. In contrast to kidneys and lungs, blockage of the AP activation did not protect against brain death-induced injury in hearts and livers. A potential explanation might be the ability of heart- and liver tissue to repair and regenerate in response to injury, in which the complement system seems to play a role. Although the exact contribution of complement in tissue repair and regeneration remains elusive, there is increasing evidence that complement-triggered pathways orchestrate regenerative responses.[24] Further research needs to focus on the complement-dependent balance between injury and regeneration in hearts and livers during brain death.

Altogether, the results from **chapter 3 and chapter 4** point towards a tailor-made approach, instead of a one-size-fits all approach, in order to protect organs against brain death-induced organ injury. A potential new strategy to reduce graft injury prior to transplantation might be the use of complement therapeutics during preservation rather than treating the entire donor. Treating only the donor organ during machine perfusion has several advantages: (i) the donor organ can be treated in an isolated manner, so it is possible to treat different donor organs with different complement therapeutics if desired, and (ii) organs are

perfused with a lower circulating volume, so lower amounts of drugs are needed, which will reduce the overall costs. So, hypothermic machine perfusion (HMP) and normothermic machine perfusion (NMP) provide a window of opportunity to administer complement therapeutics. However, the *ex vivo* set-up of machine perfusion might provoke complement activation itself due to the initial blood-to-material contact, which is also seen in other *ex vivo* circuits such as hemodialysis and cardiopulmonary bypass.[25,26] Currently, there is no literature available which demonstrates that complement is activated during HMP and NMP. A pilot study from our research group demonstrated that there is no complement activation during HMP, which is probably due to the decreased enzymatic activity at lower temperatures (data not shown). These preliminary results are in line with results from *in vivo* studies demonstrating that complement is not or to a lesser extent activated during hypothermia compared with normothermia.[27,28] Although complement is not activated during HMP, our hypothesis is that the use of a complement inhibitor during HMP could still prevent tissue injury, by priming the renal graft prior to transplantation. Our hypothesis is supported by a few recent studies, which demonstrated that the use of complement inhibitors during SCS of kidneys resulted in improved graft survival.[29–31] Especially complement inhibitors with membrane-binding properties showed beneficial results due to improved binding to the cell membrane bilayer, which allowed improved penetration into the renal tissue.[31] The penetration of complement inhibitors into the renal tissue seems to be important, since local synthesis of complement C3 plays a crucial role in complement-mediated renal ischemia-reperfusion injury as demonstrated by previous studies.[6] Therefore, the use of a complement inhibitor with membrane-binding properties during HMP might reduce renal IRI.[5,6]

NMP is the so-called 'new kid on the block' and currently tested as a preservation method in (pre)clinical studies. In theory, NMP has several advantages over HMP: (i) it mimics a near-physiological state, (ii) viability can be tested, and (iii) allows repair of injury.[32] In addition, already a few studies showed that longer periods of NMP, up to 16 hours of NMP, are superior to SCS.[32–35] So, preservation times can potentially be prolonged by the use of NMP. NMP is challenging since it requires perfusion with oxygen carriers (blood or blood components) under normothermic conditions which is intrinsically associated with increased risk for thrombus formation that in turn induces endothelial damage and an inflammatory response. To successfully implement NMP into the clinical setting, we need to gain insights into the cellular and molecular events and the underlying mechanisms related to renal injury inflicted upon *ex vivo* NMP. For this reason, **chapter 5** investigated the inflammatory response of the donor kidney during NMP. The preliminary results of **chapter 5** demonstrate that after NMP of both porcine kidneys and human discarded kidneys, there is a significant increase of complement (activation) levels in the perfusate. Complement activation during NMP might be beneficial because the complement activating capacity of the renal graft could be exhausted, which could reduce renal graft immunogenicity prior to transplantation. By doing so, complement is expected to be less activated during reperfusion post-transplantation, which could potentially improve renal allograft survival. However, these preliminary results show that complement perfusate levels are positively correlated with the perfusate levels of pro-

inflammatory mediators. Further, kidneys with high complement perfusate levels during NMP had a significant lower creatinine clearance. These results suggest that complement activation during NMP could result in tissue injury, which could potentially be reduced or prevented by the use of a complement therapeutic during NMP. To exhaust the capacity to activate complement, but to minimize tissue injury, it would be interesting to use a terminal pathway inhibitor. In this way, complement is activated up to the point of intervention, but the effector functions of complement are inhibited by the complement therapeutic.[36,37] So, NMP could be a platform which (i) reduces renal graft immunogenicity prior to transplantation by exhausting the capacity to activate complement and (ii) reduces complement-mediated injury by the use of a complement therapeutic. Lastly, this study showed that renal grafts retrieved from brain-dead donors have higher complement C3d/C3 ratio in perfusate than renal grafts derived after circulatory death. The significant higher C3d/C3 ratio might be the result of the increased immunogenicity of brain-dead donors compared to donation after circulatory death. Brain death itself results in the activation of the immune system, which results in both systemic and local inflammation.[38] Therefore, we postulate that kidneys retrieved from brain-dead donors are more likely to provoke and activate an inflammatory response during NMP.[15,39,40] So, high complement perfusate levels during NMP could imply that the complement activating capacity is exhausted. However, these high complement perfusate levels could exacerbate renal injury during NMP. Especially renal grafts from brain-dead donors could benefit from treatment with complement inhibitors during NMP.

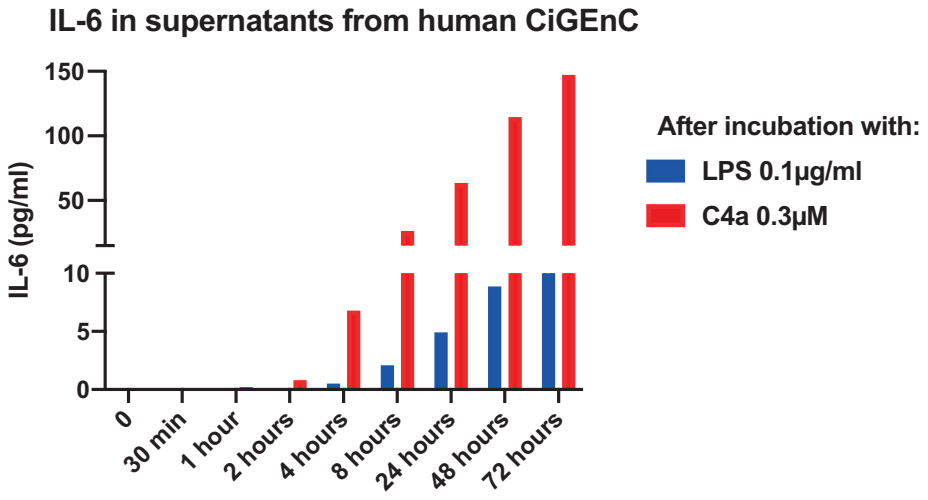
Chapter 6 provides an overview of complement therapeutics used in the multi-organ donor or during *ex vivo* preservation of donor organs. As outlined in this chapter, many complement therapeutics are under development, but only a few of them have made it to the clinics so far. One of the complement therapeutics already implemented in the clinics is C1-inhibitor (C1-INH).[41] C1-INH is well known because of the use as a prophylaxis and treatment for hereditary angioedema (HAE). C1-INH is a serine protease inhibitor that inactivates the CP via C1r and C1s and the LP via MASP-1 and MASP-2. *In vitro* studies showed that C1-INH can also downregulate the AP.[42–44] In **chapter 7**, C1-INH was tested as a therapy to reduce renal injury as a result of brain death. C1-INH was tested in an established model of brain death and intravenously administered in rats after the confirmation of brain death. C1-INH effectively reduced complement activation levels, which resulted in less histological injury and reduced systemic levels of IL-6. In addition, C1-INH preserved renal function during brain death. Recently, C1-INH was tested in a nonhuman primate model of brain death to prevent delayed graft function post-transplantation. Brain death was induced in macaque monkeys and C1-INH was administered every 3 hours as an intravenous bolus injection. After 20 hours of brain death, kidneys were procured and stored on ice for 43-48 hours and subsequently implanted in the recipients. Administration of C1-INH in brain-dead macaque monkeys significantly reduced complement activation via the CP, decreased circulating levels of TNF-α and MCP-1 and prevented delayed graft function compared to the placebo-treated macaque monkeys.[45] So, C1-INH showed promising results in animal models of brain death. Currently, plasma-derived C1-INH is tested in a phase I randomized controlled trial study as a treatment in human

brain-dead donors (NCT02435732). The use of C1-INH in transplantation may have several benefits over other complement inhibitors.[43,44] First, C1-INH is an upstream complement inhibitor, thereby inhibiting almost all effector functions of the complement system.[43] Second, C1-INH prevents the activation of the CP by donor-specific antibodies, which makes C1-INH also a promising target in later stages after transplantation.[46,47] Third, C1-INH is not an isolated complement inhibitor, it is also a major regulator of the coagulation and contact system.[44] C1-INH has shown to reduce vascular permeability and inhibit leukocyte adhesion to the endothelium, phenomena that both contribute to renal graft injury during brain death.[43] Lastly, C1-INH seems to improve the quality of multiple donor organs, including the liver and lungs.[29,48,49] Therefore, C1-INH could potentially soon be used in the donor to reduce organ injury prior to transplantation.

In **chapter 8**, a recently developed monoclonal antibody against factor B, so-called anti-FB, was tested in a murine model of brain death. Treatment of with anti-FB reduced systemic levels of complement and lowered renal complement deposition upon brain death. In addition, treatment with anti-FB preserved renal function, as reflected by significantly lower serum creatinine levels. Further, anti-FB treated rats had less renal injury and lower renal gene expression levels of pro-inflammatory mediators such as IL-6, MCP-1 and VCAM-1. So, treatment of rats with anti-FB reduces brain death-induced renal injury and inflammation. Previous studies in mice demonstrated that inhibition of factor B protected kidneys against renal IRI.[50,51] Together, these results suggest factor B might be an interesting target of intervention to reduce renal injury during transplantation. A small molecule factor B inhibitor, called LNP023, is currently in clinically tested as a treatment for a number of complement-mediated diseases, including IgA nephropathy and membranous nephropathy.[52] LNP023 is also interesting as a potential complement therapeutic in brain-dead donors. A single dose of LNP023 was shown to reduce AP activity with 80% or more within 2 hours.[53] The use of a small-molecule might be attractive, since antibodies have a larger size, low tissue penetration and high manufacturing costs.

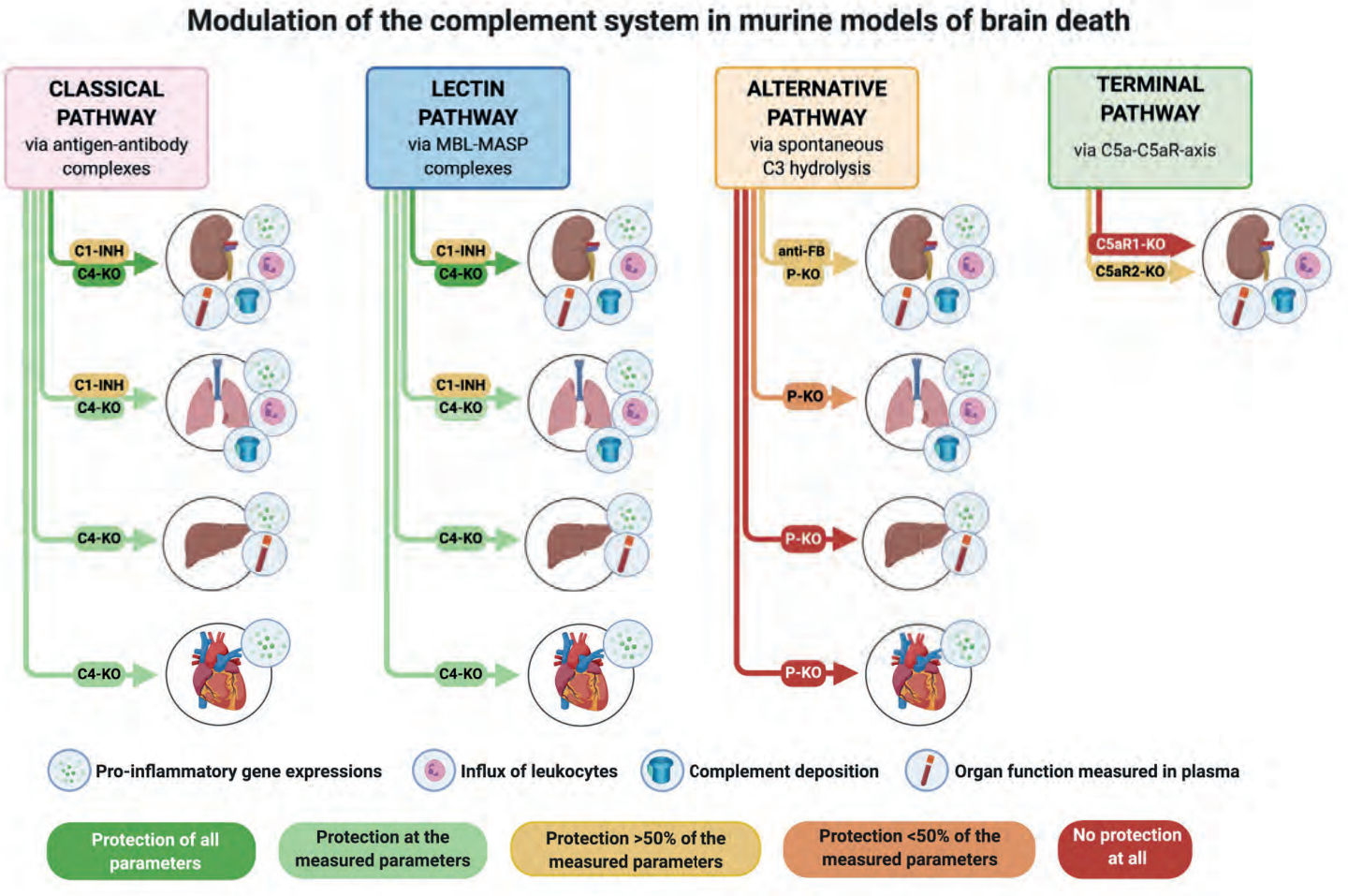
Overall, this thesis demonstrates that inhibition of each of the three complement activation pathways is effective in (partially) protecting kidneys against brain death-induced injury. However, based on our results, the AP seems to be an amplifier of complement activation, whereas the CP and/or LP are the initiators of complement activation during brain death. Therefore, inhibition of the CP and/or LP can potentially prevent brain death-induced renal injury, whereas inhibition of the AP is expected to reduce brain death-induced injury.

Supplementary Figure 1



Supplementary Figure 1. IL-6 concentrations in supernatants from human CiGEnC cells after incubation with C4a or LPS.

IL-6 concentrations in supernatants of human conditionally immortalized glomerular endothelial cell line (CiGEnC) cells with C4a (0.3µM) or lipopolysaccharide (LPS; 0.1µg/ml) up to 72 hours after incubation.


Modulation of the complement system in murine models of brain death
CLASSICAL PATHWAY
via antigen-antibody complexes
LECTIN PATHWAY
via MBL-MASP complexes
ALTERNATIVE PATHWAY
via spontaneous C3 hydrolysis
TERMINAL PATHWAY
via C5a-C5aR-axis
C1-INH
C4-KO
C4-KO
C1-INH
C4-KO
C4-KO
C1-INH
C4-KO
C4-KO
anti-FB
P-KO
P-KO
P-KO
P-KO
C5aR1-KO
C5aR2-KO
Pro-inflammatory gene expressions
Influx of leukocytes
Complement deposition
Organ function measured in plasma
Protection of all parameters
Protection at the measured parameters
Protection >50% of the measured parameters
Protection <50% of the measured parameters
No protection at all

Graphical summary of this thesis: effects of complement modulation in murine models of brain death per complement pathway and per organ.

On top, the complement pathways: the classical pathway, the lectin pathway, the alternative pathway and the terminal pathway. The arrows indicate how the pathway was blocked and in which organ. The color of the arrow indicates the effect of intervention on brain death-induced organ injury: green, protection seen for all parameters; light green, protection seen for all parameters; yellow, protection of more than 50% of the parameters; orange, protection of less than 50% of the parameters; red, no protection at all. Next to the organ, the parameters which were investigated, including: pro-inflammatory gene expression levels, influx of leukocytes, complement deposition and organ function measured in plasma. Abbreviations: anti-FB, anti-factor B; C1-INH, C1-inhibitor; KO, knock-out; P, properdin. Created with BioRender.com.

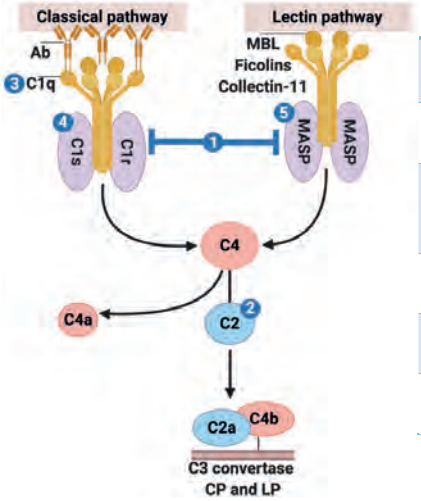
Future perspectives

Complement inhibition in brain-dead donors

This thesis demonstrated that predominantly the CP and/or LP contribute to brain death-induced organ injury and inflammation in rodents. To translate our findings to the clinics, the next step is to find the optimal complement therapeutic which fits our findings. Looking at potential complement therapeutics which can be used in brain-dead donors, it should target the CP, the LP or both. Besides target selection, other criteria should be taken into consideration, such as drug modality, drug concentration and route of delivery.

Brain death results in the systemic activation of complement that induces a systemic, and subsequently local intra-organ inflammatory response, which affects all organs. Ideally, brain-dead donors should be treated with a complement therapeutic that is administered intravenously, so it targets circulating complement components. The required dose of a complement therapeutic depends on (i) the circulating volume of the targeted complement component and (ii) the pharmacokinetics of the complement therapeutic.

Looking at the available complement therapeutics that target the CP and/or LP, there are currently 5 potential candidates (Figure 1). Two of these complement inhibitors target both the CP and LP. The first is C1-INH (Figure 1; 1), which is currently clinically tested as a therapy to reduce complement-mediated renal injury during brain death.[45,54] The second one is PRO-02 (Figure 1; 2), which is an inhibitor of complement C2. Similar to complement C4, complement C2 plays a critical role in the formation of the C3 convertase of the CP and LP.[55] A potential benefit of targeting C2 instead of C4 is the fact that serum concentrations of C2 are two times lower than serum concentrations of C4.[56] The other complement therapeutics listed in Figure 1 inhibit either the CP or the LP.[57–59] ANX005/007 is a monoclonal antibody that targets CP component C1q (Figure 1; 3). ANX005 is successfully tested in a phase 1b clinical trial in Guillain-Barré syndrome.[57] TNT009, also known as sutimlimab, is the second CP inhibitor (Figure 1; 4). TNT009 is a monoclonal antibody that targets C1s of the CP. TNT009 is currently tested in a phase 3 study in patients with cold agglutinin disease.[60] Lastly, OMS721 is listed (Figure 1; 5), which targets MASP-2 of the LP. OMS721 is currently tested in a phase 3 study in patients with IgA nephropathy. These pathway specific inhibitors could be used to differentiate between the contribution of the CP and LP in brain death-induced renal injury. It is important to differentiate between the different pathways because in human, but not in mice, MASP-3 of the LP can activate pro-factor D of the AP.[61] So, in human a link between the LP and AP. As mentioned before, the AP accounts for approximately 80% of the complement activation, as a result of amplification.[62] Therefore, there is the possibility that inhibition at the level of C2/C4 in human might not give similar results as seen in mice. If the LP initiates brain death-induced renal injury, it should be inhibited upstream from MASP-3 to avoid AP activation.



#	Name	Target	Pathway	Type	(pre)clinical phase
1	C1-INH	C1s, C1r MASP-1,-2	CP LP	Protein	FDA approved
2	PRO-02 (Prothix; Broteio)	C2	CP LP	Ab	Preclinical
3	ANX005/007 (Annexon)	C1q	CP	Ab	Phase 1B
4	TNT009 (Sutimlimab)	C1s	CP	Ab	Phase 3
5	OMS721 (Omeros)	MASP-2	LP	Ab	Phase 3

Figure 1. Overview of available complement therapeutics targeting the classical and/or lectin pathway.
The currently available complement therapeutics targeting both the classical and lectin pathway are (1) C1-INH and (2) PRO-02. Therapeutics that target the complement classical pathway are (3) ANX005/007 and (4) TNT009. (5) OMS721 inhibits the lectin pathway via MASP-2. Abbreviations: CP, classical pathway; LP, lectin pathway. Created with BioRender.com.

Complement inhibition during normothermic machine perfusion

In addition to the findings based on our murine models of brain death, this thesis showed that complement is activated during NMP of porcine and human kidneys. So, NMP could be a platform which (i) reduces renal graft immunogenicity prior to transplantation by exhausting the capacity to activate complement and (ii) reduces complement-mediated injury by the use of a complement therapeutic.

In contrast to targeting complement in brain-dead donors, complement-based therapeutics during NMP should target downstream complement components. Downstream complement components are C3, C5 or the membrane attack complex (MAC). Targeting downstream complement components will result in activation of complement up to the level of intervention, which will exhaust the complement activating capacity of the renal graft during NMP. However, the complement effector functions are inhibited by the complement therapeutic. Intervention at the level of C3 attenuates the formation of C3 and C5 convertases, which prevents the formation of the amplification loop via the AP and prevents the cleavage of C5. Currently, multiple promising drugs that target C3 are under development. One of these is TT30, which is an engineered fusion protein that consists of a complement receptor 2 domain, which binds to complement activation fragments iC3b/C3dg. In addition, TT30 consists of a factor H domain, which has complement-regulatory properties. With the complement receptor 2 domain, TT30 localizes the site of complement activation, after which the factor H domain inhibits complement activation.[30] TT30 could be administered intravenously immediately after the start of NMP (Figure 2;1). Another promising complement

therapeutic is a recently engineered mini-factor H construct, which inhibits at the level of C3.[63] Mini-factor H could infused in during NMP or the tubes can be coated with mini-factor H prior to NMP of the renal graft (Figure 2;2). In addition, intervention targeting at the level of complement C5 could be a promising strategy to reduce renal injury during *ex vivo* NMP. A well-known monoclonal antibody against complement C5 is Eculizumab, also known as Soliris.[36] However, the success of Eculizumab resulted in the development of various complement therapeutics targeting C5, C5 convertases, C5a or the C5a receptors.[36] Although the contribution of the C5a-C5aR-axis on renal injury during *ex vivo* NMP is not investigated yet, we postulate that targeting C5 could be a promising strategy. Cleavage of complement C5 results in the formation C5a and eventually the formation of the MAC. Both C5a and the MAC can trigger a plethora of pro-inflammatory signaling pathways, which contribute to renal inflammation.[10,64] Therefore, targeting C5 during *ex vivo* NMP is a promising strategy to prevent complement-mediated renal injury.

Overall, *ex vivo* NMP provides a window of opportunity to assess and recondition the quality of the renal graft prior to transplantation. The use of complement therapeutics targeting downstream complement activation products during NMP seems to be a promising to attenuate complement-mediated renal injury prior to transplantation.

Conclusion

This thesis unraveled an important role for the classical and/or lectin pathway in brain death-induced renal injury and inflammation in mice. Looking at the contribution of the C5a-C5aR-axis, only inhibition of the C5a-C5aR2-axis significantly reduced renal injury and inflammation upon brain death. However, blockage of these downstream complement components in order to prevent brain death-induced renal injury gave inferior results compared to inhibition of the upstream complement components. Therefore, targeting early complement components from the classical and/or lectin pathway might be a promising strategy to reduce brain death-induced renal injury. Inhibition of complement during normothermic machine perfusion might be a novel approach to modulate complement to reduce renal graft immunogenicity prior to transplantation.

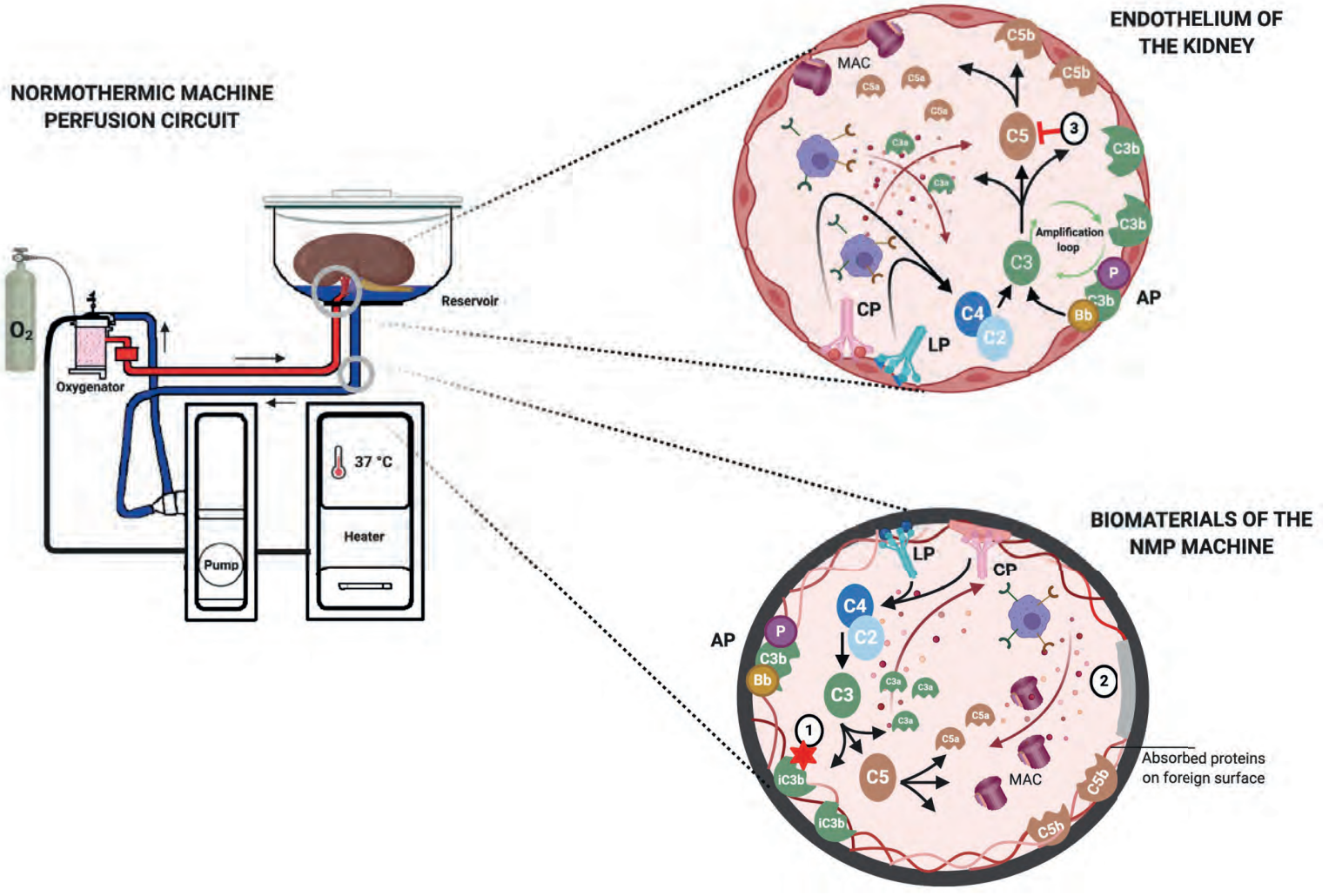

NORMOTHERMIC MACHINE
PERFUSION CIRCUIT
O_2
Oxygenator
Reservoir
Pump
37 °C
Heater
ENDOTHELIUM OF
THE KIDNEY
MAC
C5b
C5a
C5
3
C3b
C3a
Amplification
loop
C3
P
Bb
AP
CP
LP
C4
C2
BIOMATERIALS OF THE
NMP MACHINE
1
2
iC3b
Absorbed proteins
on foreign surface

Figure 2. Overview of potential strategies to inhibit complement during *ex vivo* normothermic machine perfusion.

Complement therapeutics in *ex vivo* normothermic machine perfusion should target downstream complement components. A few promising complement therapeutics are (1) TT30, which is an engineered fusion protein that binds to complement activation fragment iC3b and regulates complement activation via its factor H domain; (2) coating of the surface of the machine with a mini-factor H construct that has complement inhibiting properties at the level of C3; (3) Inhibition of complement C5. Abbreviations: AP, alternative pathway; CP, classical pathway, LP, lectin pathway; MAC, membrane attack complex, NMP, normothermic machine perfusion.

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